#AE21MAD

aquaculture europe 21

OCEANS OF OPPORTUNITY

Madeira, Portugal

October 4-7, 2021

Madeira Belongs to all

BioMar
Gold Sponsor

BLUE INVEST
Session Sponsor

Supported By

REPÚBLICA PORTUGUESA
MAR

Região Autónoma da Madeira
Governo Regional

arditi agência regional para o desenvolvimento da investigação tecnologia e inovação

Associação de Promoção da Madeira Promotion Bureau

eas european aquaculture society

www.aquaeas.org

TABLE OF CONTENTS

To find abstracts for a specific author or subject, use the pdf search features built into Adobe Acrobat.

aquaculture europe 21

ABSTRACTS

POLYCHAETE *Nereis virens* MEAL CAN INCREASE FEED EFFICIENCY IN FARMED ATLANTIC SALMON

Turid Synnøve Aas[1]*, Lars Thoresen[2], Aleksei Krasnov[3], Lene Sveen[3], Gunhild Johansson[4], Katerina Kousoulaki[2]

[1] Nofima, Sjølsengvegen 22, NO-6600 Sunndalsøra, Norway
[2] Nofima, Kjerreidviken 16, NO-5141 Fyllingsdalen, Norway
[3] Nofima, Osloveien 1, NO-1433 Ås, Norway
[4] Nofima Muninbakken 9-13, NO-9019 Tromsø, Norway
E-mail: synnove.aas@nofima.no

Atlantic salmon were fed practical diets containing 0 (control), 7.5% (7.5PC) or 15% (15PC) spray dried polychaete meal replacing fish meal in the control diet. The experimental diets were formulated to be balanced for total protein, lipids and EPA+DHA, and Lys, Met, His and Thr were balanced with crystalline amino acids. Y_2O_3 was added as an inert digestibility marker. The diets were fed to triplicate groups of salmon (salt water 32 ‰, 12 ˚C) for 63 days. Mean initial body weight was 70 g and mean final body weight was 204 g.

Feed intake was significantly higher in salmon fed the control diet than those fed 15PC, with those fed 7.5PC in an intermediate position. Specific growth rate (SGR) followed the same pattern (not significantly different). Interestingly, the feed conversion ratio (FCR) was 0.73, 0.70 and 0.68 in salmon fed control, 7.5PC and 15PC, respectively. This indicates effective utilization of the polychaete meal in salmon feed.

Apparent digestibility of energy and main nutrients was measured. The body composition was analyzed for estimation of retention of energy and main nutrients. Intestinal tissue was sampled for histologic evaluation, and for micro-array analysis of gene expression. The results from these analyses will be presented.

The polychaete meal was a nutritionally excellent feed ingredient for salmon in the present trial. Polychaeta can be produced from waste material and is therefore interesting in the context of circular economy. However, the available produced polychaete meal volumes are still low and come to a higher price as compared to fish meal. Thus, to become a commercially interesting feed ingredient, methods for production of large volumes at a competitive price need to be developed.

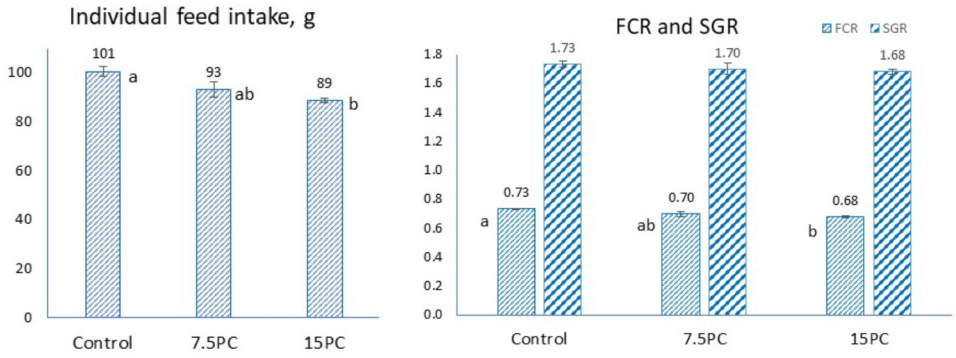


Figure 1. Individual feed intake (g; left panel), feed conversion ratio (FCR) and specific growth rate (SGR; right panel) in Atlantic salmon fed diets containing 0 % (control), 7.5% (7.5PC) or 15% (15PC) polychaete meal.

TOWARDS SUSTAINABLE JELLYFISHERIES IN EUROPEAN WATERS WITH SPECIAL EMPHASIS ON THE TRONDHEIMSFJORD AS A CASE STUDY

Aberle N*[1], Andrade C[2,3,4], Angel DL[5], Canning-Clode J[6,7], Dierking J[8], Dror H[5], Edelist D[5], Ellingsen I[9], Gueroun SKM[3,4,6], Klun K[10], Leone A[11], Majaneva, S[1], Rotter[10], A Tiller R[9], Javidpour J[12]

[1]Norwegian University of Science and Technology (NTNU), Department of Biology, Norway
[2]CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), Matosinhos, Portugal
[3]Madeira Oceanic Observatory – ARDITI/OOM, Funchal, Madeira, Portugal
[4]Mariculture Centre of Calheta, Madeira, Portugal
[5]University of Haifa, School of Marine Sciences, Israel
[6]MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI, Funchal, Madeira, Portugal
[7]Smithsonian Environmental Research Center, MD 21037, USA
[8]GEOMAR Helmholtz Center for Ocean Research, Kiel, Germany
[9]SINTEF Ocean, 7465 Trondheim, Norway
[10]National Institute of Biology, Marine Biology Station Piran, Fornače 41, 6330 Piran, Slovenia
[11]National Research Council, Institute of Science of Food Production (CNR, ISPA), Lecce, Italy
[12]University of Southern Denmark, Department of Biology, Odense, Denmark

*E-mail: nicole.aberle-malzahn@ntnu.no

***Introduction**:*
Jellyfish are an essential pelagic ecosystem component that can form dense blooms over large spatiotemporal scales. Intensity, frequency, and duration of jellyfish blooms vary, show strong species- and site-specific differences and are often considered to have negative impacts on local industries, tourism and artisanal fishery1. Demands for jellyfish products for e.g. aquaculture feed, food, cosmetics or pharmaceuticals, are increasing thus providing an opportunity to turn them into a resource2. But, a lack of ecological knowledge on the drivers of jellyfish blooms limits a sustainable, cost-efficient harvesting and management of jellyfish. Within the EU-project GoJelly, the aim was to provide a basis for ecosystem-based management taking the knowledge gaps related to jellyfish ecology, bloom dynamics, and origin of seed populations into account. More reliable predictions on jellyfish blooms and tools for more sustainable harvesting of jellyfish should be achieved by focusing on the biotic and abiotic drivers that promote jellyfish blooms.

***Material & Methods**:*
From 2018-2020, seven target jellyfish species with broad spatiotemporal distribution ranges in European waters were harvested in several GoJelly study areas (Norwegian Sea, Baltic Sea, Mediterranean Sea and Eastern Atlantic). Data on the fishing methods (e.g. vessel, fishing gear, fishing effort) and activities (e.g. harvesting period, fishing duration, target species and life-stages, post-harvesting biomass processing) were recorded evaluating jellyfish fishery processes.

***Results**:*
Overall, three tons of jellyfish were harvested and 60% of the total biomass was harvested in the Trondheimsfjord, Norway. Interannual variation in harvested biomass occurred due to differences in harvesting efforts and bloom intensities of the target species (Figure 2A).

Nine jellyfish species were harvested (Figure 2B), three of them representing 80% of the total catch (44% *Periphylla periphylla,* 18% *Rhizostoma pulmo,* 17% *Aurelia* spp.). Different types of fishing vessels were used (from small-sized open boats to medium-sized research vessels). Jellyfish were harvested using six gear types (gillnets, bottom trawls, pelagic trawls, landing nets, hand nets, 20-L buckets). The mesh size varied from 0.35 to 50 mm. The gear type reflected the harvesting depth as well as the vessel type.

Discussion & Conclusion:
Jellyfish resources in Europe are currently not managed, in comparison to e.g. finfish fisheries thus hampering the sustainable, cost-effective harvesting of this biomass. In GoJelly we employed an ecosystem-based management approach based on jellyfish ecology, bloom dynamics, and the origin of seed populations. The aim was to analyse biotic/abiotic drivers that promote jellyfish blooms (e.g. in Trondheimsfjord), establish bloom predictions and sustainable harvesting tools. In this study, we address the knowledge gaps related to jellyfish fisheries and demonstrate future needs towards ecosystem-based management of local jellyfish resources.

(Continued on next page)

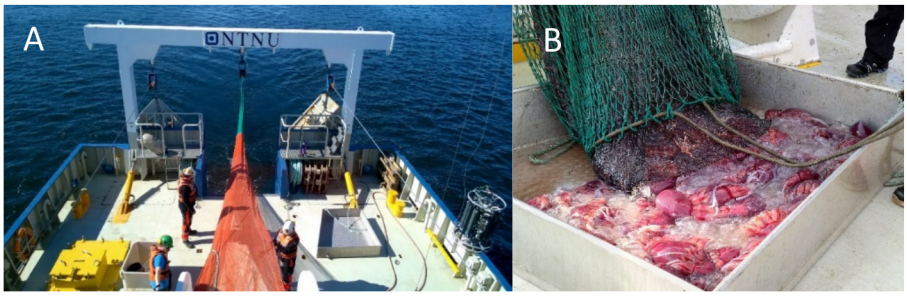


Figure 1 (A) Jellyfish fisheries in the GoJelly case area Trondheimsfjord, Norway, and (B) Jellyfish catch using bottom trawling.

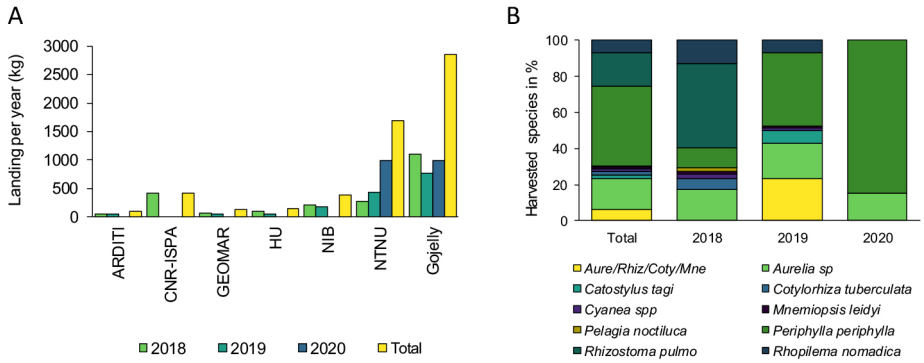


Figure 2 Total biomass (A) and proportion (B) of harvested jellyfish biomass by GoJelly partners from 2018-2020. Abbreviation: Aur., Aurelia sp, Rhiz., Rhizostoma pulmo, Coty., Cotylorhiza tuberculata, Mne., Mnemiopsis leidyi.

References:

[1] Purcell, J. E., Uye, S.-I., and Lo, W. T. (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review, *Mar Ecol Prog Ser 350*, 153-174.

[2] Bosch-Belmar, M., Milisenda, G., Basso, L., Doyle, T. K., Leone, A., and Piraino, S. (2020) Jellyfish Impacts on Marine Aquaculture and Fisheries, *Reviews in Fisheries Science & Aquaculture 29*, 242-259.

MARITIME SPATIAL PLANNING PRINCIPLES AND SUSTAINABLE AQUACULTURE DEVELOPMENT: CASE STUDIES IN MADEIRA AND CANARY ISLANDS

A. Abramic[1], S. Kaushik[1], A. García Mendoza[1], N. Nogueira[2,3,4], C. Andrade[2,3,4], R. Haroun[1]

[1]BIOCON, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Spain
[2]Mariculture Center of Calheta, Fisheries Directorate, Madeira, Portugal
[3]CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal
[4]Secretaria Regional de Mar e Pescas, Direção Regional do Mar, Madeira, Portugal
andrej.abramic@ulpgc.es
Scientific & Technological Marine Park, Universidad de Las Palmas de Gran Canaria, Ctra. de Taliarte s/n, E-35214 Telde, Spain

Introduction

Accessibility to marine space is one of the major bottlenecks for the marine aquaculture sector. A methodology was defined for the implementation of MSP processes associated with marine aquaculture facilities in two Eastern Atlantic archipelagos: Madeira & the Canary Islands. The search for the most suitable marine locations is linked to 5 parameter clusters: oceanographic potential; environmental sensibility; restrictions related to marine conservation; Land-Sea interactions and avoidance of potential conflicts with extant maritime and coastal activities.

Materials and methods

A methodological approach has been designed to identify suitable marine areas with significant potential considering physical oceanographic parameters (temperature, depth…), minimizing impact on the marine environment (structured as Good Environmental Status defined by Marine Strategy Framework Directive 2008/56/EC), (in)compatibility with marine conservation (marine protected areas under the Natura 2000) and avoiding conflicts with operative maritime and coastal sectors (as coastal tourism, fisheries, aquaculture, maritime transport, etc.). We have applied this framework in the cases of Madeira (Portugal) and Canary Islands (Spain) in the Eastern Atlantic Ocean.

Results

Aquaculture Suitability maps were developed based on the outputs of the Decision Support System of INDIMAR, which analyzed potential aquaculture production sites in relation with each of the 5 cluster parameters, introducing weights as appropriate calculated using an Analytical Hierarchy Process. The applied approach strikes a balance for all the five clusters reflecting on Ecosystem Based Management components that should be mirrored in the Maritime Spatial Planning strategy, including options with trade-offs regarding sectoral growth, conflict prevention and environmental protection and conservation.

Finally, results are compared with the extant aquaculture areas included in both the Madeira Zones Areas of Interest for Aquaculture that were adopted by the National Spatial Maritime Plan, based on the study delivered by Torres and Andrade (2010), and the Regional Plan of Management of the Canarian Aquaculture approved in 2018.

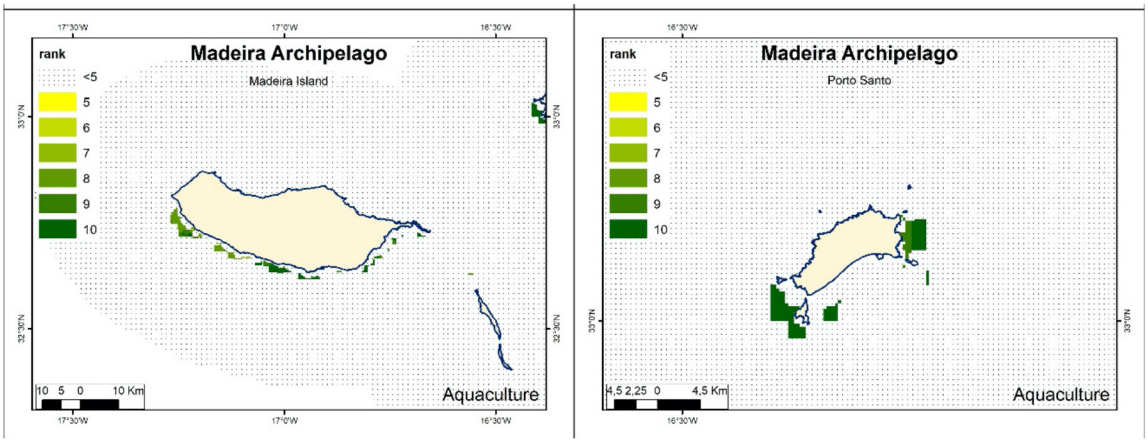


Figure 1: Aquaculture suitability maps for the Madeira and Porto Santo. Analysis delivered by INDIMAR Decision Support System considering physical oceanographic parameters, marine environment & conservation, avoiding conflicts with operative maritime and coastal sectors.

(Continued on next page)

Acknowledgements
This research was funded through the Interreg project PLASMAR (MAC/1.1a/030) and finalized within the project PLASMAR+ (MAC2/1.1a/347) with the support of the European Union (EU), and mainly financed by the European Regional Development Fund (ERDF) and the INTERREG V-A Spain-Portugal MAC 2014–2020 (Madeira-Azores-Canarias).

References
Torres, C. and Andrade, C. (2010) Spatial decision Analysis Process for selection Marine Aquaculture suitable zones: The example of Madeira Island. Journal of Integrated Coastal Zone Management, 10(3): 321-330.

GROUNDWORK FOR OFFSHORE AQUACULTURE IN AN ISLAND NATION, THE CASE OF THE REPUBLIC OF CYPRUS

R. Abualhaija[1*], S. Charalambous[2], M. Demetriou[3], G. Triantaphyllidis[4], G. Triantaphyllou[5], M. Menikou[6], V. Papadopoulos[7], I. Kyriakides[4]

[1]Cyprus Subsea Consulting and Services C.S.C.S. Ltd, Cyprus
[2]Geomatic Technologies Ltd, Cyprus
[3]Oceanography Centre- University of Cyprus, Cyprus
[4]University of Nicosia Research Foundation, Cyprus
[5]Hellenic Centre of Marine Research, Greece
[6]Frederic Research Centre, Cyprus
[7]Department of Fisheries and Marine Research, Cyprus
*rana.a@cyprus-subsea.com

Introduction

The operation of open sea aquaculture (OSA) should be ecosystem-based, balancing ecological, economical, and social objectives for sustainable development. To tackle these issues we formed a consortium of six partners in a project that will and lay the groundwork of OSA in Cyprus. The project (OS-AQUA, 2020-2023) addresses marine spatial planning, the legislation framework, location selection through modeling, offshore aquaculture technologies, finfish species selection, environmental monitoring, and business development. The overall aim is to lead the way to economically and ecologically sustainable OSA business in Cyprus. This document discusses the milestones and developments of the project.

Setting the Scene: Marine Aquaculture in Cyprus

The first commercial-scale marine aquaculture unit in Cyprus was opened in 1989. Increased water temperatures allow the faster development of farmed species and production cycles can cover all seasons. The ultra oligotrophic waters of the Eastern Mediterranean Basin offer excellent water quality conditions. Cypriot coastline is unaffected by human nuisance or pollution and provides natural weather protection to OSA operations as opposed to the southern rim of EMED. Today, aquaculture in Cyprus accounts for about 85% of the total amount of Cypriot fishery production both in quantity and value.

Marine Spatial Planning and Legal Framework

National authorities use marine spatial planning to allocate space among the multiple users of the sea. The project sourced geospatial data of and planned project in the coast of Cyprus. The information was incorporated into a customized geographic information system. The system will be the basis of a reviewed National Marine Spatial Plan which will pave the way to a legal and regulatory framework to designate zones and facilitate the operation of OSA.

Ecological implications of OSA development

The HCMR Aquaculture Integrated Model (AIM) has been used to improve planning and management of OSA in Cyprus. Three nested submodels at 500m resolution were implemented to evaluate the effect of existing fish farms on the coastal environment of Cyprus (Kalaroni et al. *in-prep*). A finer-scale sub-model of targeted areas will be implemented to assess the future OSA units. The area most suited for the installation of an OSA unit in Cyprus will be further studied. An onsite survey will collect data in-line with the national regulation for aquaculture environmental assessment. A deep-water buoy will be placed as part of the suggested ongoing monitoring network for the development of OSA in Cyprus. In situ data will be used to ground truth modeling and satellite data.

Aquaculture unit design

Husbandry, robotics, artificial intelligence, and innovative materials have aided in advancement of OSA operation. Existing and new finfish species, cage and mooring designs, feeding systems, operational vessels, as well as innovative cage and fish monitoring systems, along with a new cage designed for the local conditions will be assessed for their use in Cyprus. The final proposals from this exercise will be taken into consideration in the bankable projects.

Bankable Projects

A risk analysis will reveal the economic performance of varying input parameters from sections 3, 4 and 5. A selection of the bankable demonstration projects will become the basis of detailed Business Plans which would inform industry and financing institutions interested in investing in the OSA in Cyprus.

(Continued on next page)

Challenges and Opportunities

The European Union supports sustainable aquaculture development financial and regulatory incentives. In line with these strategies, Cyprus includes open sea aquaculture and the opening of new markets as priority goals for the next decade (DFMR 2020). Nevertheless, the interdependency of parameters and the interests of multiple marine users need to be taken into consideration. To bridge this gap, stakeholders, regulatory bodies and investors will be brought together to discuss, offer opinions and solutions to this complex issue.

Conclusion

Given proper planning and encouragement, growth of the aquaculture sector in Cyprus will generate jobs and strengthen small companies that will design, install, operate, maintain and monitor the marine facilities, and attract investors to the island. Succeeding in this endeavor would significantly expand a profitable business sector leading to significant increase in food exports. The EAS conference is an excellent forum to gain insight and inform project outcomes.

References

Department of Marine Research -DFMR (2020) Multiannual National Aquaculture Plan, Cyprus (2021-2030)

Kalaroni S., Tsiaras K. Triantaphyllidis G., Pollani A., Triantafyllou, G. Environmental assessment model for ecosystem and oceanographic modelling for site selection in offshore areas in Cyprus. (*in-prep*)

Trichoderma viride PEPTAIBOL ACCELERATES APOPTOSIS IN *Aspergillus niger* INFECTING FISH IN AQUACULTURES

Iman Kamel Abumourad[1], Marwa Gamal[2], Mohamed Abou Zaid[2], Al-Zahraa Ahmad Karam El Deen[3], Hussein Abd El Kareem[2], Ola M. Gomaa[2*]

[1]Veterinary Division, National Research Center, [1]Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), [3]Microbiology Department, Faculty of Science, Ain Shams University.

Introduction

aquaculture fish activity encounters many environmental problems. Two of environmental challenges fish farming faces are 1) fungal infection of fish and 2) lead contamination of aquaculture water.

Materials and methods

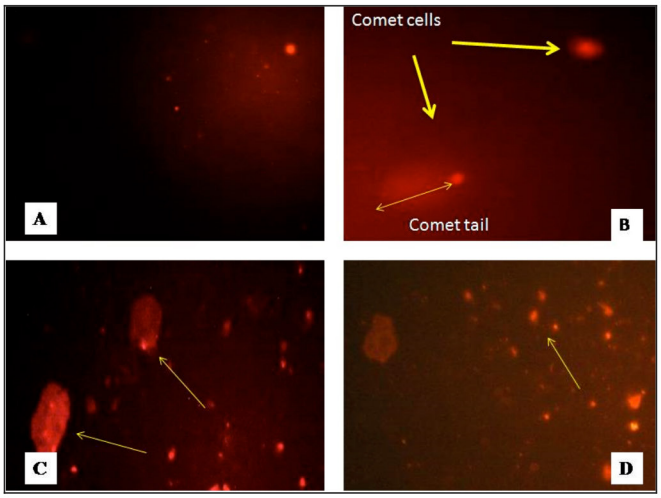
Trichoderma viride isolated from soil was used to produce peptaibol under shaking conditions at pH 6, temperature 30°C for 7 days in malt extract media.

Results

Incubating the extract with the *A. niger* induced catalase production, protein content and lipid peroxidation. The caspase 3 level was increased in fungus incubated with the fungal extract. The extract was characterized by boiling, Fourier Transform Infra-red Spectroscopy (FTIR) and Liquid Mass (UPLC/MS/MS) Chromatography 1294 m/z, the results show that the extract is a non-enzymatic protein that closely resembles peptaibol in structure. The extract was incubated with fish cell line and the results of DNA showed that 20 μl of the extract was effective in inducing apoptosis in *A. niger* and at the same time no changes were induced in the fish. This proves that the extract is safe to use in aquacultures.

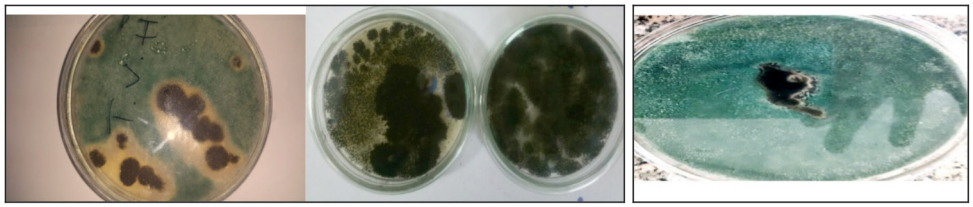
Conclusion

The extract of *Trichoderma virde* has proved an efficacy in causing apoptosis, not mycostatic effect, in *Aspergillus niger* which is one of the main pathogens infecing fish in aquaculture. The results show that the extract defined as peptaibol was effective under optimized conditions. The extract also did not affect the fish or caused any toxicity which is a very important aspect when using a biocontrol agent. This work is part of another work that involves the use of *Trichoderma virde* in removal of lead in aquaculture which is another major problem that causes loss of fish in Aquaculture in Egypt.



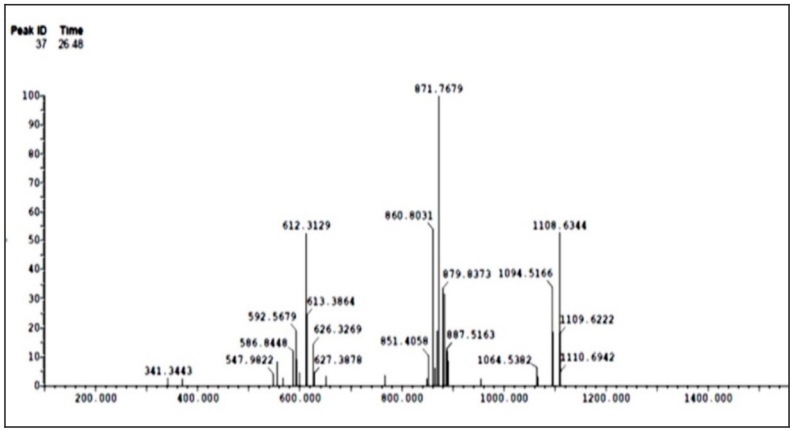
Fig(5): Comet cells in *Tilapia* fish blood showing ; A: Control blood; B: comet cells with long tails; C & D: Arrows pointing to different numbers of comet cells.

(Continued on next page)

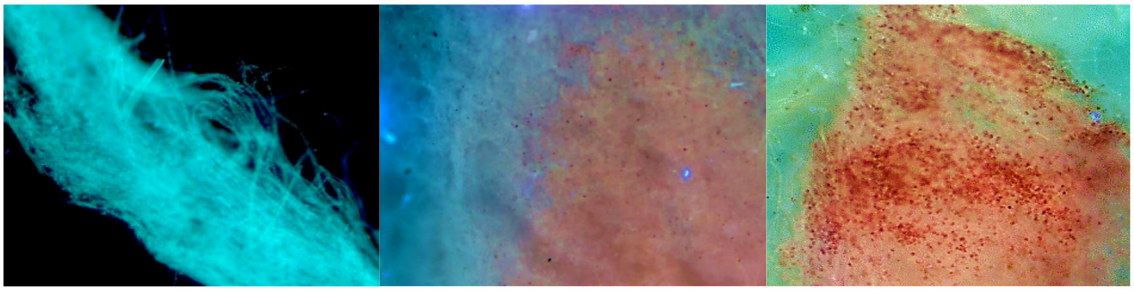


A (B) (C)

Fig(1): Antimicrobial activity of *T.viride* cell free culture filtrate on A.niger using concentration 25% v/v (B), 50% v/v (C) as compared to A.niger in the absence of T.viride culture filtrate (A).



Fig(2): Liquid chromatography Mass spectrometry (LC/ESI/MS) for Extra Cellular Fluid of *T.viride*



Fig(3): Detection of apoptosis in *A.niger* using mitotacker assay (A) *A.niger* as control, (B) Treated *A.niger* with 25 % *T.viride* CF and (C) Treated *A.niger* with 50 % *T.viride* CF.

PHYLOGENETIC RECONSTRUCTION AND HISTOPATHOLOGICAL CHARACTERIZATION OF VIRULENT *Nocardia brasiliensis* EXPERIMENTALLY INFECTED IN MEAGRE (*Argyrosomus regius*)

Félix Acosta[1*], Belinda Vega[1, δ], Daniel Montero[1, δ], Luis Monzón-Atienza[1], José Ramos–Vivas[2], Jorge Galindo-Villegas[3]

[1] Grupo de Investigación en Acuicultura (GIA), Instituto Ecoaqua, Universidad de Las Palmas de Gran Canaria, PCTM, Crta. Taliarte s/n, 35214 Telde, *Spain*.
[2] Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla and Instituto de Investigación Marqués de Valdecilla (IDIVAL), Santander, Cantabria, *Spain*.
[3] Faculty of Biosciences and Aquaculture, Nord University, Bodø, 8049, *Norway*.
δ Authors with equal contribution
*Email: felix.acosta@ulpgc.es

Introduction
Nocardiosis caused by diverse, cosmopolitan aerobic actinomycetes species of the genus *Nocardia* is a common disease in several aquaculture species. In the Canary Islands, Spain, variated internal granulomas resembling those produced by nocardiosis were unexpectedly observed in cultured meagre (*Argyrosomus regius*). Hence, we aimed to identify the causative species and experimentally reproduce the disease.

Material and Methods
Following classical methods, the bacterium was isolated from granulomas in the liver of several specimens and identified using 16S-rRNA gene sequencing and MALDI-TOF MS. The local *in silico* alignments with known 16S-rRNA sequences revealed the target identity as *Nocardia brasiliensis*, a species never before reported affecting fish. The virulence of this species was experimentally characterized *in vivo*. Juvenile meagre intraperitoneally infected with *N. brasilensis* develop internal micro granulomas without gross external sign or significant mortalities. Fish exposed to concentrations lower than 10^6 CFUs did not die at all within the two months trial. Five types of microscopic granulomas with classic necrotic centers and macrophages arranges were recorded in a dose-dependency fashion. Besides, immunofluorescence revealed the presence of live bacteria withing some granulomas.

Results and Conclusion
To our best knowledge, this is the first report of *Nocardia brasilensis* as disease agent in marine cultured fish. Besides, these results show that *N. brasilensis* successfully colonizes internal fish organs and is sufficient to develop chronic granuloma disease in meagre.

Acknowledgements
This work has been funded by the EU seventh Framework Programme by the DIVERSIFY project 7FP-KBBE-2013-GA-602131.

THE RESPONSE OF MACROZOOBENTHOS ASSEMBLAGE ON LONG-TERM EXTREME FLOW RATE FLUCTUATIONS IN A CARP POND INFLOW CANAL

Zdeněk Adámek[1*], Jana Konečná[2], Petr Karásek[2], Lucie Všetičková[3], Jana Podhrázská[2], Antonín Zajíček[2]

[1] University of South Bohemia in *České* Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic, zadamek@frov.jcu.cz
[2] Research Institute for Soil and Water Conservation, Žabovřeská 250, 156 27 Praha 5 – Zbraslav, Czech Republic
[3] University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

Introduction

Between 2014 and 2019, Central Europe was subject to unusually high temperatures and periods of drought due to climate change, with a subsequent negative impact on stream flow regimes, including the canals, providing water inflow to carp ponds. We examined changes in macrozoobenthos community composition and diversity in a small fishless brook (Němčický potok) supplying water to 0.5 ha carp pond in highland during the last decade. As temperatures rise and rainfall declines, many small water courses are likely to shift from permanent streams to intermittent (riffle-pool sequences replaced by isolated pools) or ephemeral (periods characterised by regular occurrence of dry riverbed) streams during periods of drought, often for periods of several weeks or longer (Bonada et al. 2006).

Material and Methods

While flow rates were relatively constant prior to 2014, the hydrological regime was significantly influenced by periods of severe drought between 2017 and 2019, with periods of zero discharge (ZD) and dry riverbed (DR) days. As this time period was characterized by dramatic decline of flow rates and by onset of atypical events of flow cessation and dry riverbed, the community of benthic macroinvertebrates (MIV) passed significant changes.

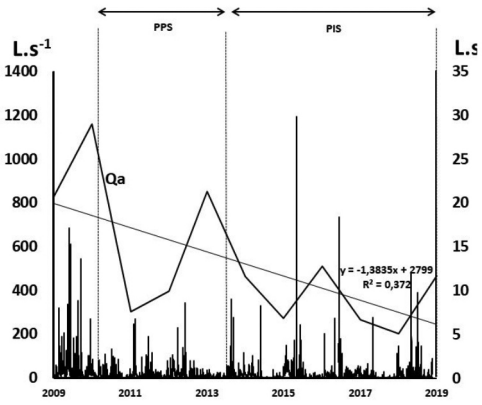


Fig 1. The course of recorded discharge rates (L.s^{-1}, left y axis) and mean annual discharge rates (Qa in L.s^{-1}, right y axis) during the periods of perennial (PPS) and intermittent stream (PIS – ZDs and DR days occurrence).

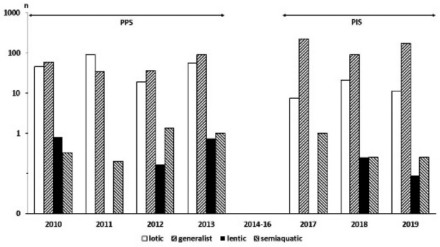


Fig. 3. Abundance (n) of habitat-associated groups during perennial (PPS) and intermittent (PIS) stream periods. Note: x axis in $\log_{10}$ scale.

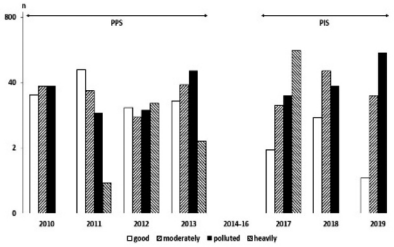


Fig. 4. Abundance (n) of water quality (saprobity) associated groups during perennial (PPS) and intermittent (PIS) periods. Note: x axis in $\log_{10}$ scale.

(Continued on next page)

Benthic MIV samples were obtained semi-quantitatively using a 1-minute hand-disturbance and sweep approach using a 0.5 mm mesh hand net (200x100 mm aperture). After laboratory processing, each taxon was assigned to one of four groups based on hydrological preferences, i.e. lotic, generalist, lentic or semi-aquatic. The classification by Sládeček [16], expressed as a saprobic index (S_I) was applied for assigning taxa into appropriate water quality (saprobity) categories in accordance with Czech National Standard [38], i.e. good, moderately polluted, polluted and heavily polluted.

Results

Hydrological patterns

We recorded no ZDs during the period between 2010 and 2011, with the first events being recorded in 2012 and 2013, though still with no DR occurrence - this was considered as a period of a perennial stream (PPS). The first eleven DR days occurred in 2014, persisting throughout the whole period until 2019. The highest DR frequency was recorded in 2015, with 110 ZDs and 81 DRs, and 2017, with 108 ZDs and 57 DR days which represented a period of an intermittent stream (PIS).

The average annual discharge rates (Qa) ranged between 7.57 to 28.96 $L.s^{-1}$ during the period 2010–2013, and 5.06 to 12.78 $L.s^{-1}$ during the period 2014–2019 (Fig. 1). Overall, Qa showed a declining trend ($R^2 = 0.372$, $p = 0.046$) between 2010 and 2018 with Qa dropping from 28.96 $L.s^{-1}$ to 5.06 $L.s^{-1}$ (Fig. 1).

The response of benthic MIV in the NEM brook was clearly dependent on the flow regime, and particularly on the frequency and duration of the ZD period. Taxa associated with lotic-habitats (e.g. *Gammarus fossarum*, *Baetis vernus* and *Micropsectra* sp.) dominated during the PPS period without ZDs (Fig. 2). During the dryer PIS period, however, lotic-habitat indicators had dropped, while the number of generalist taxa increased.

Conclusion

The macroinvertebrate community structure in a small fishless pond inflow canal was shaped by a transition from permanent to intermittent discharges resulting in waterless periods. The clearest impacts of this transition were a dramatic reduction (almost disappearance) in the population of *G. fossarum* and the prevalence of oligochaetes and dipteran larvae. Overall, the structure of the benthic macroinvertebrate assemblage shifted from being dominated by lotic taxa and good water quality indicators to one dominated by generalists and more tolerant taxa associated with more polluted environments.

Acknowledgement

This research and the article were funded by the Ministry of Agriculture CR in the frame of the project QK1910282 and the Institutional support MZE-RO0218.

References

Bonada, N.; Rieradevall, M.; Prat, N.: Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. *J N Am Benthol Soc* **2006**, *25*, 32-43.

ČSN 75221. *Water quality – Classification of surface water quality*. ČNI Prague, **2017**, 10 p.

Sládeček, V. System of Water Quality from the Biological Point of View. *Adv Limnol* **1973**, *7*, 1-218.

GREAT CORMORANT (*Phalacrocorax carbo sinensis*) OCCURRENCE PATTERN IN CARP POND AQUACULTURE: CASE STUDY FROM THE SOUTH BOHEMIA (CZECH REPUBLIC) POND REGION IN 2019

Zdeněk Adámek

University of South Bohemia in *České* Budějovice, Faculty of Fisheries and Protection of Waters
South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses
Zátiší 728/II, 389 25 Vodňany, Czech Republic
zadamek@frov.jcu.cz

Introduction

The great cormorant (*Phalacrocorax carbo sinensis*) has fully recovered from an endangered status in 1980s and is now present throughout Europe. As fish are its almost exclusive prey, cormorant is considered as a conflict species in concern to fisheries, both to the management of open waters and aquaculture enterprises.

Carp ponds, which usually replaced the original wetlands, create a complex mosaic of habitats which often represent unique refuges for both plant and animal communities. As such, they are often considered to be 'hotspots' of aquatic biodiversity and cormorants are to be seen as an integral part of their ecosystems. On the other side, however, cormorant predation on ponds which are primarily destined for commercial culture of common carp (*Cyprinus carpio*) under specific conditions of carp pond aquaculture, is steadily considered as one of substantial negative factors influencing the production profits.

Currently, the numbers of cormorants in the Czech Republic were estimated as 813 breeding and 13148 migrating and wintering birds in 2019 (CFFU, unpubl.), which caused a loss in aquaculture production amounting to approximately ~ 4 million EUR. While birds in breeding colonies contribute ~ 0.5 million EUR to the damage, the losses caused by non-resident cormorants amount to ~ 3,5 million EUR.

The 2019 data on cormorant occurrence on carp aquaculture ponds in the Třeboň Biosphere Reserve were elaborated with respect to various aspects – namely pond size, annual course of occurrence, distance from the cormorant breeding colony and economic issues.

Material and Methods

Study area

The Třeboň Biosphere Reserve represents an old pond district with ca. 600 fishponds surrounded by agricultural and forrested landscape. Currently, one regularly occupied breeding colony with 139 nests (3.4 chicks per nest) exists in the reserve.

Data resource

The data on cormorant numbers were regularly monitored by the Třeboň Fisheries Co. as the maximum current daily counts for the purposes of the request for recompensation. The average daily numbers, expressed as cormorant/days (corm/d), in weeks 1 – 52 of the year 2019 were applied for further evaluation in this study. Altogether, data from 118 monitored ponds were elaborated. The ponds were divided into 5 groups according to their area - < 5 ha (24 ponds, 0.58 – 4.58 ha), 5 - 20 ha (44 ponds, 5.09 – 18.54 ha), 20 – 50 ha (24 ponds, 20.12 – 46.24 ha), 50 – 100 ha (15 ponds, 50.38 – 99.97 ha) and > 100 ha (11 ponds, 104.71 – 644.74 ha). Pond stocks consisted of carp pond polyculture species with the dominance of one- to three-year-old scaly and mirror common carp, which share ranged mostly between 80 – 100 %.

(Continued on next page)

Fig. 1. The course of cormorant attendance at ponds during the year 2019.

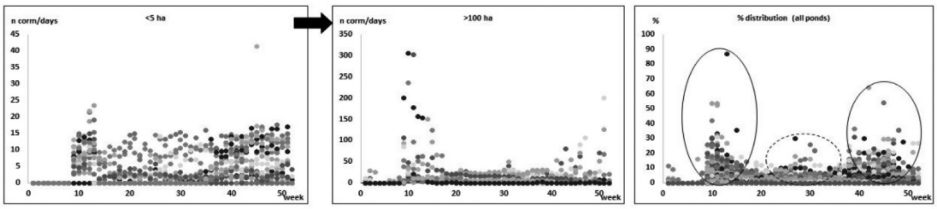


Fig.2. The relationship between cormorant numbers (corm/d) and colony distance at ponds of various size

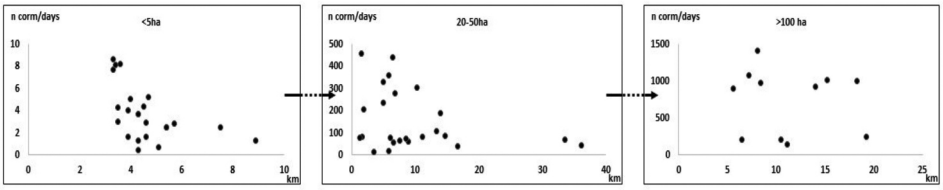


Table 1. Average numbers and economic losses in 2019. Note: n – number of ponds, Σ c/d – total number of cormorant/day, Σ kg – total fish losses in kg, Σ EUR – total losses in EUR, p – per pond, ha – per 1 ha of a pond

pond area	n	Σ c/d/p	Σ c/d/ha	Σ kg/p	Σ kg/ha	Σ EUR/p	Σ EUR/ha
< 5 ha	17	1327	475	663	238	1599	515
5-20 ha	44	1058	89	528	44	1255	105
20-50 ha	24	1031	32	515	16	1162	37
50-100 ha	15	2074	29	1037	15	2237	32
> 100 ha	11	5091	19	2544	10	6031	23

Results and Discussion

The annual pattern of cormorant abundance differed with respect to the pond size. While its course is rather even at ponds < 5 ha nearby the colony, the occurrence of spring and autumnal migratory birds is of increasing importance with increasing pond size. However, when taking into account the percentage distribution of annual occurrence, a smaller summer peak is also noticeable at all ponds due to appearance of young birds leaving the nests (Fig. 1).

The distance of a pond from the breeding colony proved to be another important factor influencing the attendance by cormorants. In this respect, the heaviest hunting pressure was focused on the ponds <50 ha within the 10 km distance while it was rather more evenly distributed at bigger (>50 ha) ponds (Fig.2).

Total numbers of cormorant appearance per day (corm/day) were highest on biggest ponds (> 50 ha) but when considering their counts per ha, small ponds (< 5 ha) were under strongest hunting pressure. The same applies to values expressing the losses both in fish biomass and, in particular, in financial consequences. Economic losses in smaller ponds were increased evidently due to the higher price of young fish cultured in them (Table 1).

Acknowledgement

This research and poster presentation were funded by the Ministry of Agriculture CR in the frame of the project QK1920102.

STUDY OF LESIONS PRESENT IN *Crassostrea angulata* AND *Crassotrea gigas* FROM DIFFERENT AREAS IN PORTUGAL

Pires, D.*[1], Grade, A.[2], Ruano, F.[2], Afonso, F.[1]

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine
University of Lisbon. Av. da Universidade Técnica
1300-477 Lisboa
*dspires@fmv.ulisboa.pt

[2]IPMA - Instituto Português do Mar e da Atmosfera
Av. Alfredo Magalhães Ramalho, 6
1495-165 Lisboa

Introduction

Oysters are among the most appreciated seafood products and there has been an increase in their production worldwide, being among the five most valued species farmed in the EU (FAO, 2019). The culture of bivalve molluscs is an activity with high expression in Portuguese aquaculture. It represents 55% of the whole production, being the main species, clams, oysters and mussels (INE, 2019). The Portuguese oyster, *Crassostrea angulata*, is a very important resource, not only because of its socioeconomic value but also because of its genetic heritage. This species declined and disappeared from the estuaries on 1970s due to massive mortalities, but currently this resource seems to be recovering and is mainly found in wild beds, with particular edaphoclimatic conditions.

The marine environment is known to provide several sources of stress to oysters during their life cycle, including changes in environmental parameters, changes in the availability of food and the display of various toxic pollutants (Lacoste *et al*., 2001; Kennedy *et al*., 1996; Garnier *et al*., 2007). Aquatic ecosystems can be modified by human activity, namely due to overharvesting and destruction of substrates (Pogoda, B 2019). Also, transfers of oysters between countries and regions can spread diseases and invasive species.

Aquatic animals can transmit infection to the consumer. Nowadays, zoonosis have a worldwide distribution and the consumption of aquatic animals, including oysters, has been growing. There are parasites that are responsible for zoonosis and other species that are not transmissible to humans but can have repugnant aspect and will not be consumed.

The identification of lesions and pathogenic agents in aquatic animals, namely in oysters, for human consumption represent an important information for sanitary control. For the study of pathologic lesions it is necessary to perform necropsies of animal samples to identify lesions and etiologic agents. This work shows the importance of necropsy and the anatomohistopathological exam for the sanitary control, to identify lesions and pathologic agents that wouldn't be recognized and observed by the consumers.

Materials and Methods

Oysters were sampled from four different sites of the Portuguese coast. Portuguese oyster from Sado estuary (n=30) and Mira estuary (n=30) and Japanese oyster from Alvor lagoon (n=30) and Aveiro lagoon (n=30). Oysters were collected from populations of Japanese oyster cultivated in a strong ocean-influenced environment and from populations of Portuguese oyster cultivated in wild-beds with distinct edaphoclimatic conditions than those found in production nurseries. To survey the presence of lesions and diseases anatomo and histopathological examinations were used as main diagnostic methods. Tissue samples were prepared for histopathology processing, following the protocol used in Pathology laboratory of IPMA. Samples were fixed in Davidson's fixative for 48h, dehydrated and embedded in paraffin. Sections with 5μm thick were stained with Hematoxylin-Eosin and mounted on a microscopic glass slide.

Histological preparations were carefully examined under light microscopy (Motic BA-410), looking for the presence of lesions and pathogens in oysters.

(Continued on next page)

Results

The macroscopic lesions and microscopic lesions were recorded in the specimens collected in wild beds (*C. angulata*) and farmed oysters (*C. gigas*). Samples of *C. angulata* from Sado Estuary show the following results: 20% presented mud blisters, mainly caused by *Polydora sp.*; 33% with gills lesions; 10% had shell disease associated with the presence of the fungus *Ostracoblabe implexa*. Samples from Mira Estuary showed mud blisters (96%), 50% had gills lesions, 10% presented edema and 10% shell disease. In samples of *C. gigas* from Aveiro Lagoon, it was observed only 3% of oysters with edema and 3% with shell disease. Finally, in samples of *C. gigas* from Alvor Lagoon 10% presented mud blisters.

Discussion and conclusion

The aim of the present work was to study the evolution of the sanitary condition in oyster production in four different populations of oysters were sampled. Lesions of the internal organs, such as metaplasia, may reflect physiological stress, contaminants or the presence of large parasitic loads (Ruano, 1997). Hemocytosis and edema was mainly present in the interstitial connective tissue. The lesions observed in the epithelium of the diverticula of the digestive gland, the hemocytic infiltration and necrosis were usually associated and could be related with the presence of the observed pathogenic agents. The low number of lesions found in oysters reflects the good sanitary condition.

The lesions observed in the epithelium of the diverticula of the digestive gland, the hemocytic infiltration and necrosis were usually associated and could be related with the presence of pathogenic agents observed. No organisms potentially pathogenic to humans were identified. The identification, characterization and registration of pathologic processes in oysters constitutes important measures for sanitary control and an appropriate for the sustainability of oyster populations management.

References

INE, 2019. Fisheries Statistics 2018.

FAO, 2019. Fisheries and aquaculture information and statisticsbranch. http://www.fao.org/fishery/statistics/global-production/en

Kennedy, V., Newell, R.,Eble, F., 1996. The eastern oyster *Crassostrea virginica*. Maryland Sea Grant College, College Park.

Labreuche, Y., Lambert, C., Soudant, P.,Boulo, V.,Huvet, A., Nicolas, J., 2006. Cellular and molecular hemocyte responses of the Pacific oyster, *Crassostrea gigas*, following bacterial infection with *Vibrio aestuarianus* strain 01/32. *Microbes and Infection*, 8, 12-13: 2715-2724.

Lacoste, A., Jalabert, F., Malham, S., Cueff, A., Poulet, S., 2001. Stress and Stress-Induced Neuroendocrine Changes Increase the Susceptibility of Juvenile Oysters (*Crassostrea gigas*) to *Vibrio splendidus*. 67(5): 2304–2309.

Pogoda, B., 2019. Currente Status of European oyster decline and restoration in Germany.

Ruano, F., 2008. A health perspective of the mollusc farming sector in Portugal. In H. A. Silva e I. Baptista (Eds), Produção, Salubridade e Comercialização de Moluscos Bivalves em Portugal, pp. 62-73. Lisboa: *Publicações avulsas do IPIMAR*.

Ruano, F. and Dias, M., 1994. Contribution to support the mollusc farming in Sado Estuary and Albufeira Lagoon, pp. 195-205. Lisboa: *Publicações avulsas do IPIMAR*.

Ruano, F., 1997. Fisheries and farming of important marine bivalves in Portugal. *NOAA Tech. Rep. NMFS*, pp. 191–200.

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

ENRICHMENT OF BLACK SOLDIER FLY MEALS WITH CHITINASE AND LONG-CHAIN POLYUNSATURATED FATTY ACIDS IS AN EFFECTIVE FISH MEAL ALTERNATIVE FOR IMPROVING GROWTH IN RELATION TO PHYSIOLOGICAL AND IMMUNE MODULATIONS IN NILE TILAPIA

P. S. Agbohessou[1,2*], S. N.M. Mandiki[1], V. Cornet[1], A. Gougbédji[2,3], R. C. Megido[3], F. Francis[3], P. A. Lalèyè[2], P. Kestemont[1]

[1]Research Unit in Environmental and Evolutionary Biology (URBE), Institute of Life, Earth and Environment (ILEE), University of Namur, Belgium
[2]Laboratory of Hydrobiology and Aquaculture (LHA), Faculty of Agricultural Sciences, University of Abomey-Calavi, Cotonou, Republic of Benin
[3]Functional and Evolutionary Entomology-Gembloux Agro-Bio Tech (University of Liège), Gembloux, Belgium
Email: agbohessou.pamphile@yahoo.fr

Introduction

Total or partial replacement of fishmeal (FM) with black soldier fly (BSF) pre-pupal meal induced a growth reduction in many species such as Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus* and zebrafish (*Danio rerio*) (Zarantoniello et al., 2019; Adeoye et al., 2020; Agbohessou et al., 2021). In our previous experiment, total substitution of FM and fish oil (FO) with BSF larval meal enriched with long-chain polyunsaturated fatty acids (LC-PUFAs) combined with palm oil improved the essential fatty acid content of the whole body of the fish, but decreased the protein digestibility and consequently the growth rate of Nile tilapia (Agbohessou et al., 2021). Chitin in insect meals may affect protein availability and digestibility and thus growth performance. Fish consuming insects have an important adaptation that relates to the digestive tract system of carnivorous and omnivorous fish; the gastrointestinal tract of many fish displays chitinase activity to facilitate insect consumption (Nogales-Mérida et al., 2019). Chitinase can hydrolyze chitin into chitin oligosaccharides, including oligomers, chitobiose and N-acetyl-glucosamine, which may play the role of antibacterial agents, lysozyme inducers and immunostimulators (Zhang et al., 2012). The present study aimed to determine the combined effects of LC-PUFAs and/or chitinase enrichment of BSF-based diets on the growth, digestibility, digestive enzymes, gut microbiota and immune status in Nile tilapia juveniles.

Materials and methods

Two types of BSF meals enriched with either a-linolenic acid (ALA) or ALA + eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) were produced using BSF pre-pupae cultured on vegetable substrates (VGS) or fish offal substrates (FOS), respectively. Seven diets were formulated, namely a control FMFO diet and two other control diets BSF/VGS0 and BSF/FOS0 containing the meals of each type of BSF meal as total replacement for FM and FO, as well as four diets supplemented with chitinase. Two doses of commercial chitinase from *Aspergillus niger* (Creative Enzymes, USA) (2g/Kg and 5g/Kg of feed) were supplemented to the other diets containing BSF/VGS and BSF/FOS meals to formulate BSF/VGS2, BSF/VGS5, BSF/FOS2 and BSF/FOS5 diets. Extruded pellets were prepared and a set of 630 sex-reversed male Nile tilapia juveniles (5.57 ± 0.05g) were randomly distributed in 21 tanks with a density of 30 fish per tank, and three tank replicates per feeding treatment. Fish were hand-fed to apparent satiation three times daily for 53 days to evaluate growth performance and feed utilization.

Table 1. Growth performance and feed utilization of juvenile Nile tilapia fed with FM and FO or BSF meals enriched with (LC)-PUFA and supplemented with chitinase for 53 days (mean ± S.D., n = 3). Values from the same line with different superscript letters are significantly different ($P < 0.05$).

Parameters	FMFO	BSF/VGD0	BSF/VGD2	BSF/VGD5	BSF/FOD0	BSF/FOD2	BSF/FOD5
Specific growth rate (SGR, % d^{-1})	3.61 ± 0.14^{a}	2.98 ± 0.19^{c}	3.11 ± 0.16^{bc}	2.96 ± 0.17^{c}	3.20 ± 0.06^{bc}	3.17 ± 0.12^{bc}	3.43 ± 0.26^{ab}
Feed intake (FI, g/fish/day)	0.63 ± 0.05	0.55 ± 0.03	0.58 ± 0.06	0.53 ± 0.02	0.63 ± 0.08	0.59 ± 0.06	0.61 ± 0.09
Feed efficiency (FE)	0.96 ± 0.05^{a}	0.74 ± 0.10^{b}	0.77 ± 0.03^{b}	0.75 ± 0.08^{b}	0.74 ± 0.06^{b}	0.78 ± 0.03^{b}	0.91 ± 0.06^{a}
Protein efficiency ratio (PER)	3.03 ± 0.02^{a}	2.32 ± 0.31^{b}	2.43 ± 0.12^{b}	2.37 ± 0.26^{b}	2.33 ± 0.20^{b}	2.45 ± 0.09^{b}	2.87 ± 0.20^{a}

(Continued on next page)

A digestibility test was performed on the same fish used for the growth test, but three weeks after feeding the different diets. Fish were fed a diet containing chromium oxide (Cr_2O_3) as an inert marker at a level of 1% compared to the previous formulation, and the fish were fed under the same conditions as the growth experiment. At the end of the feeding trial, the total number of fish and their body weight were recorded to determine the survival (SR) and growth rates. Blood, kidney and spleen samples were collected from 6 fish per aquarium and stored at -80°C for immune analysis. Stomach and intestine samples were collected for digestive enzymes, microbiota and histopathology analysis. A challenge test with bacterial lipopolysaccharide (LPS) was performed and 24 h later, blood, kidney and spleen samples were collected to evaluate the response of fish after LPS stimulation. Lysozyme activity, total peroxidase activity and plasma hemolytic alternative complement (ACH50) activity will be assessed in plasma before and after LPS stimulation. Immune gene expressions will be analyzed by Real-time qPCR in kidney and spleen samples before and after LPS stimulation while chitinase gene expressions will be analyzed in the intestines. The activities of digestive enzymes such as pepsin, amylase and chitinase will be analyzed in the stomach, and trypsin, alkaline phosphatase and aminopeptidase will be analyzed in the intestine. For histopathological analysis, the samples of anterior intestine were fixed in 10% neutral buffered formalin. Histological images will be acquired using a digital color camera combined with the microscope. For the description of the gut microbiota, we will use the next generation sequencing (Illumina HiSeq 2500). The hypervariable V1-V3 region 16S rRNA gene will be sequenced to assess the taxonomic composition and diversity index of bacteria.

Results and discussion

After 53 days of feeding, the BSF/FOD5 diet induced a growth performance (SGR) similar to the one of the FMFO control diet, while a significant decrease ($p < 0.05$) was observed for the other insect-based diets in relation to a lower feed efficiency (FE) and protein efficiency ratio (PER) ($p < 0.05$). However, the feed intake (FI) and the survival rate which varied from 82.22 to 93.33% were not significantly affected by the different diets (Table 1).

Fish fed the BSD/FOD5 diet, rich in ALA, EPA and DHA and supplemented with 5g of chitinase per Kg of feed showed growth rates comparable to those of fish fed the FMFO control diet, with higher values than those of fish fed other BSF meal diets. The combined effect of 5g of chitinase per Kg of feed and LC-PUFA enrichment of BSF meal improved the growth and feed efficiency of fish consuming the BSF/FOD5 diet.

Subsequent biochemical analyses are in progress, including diet digestibility, digestive enzymes, gut microbiota, gut histopathology, immune defense capacity and the response after LPS injection of fish fed the different diets. The results of these different analyses will be available for the oral communication.

References

Adeoye, A. A., Akegbejo-Samsons, Y., Fawole, F. J., & Davies, S. J. (2020). Preliminary assessment of black soldier fly (Hermetia illucens) pre-pupal meal in the diet of African catfish (Clarias gariepinus): Impact on growth, body index, and hematological parameters. *Journal of the World Aquaculture Society*, *51*(4), 1024-1033.

Agbohessou, P. S., Mandiki, S. N., Gougbédji, A., Megido, R. C., Hossain, M. S., De Jaeger, P., ... & Kestemont, P. (2021). Total replacement of fish meal by enriched-fatty acid Hermetia illucens meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles. *Aquaculture Nutrition*.

Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., ... & Józefiak, A. (2019). Insect meals in fish nutrition. *Reviews in Aquaculture*, *11*(4), 1080-1103.

Zarantoniello, M., Randazzo, B., Truzzi, C., Giorgini, E., Marcellucci, C., Vargas-Abúndez, J. A., ... & Olivotto, I. (2019). A six-months study on Black Soldier Fly (Hermetia illucens) based diets in zebrafish. *Scientific reports*, *9*(1), 1-12.

Zhang, Y., Feng, S., Chen, J., Qin, C., Lin, H., & Li, W. (2012). Stimulatory effects of chitinase on growth and immune defense of orange-spotted grouper (Epinephelus coioides). *Fish & Shellfish Immunology*, *32*(5), 844-854.

YEAST AS A PROTEIN SOURCE WITH HEALTH BENEFICIAL EFFECTS IN DIETS FOR ATLANTIC SALMON (*Salmo salar*)

J. O. Agboola*, B. Morales-Lange, J. Ø. Hansen, L. T. Mydland, M. Øverland

Department of Animal and Aquaculture Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, P. O Box 5003, NO-1432, Ås, Norway
Jeleel.opeyemi.agboola@nmbu.no

Introduction

Yeasts are gaining increasing focus as major protein ingredients in fish feeds. This is in part due to their ability to convert non-food biomass of low industrial applications into high valuable resources. In addition, yeast cell wall contains bioactive components such as β-glucan, mannan and chitin, which are known for their health beneficial effects in fish. Yeasts also contain about 50-60% of crude protein with favourable amino acid composition. Therefore, in the present study, we examined the nutritional value and health effects of three non-*saccharomyces* yeasts exposed to different down-stream processing conditions in diets for Atlantic salmon.

Materials and methods

Cyberlindnera jadinii (CJ), *Blastobotrys adeninivorans* (BA) and *Wickerhamomyces anomalus* (WA) yeasts produced from wood sugars and chicken hydrolysates were either heat inactivated (I) or autolyzed (A) and the feeding quality of the resulting yeast products were evaluated through two studies with Atlantic salmon. The first trial assessed the ability of yeasts to restore intestinal health in Atlantic salmon fry exposed to a dietary soybean meal challenge. The experimental diets consisted of a fish meal-based control (FM), a challenge diet with 40% soybean meal (SBM), and 6 test diets with 40% SBM and 5% of each of the yeast products (ICJ, ACJ, IBA, ABA, IWA and AWA). Fish were fed one of the experimental diets for 37 days, after which distal intestine, head-kidney and spleen were collected for histological and immune response analyses. The second trial evaluated the nutrient digestibility of the different yeast products using experimental diets containing 700g kg^{-1} of a FM reference diet and 300g kg^{-1} of each of the yeast products.

Results and discussion

The crude protein content of the three yeasts ranged from 38-53% and the yeasts contain cell wall components that support growth and health of Atlantic salmon. Autolysis modified the ultrastructure layers of the yeast cell wall (Figure 1a). Atomic force microscopy analysis demonstrated that the ability of yeast mannoprotein to bind to cell surfaces changed with the autolysis process. Histological evaluation of distal intestine based on widening of lamina propria, showed that AWA was effective in restoring gut health, while only limited effects were observed for the other yeasts (Figure 1b). As determined by indirect ELISA, inclusion of yeasts in Atlantic salmon diets induced production of TNFα and a concomitant reduction in Annexin 1 level compared to SBM fed fish (Figure 2a), which suggests a modulation of the immune response. Protein digestibility in inactivated yeast ranged from 63-72% and increased by 9-12% after autolysis (Figure 2b).

Conclusion

Based on the results of this study, yeast has a high nutritional value and can give positive health effects, and could thus be used as major protein ingredient in fish feeds. Ongoing analyses will evaluate the ability of the different yeasts to modulate gut microbiota of Atlantic salmon.

The immunological markers are presented as fold change relative to SBM, and the digestibility as percentage.

(Continued on next page)

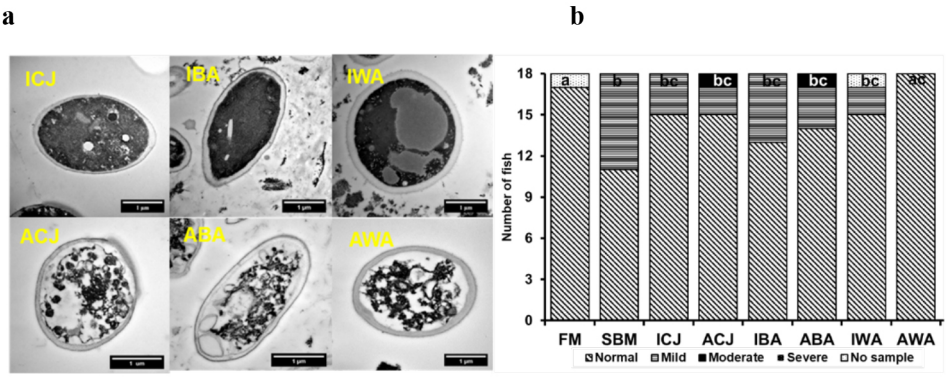


Figure 1. **a**. Micrograph showing the impact of autolysis on the ultrastructure layers of the yeast species. **b**. Histological evaluation of distal intestine based on scoring of lamina propria.

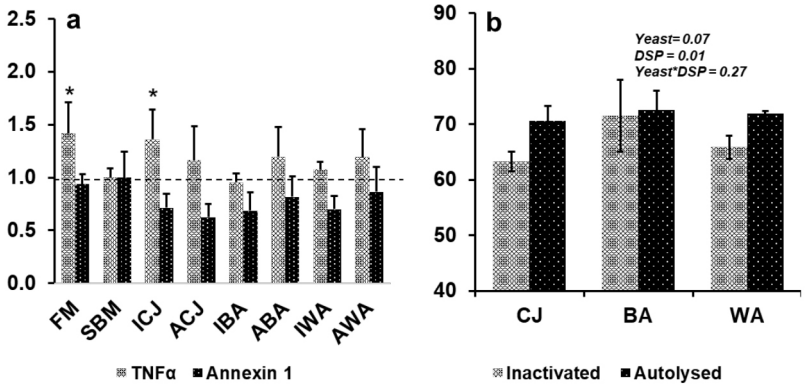


Figure 2. **a.** Detection of immunological markers by indirect ELISA in distal intestine of Atlantic salmon fed different yeast species. **b.** Protein digestibility (%) of three different non-*saccharomyces* yeast species produced from media containing wood sugars and chicken hydrolysates.

References

Agboola, J.O., Øverland, M., Skrede, A., Hansen, J.Ø., 2020. Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. Reviews in Aquaculture, 1-22.

Agboola, J.O., Schiavone, M., Øverland, M., Morales-Lange, B., Lagos, L., Arntzen, M.Ø., Lapeña, D., Eijsink, V.G., Horn, S.J., Mydland, L.T., François, J.M., Mercado, L., Hansen, J.Ø., 2021. Impact of down-stream processing on functional properties of yeasts and the implications on gut health of Atlantic salmon (*Salmo salar*). Scientific Reports. 11, 1-14.

BUILDING A GROWTH MODEL FOR *Hediste diversicolor* AQUACULTURE

F. Aguado-Jiménez[1*], B. García-García[2], I. E. Martín-Montero[1] and I. Rasines-Pérez[1]

[1] Spanish National Research Council. National Center Spanish Institute of Oceanography. Oceanographic Centre of Santander. "El Bocal" Marine Aquaculture Station. Monte-Corbanera, 39012, Santander, Spain
E-mail: felipe.aguado@ieo.es
[2] Murcian Institute for Agri-Food Research & Development. Department of Bioeconomy, Water and Environment. C/. Mayor s/n. La Alberca, 30150 Murcia, Spain.

Introduction

The "ragworm" *Hediste diversicolor* is a euryhaline and eurytherm polychaete with a great feeding plasticity. Its demand for baitfish and as food for fishes and crustaceans makes him a good candidate for the aquaculture industry (Batista et al., 2003). Modelling growth is basic for aquaculture purposes from a husbandry point of view, in order to establish a correct planning and optimization of production performance. In this work we show the results obtained to date of an experience in progress whose main objective is to build a growth model of *H. diversicolor* with the body weight and water temperature as descriptive variables.

Material and methods

Ragworms of nine different size classes (< 25 mg; 25-50 mg; 50-75 mg; 75-100 mg; 100-150 mg; 150-200 mg; 200-250 mg; 250-300 mg; 300-400 mg) coming from a captive population stock were placed in experimental units (EU) which consisted of a cylindrical PVC structure (height: 20 cm; internal Ø: 11.3 cm) whose wall and base were 335 μm mesh. EUs were filled with sifted sand (0.25-1 mm grain size) to a height of 12 cm. The substrate was always submerged. Ragworm density was about 1,000 ind m^{-2} (10 ind. per EU). Each size class was in triplicate. Groups of 6 EUs were placed inside polycarbonate trays (width: 35 cm; height: 30 cm; length: 54 cm) which were part of a Recirculating Aquaculture System (RAS) with a daily water renewal rate of around 10 %. The RAS had biological and mechanical filtration, UV sterilization and temperature control. Salinity was around 36 ‰ and photoperiod was 16L:8D. Ragworms were weighed (wet body weight: *BW*) to the nearest mg at the beginning (BW_i) and at the end (BW_f) of the experimental period of 15 days during which they were fed to apparent satiety with sole (*Solea senegalensis*) weaning feed pellets (0.35-0.50 mm in size) once a day. Dissolved oxygen was always close to 100 % saturation, and ammonium level below 0.1 ppm. The assay was conducted at three different temperatures (*T*): 10-11 °C, 15-16 °C, and 19-20 °C. Absolute growth rate ($AGR = \frac{BW_f - BW_i}{t}$) was calculated for each EU. A natural-log relationship between *BW* and growth has been clearly established for most animals (Aguado-Giménez & García-García, 2002). In order to explain variations in growth according to *BW* and *T*, data were empirically fitted by mean of multiple regression analysis (MRA) to equations such as the following:

$$LnAGR = Ln\ a + b \times LnBW + c \times LnT \quad \text{(Eq. 1)}$$

$$LnAGR = Ln\ a + b \times LnBW + c \times T \times LnBW \quad \text{(Eq. 2)}$$

Eq. 1 predicts that maximum *AGR* will occur around an optimum *T* and it is independent of *BW*, while Eq. 2 predicts that the optimum *T* for *AGR* changes with *BW*. Estimations of *AGR* were carried out with both models. Residual analysis was performed by linear regression to compare the goodness of fit of both models.

Results and Discussion

Variability explained by both models was very high, and the estimation error was low for both models (Table 1). As it was expected, MRA showed that *BW* and *T* have a strong influence on *H. diversicolor* growth: the larger the ragworm and the higher the temperature, the higher the growth. The coefficient of determination R^2_{adj} and the goodness of fit were slightly better for Eq. 1 (Table 1; Figure 1C), but the differences in estimating *AGR* were minimal (Figure 1 A-C). We go on conducting trials with worms of the same size classes and higher, and at temperatures above and below those set out in this communication. It would be expected that expanding the range of *BW* and *T*, the combined effect of weight and temperature on growth may be highlighted, being able to define the optimum *T* for growth depending on the worms size.

(Continued on next page)

Table 1: Results of the Multiple Regression Analyses for *AGR* (a-b-c: coefficients; s.e. standard error; RSE: residual standard error). *** $P < 0.001$

	a s.e. *P*-value	b s.e. *P*-value	c s.e. *P*-value	R R^2_{adj} RSE	ANOVA *F* *P*-value
Eq. 1	-3.9927 0.1763 ***	0.6873 0.0193 ***	0.9585 0.0591 ***	0.9584 95.72 % 0.1312	851.7 ***
Eq. 2	-1.4574 0.1094 ***	0.5034 0.0263 ***	0.0125 0.0008 ***	0.9492 94.78 % 0.1450	691.2 ***

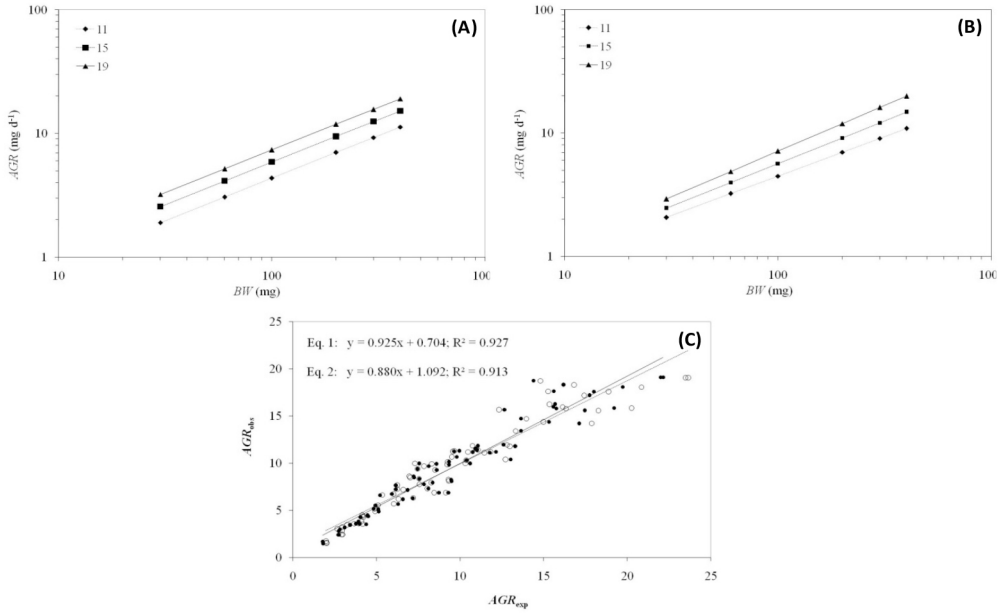


Figure 1: Simulations of *AGR* with Eq. 1 (A) and Eq. 2 (B), and observed *vs*- expected residual analysis (C).

References

Aguado-Giménez, F. & García-García, B. Growth and food intake models on *Octopus vulgaris* Cuvier (1,797): influence of body weight, temperature, sex and diet. Aquac. Int. 10: 361-377.

Batista, F.M., Fidalgo e Costa, P., Ramos, A., Passos, A.M., Pousão Ferreira, P., Cancela da Fonseca, L. (2003). Production of the ragworm *Nereis diversicolor* (O.F. Müller, 1776), fed with a diet for gilthead seabream *Sparus aurata* L., 1758: survival, growth, feed utilization and oogenesis. Bol. Inst. Esp. Oceanogr. 19(1-4): 447-451.

Acknowledgments

This study is co-funded (75 %) by the European Maritime & Fisheries Fund (EMFF) and the CN-IEO-CSIC.

INTERANNUAL VARIATION IN ENERGY CONTENT OF MARINE COSTAL SESTON DETERMINED BY DIRECT CALORIMETRY

T. Strohmeier*, R. Filgueira, P. J. Cranford, L. E. Steeves, A. Aguera, C. Krogness and Ø. Strand

*Institute of Marine Research, PO Box 1870 Nordnes N-5817 Bergen Email: tores@imr.no

Suspension feeders (mollusks) are currently by far the world's largest marine non-fed cultured production of animals [14]. This vast production capacity relies on their capacity to exploit the largest trophic resource in the marine environment, the seston. The seston comprises plankton and inorganic and organic matter in suspension. This multifaceted food resource varies on spatial and temporal scales with respect to concentration, biopolimeric composition, energy content and nutritional value [2-7, 9].

The seston food source, and the ecological and physical processes that control this food supply, fuels growth, sets the production capacity and determines the ecological carrying capacity of low trophic animal aquaculture [7, 8, 10, 11]. However, a unifying approach for quantifying this food resource that can explain growth, production and ecological carrying capacity is currently lacking.

Proxies of seston abundance and nutritional quality are typically converted to energy content units (J g^{-1}) using a constant factor to estimate the capacity of the food supply to support bivalve production. The factors commonly used are 47.7 J mg^{-1} for phytoplankton carbon [16] and 23.4 J mg^{-1} for living organic "particle" [15]. There are few published studies on the energy content of mixed seston [1, 6, 12, 13, 17, 18] and to our knowledge none that has directly measured the temporal energy content of seston. Since the temporal variation in the relationship between the food proxy and the actual energy content of the seston is not known, there is a large potential for erroneous interpretation of the capacity of the ambient food resource to support aquaculture.

The presentation will summarize the temporal (interannually) variation in energy content of marine costal seston as determined by direct calorimetry.

1. Berg JA, Newell RIE (1986) Temporal and spatial variations in the composition of seston available to the suspension feeder *Crassostrea virginica*. Estuarine Coastal Shelf Sci 23:375-386
2. Bernard I, De Kermoysan G, Pouvreau S (2011) Effect of phytoplankton and temperature on the reproduction of the Pacific oyster Crassostrea gigas: investigation through DEB theory. J Sea Res 66:349-360
3. Cranford PJ (1995) Relationships between food quantity and quality and absorption efficiency in sea scallops *Placopecten magellanicus* (Gmelin). J Exp Mar Biol Ecol 189:123-142
4. Cranford PJ, Hill PS (1999) Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Mar Ecol-Prog Ser 190:223-239
5. Danovaro R, Fabiano M (1997) Seasonal changes in quality and quantity of food available for benthic suspension-feeders in the Golfo Marconi (north-western Mediterranean). Estuarine Coastal and Shelf Science 44:723-736
6. Fegley SR, Macdonald BA, Jacobsen TR (1992) Short-term variation in the quantity and qualtity of seston available to benthic suspension feeders. Estuarine Coastal and Shelf Science 34:393-412
7. Filgueira R, Grant J (2009) A Box Model for Ecosystem-Level Management of Mussel Culture Carrying Capacity in a Coastal Bay. Ecosystems 12:1222-1233
8. Filgueira R, Comeau LA, Guyondet T, McKindsey C, Byron C (2015) Modelling carrying capacity of bivalve aquaculture: a review of definitions and methods. Springer
9. Filgueira R, Guyondet T, Comeau LA, Tremblay R (2016) Bivalve aquaculture-environment interactions in the context of climate change. Global Change Biology:n/a-n/a
10. Grant J, Filgueira R (2011) Shellfish aquaculture and the environment. The application of dynamic modelling to prediction of production carrying capacity in shellfish farming. Wiley-Blackwell Science Publishers, Iowa
11. Kluger LC, Filgueira R, Wolff MJE (2017) Integrating the concept of resilience into an ecosystem approach to bivalve aquaculture management. 20:1364-1382
12. Navarro JM, Clasing E, Urrutia G, Asencio G, Stead R, Herrera C (1993) Biochemical-composition and nutritive-value of suspended particulate matter over a tidal flat of southern Chile. Estuarine Coastal and Shelf Science 37:59-73
13. Navarro JM, Thompson RJ (1995) Seasonal fluctuations in the size spectra, biochemical-composition and nutritive-value of the seston available to a suspension-feeding bivalve in a sub-arctic environment. Mar Ecol-Prog Ser 125:95-106
14. Olsen Y (2015) How can mariculture better help feed humanity? Frontiers in Marine Science 2:46
15. Parsons TR, Takahashi M, Hargrave B (1977) Biological oceanographic processes. Pergamon press
16. Platt T, Irwin B (1973) Caloric content of phytoplankton. Limnology and Oceanography 18:306-310
17. Sarà G, Leonardi M, Mazzola AJC, Ecology (1999) Spatial and temporal changes of suspended matter in relation to wind and vegetation cover in a Mediterranean shallow coastal environment. 16:151-173
18. Widdows J, Fieth P, Worrall CM (1979) Relationships between seston, available food and feeding-activity in the common mussel *Mytilus edulis*. Mar Biol 50:195-207

PHYSIOLOGICAL PLASTICITY OF BLUE MUSSEL INDIVDUALS UNDER NATURAL ENVIRONMENTAL CONDITIONS

A Agüera[1], T Strohmeier[1], R Filgueira[1,2], L Steeves[3], C Krogness[1], Ø Strand[1]

[1] Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway
[2] Marine Affairs Program, Dalhousie University, 1355 Oxford St., Halifax, NS B3H 4R2, Canada
[3] Dalhousie University, Department of Biology, 1355 Oxford Street, Halifax, NS, B3H 4R2, Canada

e-mail: antonio.aguera@hi.no

Blue mussels, *Mytilus edulis* L., are an important natural and cultured resource in coastal areas around the world. As such, there has been a wide interest in describing the physiological performance of blue mussels in varying environments. However, our understanding of the individual plasticity as environmental conditions change is still limited.

High temporal resolution measurements of feeding physiology and metabolism are necessary to gain further insight on how sensitive blue mussels are to natural changes. Key physiological aspects such as the adaptation to different seasons, plasticity under variable combinations of food and temperature or individually predetermined physiological performance are still poorly understood. To increase our knowledge in these aspects we measured growth, feeding physiology (clearance, retention rates) and metabolism (respiration rate) of the same 20 mussels exposed to the natural variation of food, temperature and salinity. An automatised system performed measurements of feeding every 2 hours and respiration every 4 hours during one year.

Over 17000 respirometries and 34000 measurements of clearance and retention were collected over that year. Preliminary results show that mussels exhibited a wide variability in both metabolism and feeding physiology that reflected in growth and overall performance. Individuals could not be classified as either slow or fast growers/feeders but a continuous range of performance was observed that resulted in larger variability of measured growth, feeding and metabolism as the experiment progressed. Food was the main driver of both feeding physiology and metabolism variability during the year, with little effect of temperature in modulating these within the observed temperature range (5 - 18°C).

BEHAVIOURAL FEVER PROMOTES AN INFLAMMATORY REFLEX CIRCUIT IN ECTOTHERMS

Andrea Aguilar[1], Nataly Sanhueza[1], Sebastian Boltaña[1]

[1]Centro de Biotecnología, Departamento de Oceanografía, Universidad de Concepción, Concepción 4030000, Chile

The communication between the brain and the immune system is a cornerstone in animal physiology. This interaction is mediated by immune factors acting in both health and pathogenesis, but it is unclear how these systems molecularly and mechanistically communicate under changing environmental conditions. Behavioural fever is a well-conserved immune response that promotes dramatic changes in gene expression patterns during ectotherms' thermoregulatory adaptation, including those orchestrating inflammation. However, the molecular regulators activating the inflammatory reflex in ectotherms remain unidentified. Methods: We revisited behavioural fever by providing groups of fish a thermal gradient environment during infection. Our novel experimental setup created temperature ranges in which fish freely moved between different thermal gradients: (1) wide thermoregulatory range; $T^{o} = 6.4\ ^{o}C$; and (2) restricted thermoregulatory range; $T^{o} = 1.4\ ^{o}C$. The fish behaviour was investigated during 5-days post-viral infection. Blood, spleen, and brain samples were collected to determine plasmatic pro- and anti-inflammatory cytokine levels. To characterize genes' functioning during behavioural fever, we performed a transcriptomic profiling of the fish spleen. We also measured the activity of neurotransmitters such as norepinephrine and acetylcholine in brain and peripheral tissues. Results: We describe the first set of the neural components that control inflammatory modulation during behavioural fever. We identified a neuro-immune crosstalk as a potential mechanism promoting the fine regulation of inflammation. The development of behavioural fever upon viral infection triggers a robust inflammatory response in vivo, establishing an activation threshold after infection in several organs, including the brain. Thus, temperature shifts strongly impact on neural tissue, specifically on the inflammatory reflex network activation. At the molecular level, behavioural fever causes a significant increase in cholinergic neurotransmitters and their receptors' activity and key anti-inflammatory factors such as cytokine Il10 and Tgfβ in target tissues. Conclusion: These results reveal a cholinergic neuronal-based mechanism underlying anti-inflammatory responses under induced fever. We performed the first molecular characterization of the behavioural fever response and inflammatory reflex activation in mobile ectotherms, identifying the role of key regulators of these processes. These findings provide genetic entry points for functional studies of the neural–immune adaptation to infection and its protective relevance in ectotherm organisms

EFFECT OF DIFFERENT DOSES OF DIETRY EUROPEAN OLIVE OIL PROCESSING BY-PRODUCT ON THC AND DHC OF PACIFIC WHITE SHRIMP (*Penaeus vannamei*)

Sara Ahani[1]*, Mehdi Shamsaie Mehrgan[1], Aghil Dashtiannasab[2], Farhad Foroudi[3]

Department of fisheries Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
Shrimp Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bushehr, Iran
Department of Animal Varamin-Pishva Branch, Islamic Azad University, Tehran, Iran
E-mail: Sara.Ahani@yahoo.com

Introduction

Pacific white shrimp as a commercial resources in aquaculture has been always effected by many infectious diseases. The use of the plant immunostimulants in compare with antibiotics, which are harmful for human, animals and also environment, is an alternative for disease management and has become a common practice recently. Phenolic compounds in olive oil processing by-products, regarding to phytotoxic and antimicrobial activities can be a great risk for the organisms in the case of sludge accumulation in the environment (Jerman Klen et. al., 2015). Thus using them in animals diet can profited them from antioxidant properties (Artajo et. al., 2006) and reduce the damaging organic load to the environment. The innate immune system of the shrimps mostly involve hemocytes and are consist of hyalinocytes, granulocytes and semi-granulocytes which have biological functions against pathogens. Hyaline and semi-granular cells are in charge of phagocyte activity (Johansson et. al., 2000), while granular cells are more responsible for prophenoloxidase and cytotoxicity functions. One part of this comprehensive study has been specified to study the innate immune system and the levels of THC and DHC of the shrimps using different amounts of olive pomace in the diet.

Materials and Methods

For this purpose 225 shrimps weighing 8.09±0.04g divided into 5 groups in triplicates after adaptation at 28 °C, pH 7.55, dissolved oxygen 7.37. Five experimental diet consisting 0, 3.5, 7, 10.5 and 14 percent olive pomace (OP), were formulated to be iso-nitrogenous (421 g/kg crude protein) and iso-lipidic (121 g/kg crude lipid) according to NRC nutrient values of Pacific white shrimp. Approximate composition of diets were determined by AOAC, 2000.After 60 days, THC and DHC of each treatments were determined. For this purpose sterile syringe were used to withdraw 1-ml of haemolymph from the ventral sinus of shrimps and were diluted individually in Alsever anticoagulant solution (ratio 1:1). A drop of this mixture was loaded on a haemocytometer to count the THC and DHC.

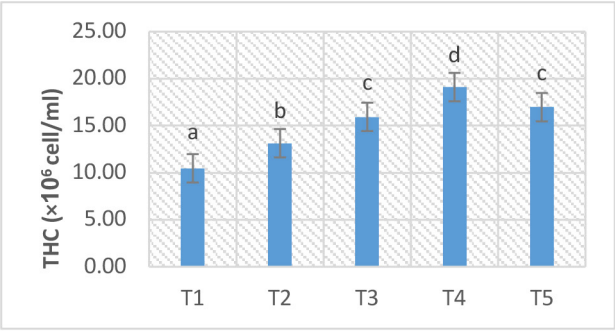


Fig 1- Total Hemocyte Count (THC) of five different doses of the five treatments

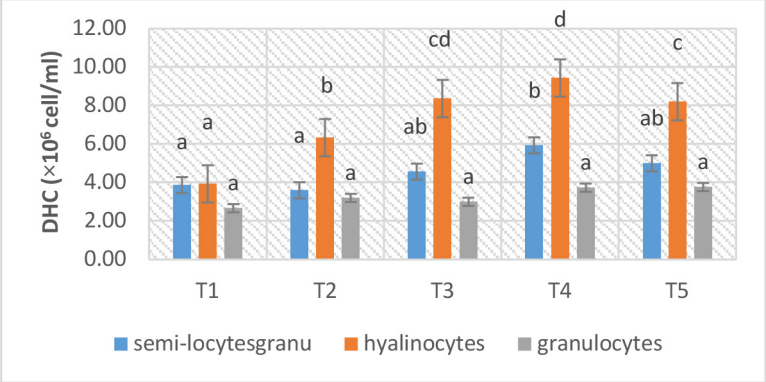


Fig 2- Differential Hemocyte Count (THC) of the five treatments

(Continued on next page)

Result

The results show that the control treatment which consisted no amount of olive pomace had the lowest level of THC (10.47×106cell/ml) sixty days after consuming the diet, while the highest level of THC belonged to the fourth treatment, OP10.5 (19.1×d106cell/ml) with a significant difference ($P<0.05$)(fig. 1). Comparing the amount of different hemocytes revealed that hyalinocytes are the most abundant cells while the granulocytes are the least cells in each treatment. Differential hemocyte count (DHC) showed the most level of hyaline cells and semi-granular cells in the treatment of 10.5% OP (treatment 4) with 9.43×106 and 5.93×106 cell/ml respectively and the least hyaline cells and semi-granular cells in the control treatment with no olive pomace (fig. 2). Granular cells however are almost the same in every group with no significant difference ($P>0.05$). Considering the hemocytes in this survey, using 10.5% of olive pomace in the diet of the shrimps had the best effect in boosting its immune performance.

References:

AOAC (2000). Official methods of analysis of AOAC International, (17th ed.), Gaithersburg, MD, USA.

Jerman Klen, Tina, Golc Wondra, A., Vrhovšek, U. and Mozetič Vodopivec, B. (2015). Phenolic profiling of olives and olive oil process-derived matrices using UPLC-DADESI-QTOF-HRMS analysis. Journal of Agricultural and Food Chemistry, 63(15), PP: 3859–3872.

Artajo L.S., Romero M.P., Morelló J.R., Motilva M.J. (2006). Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index. Journal of Agricultural and Food Chemistry 54(16), PP: 6079-6088

Johansson M. W., Keyser P., Sritunyalucksana K. and Söderhäll K. (2000). Crustacean hemocytes and haematopoiesis. Journal of Aquaculture, 191(3), PP: 45-52.

DEVELOPMENT AND APPLICATION OF GENOMIC TOOLS FOR TRACKING VIRAL OUTBREAKS IN TILAPIA

Shayma Alathari*, Charles R. Tyler Richard Paley, David V. Jeffreys, and Ben Temperton.

Geoffrey Pope building, University of Exeter, Stocker Road, Exeter, EX4 4QD, U.K.
Cefas (Centre for Environmental fisheries and Aquaculture Science) The Nothe, Barrack Road, Weymouth, Dorset DT4 8UB Tel: +44 (0) 1305 206600
Email: sa655@exeter.ac.uk

Introduction

Tilapia farming is one of the most important finfish species cultured worldwide with a total global production estimated at more than 6.6 million tonnes (in 2016 ;FAO 2018). Recently, a novel strain of infectious spleen and kidney necrosis virus (ISKNV) has been identified that is an agent of high morbidity and mortality threatening tilapia aquaculture. In tilapia farms in Lake Volta, Ghana, molecular diagnostics of regional fish farms have suggested rapid spread of the disease (in a matter of months), with losses of more than 10 tonnes of fish per day following infection (Ramírez-Paredes et al. 2019). A primary objective of this work in this study is developing methods for long read sequencing to enable field-based, real-time genomic surveillance of ISKNV phylogeography, adopting a similar approach to the recent work by the Artic Network group on Ebola, Zika virus and SARS-CoV-2. Whole genome sequencing captures all variation across the full genome, providing greater resolution of emerging diversity. Phylogeographic analysis can be used to reconstruct a detailed spatial history of virus spread from the origin of an outbreak. With the information obtained from genomic surveillance and epidemiological data, it is possible to reconstruct chains of transmission (Quick et al. 2020), (Gardy et al. 2015).

Materials and methods

This study was performed on samples collected by the Centre for the Environment, Fisheries and Aquaculture Sciences (Cefas), from seven farms in Lake Volta/Ghana, during an outbreak of ISKNV between October/2018- July/2019. We performed whole genome sequencing of ISKNV from infected fish using a tiled PCR approach, with 2000 nt amplicons spanning the genome. Library preparation was conducted on the generated amplicons. Samples were multiplexed and sequenced using the MinION sequencer. High accuracy base calling was carried out after sequencing from the fast5 files using the Oxford Nanopore Guppy tool *(v.* 5.0.7*)*. A consensus genome was generated according to the ARTIC bioinformatics pipeline (artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). Geneious Prime software (Biomatters, Auckland, New Zealand) was used to explore the ISKNV strains collected from Ghana, and visualise gene variability and single nucleotide variants (SNV). The generated consensus sequences were aligned with previously ISKNV published genomes to create a phylogenetic tree.

Results and discussion

In this study, we were able to recover 85%- 89% of the ISKNV genome, directly from fish samples collected from Ghana during an outbreak, using the tiled PCR protocol. Approximately 125 SNVs were detected, when compared with the original reference genome, and it has shown that changes were sufficient to detect a phylogeographic signal during this outbreak. This work shows that PCR tiling approaches used successfully to track the evolutionary rate, signatures of host adaptation, and transmission patterns of **RNA viruses** (Quick et al. 2016) can also be applied to monitor infections of a **large dsDNA** fish viruses. Thus, this method can be utilised as a surveillance tool for other viral infections threatening the growth of the aquaculture industry. RAMPART (Artic Network), (https://artic.network/rampart) software was used to determine coverage in real-time for samples collected from four different farms.

Future research

Further studies will include determining the sensitivity of the Artic-Network protocol across a range of viral loads. We aim to use the qPCR to monitor viral concentration in the surrounding water bodies, and neighbouring aquaculture sites to assist in the monitoring and management of viral outbreak threats. Through detecting the gradient of virus concentration in the water, moving away from infected farms we propose to evaluate the possible threat to stocks to other farms and to assess whether the spread of the virus is operating through water flow or movement of livestock. Determining the concentration of ISKNV from water samples, and via amplicon sequencing, may enable us to monitor the ISKNV variants present in a farm without destructive sampling. Finally, we aim to adapt this protocol to track other important fish viruses threatening aquaculture, and establish how this method may be applicable in the field.

(Continued on next page)

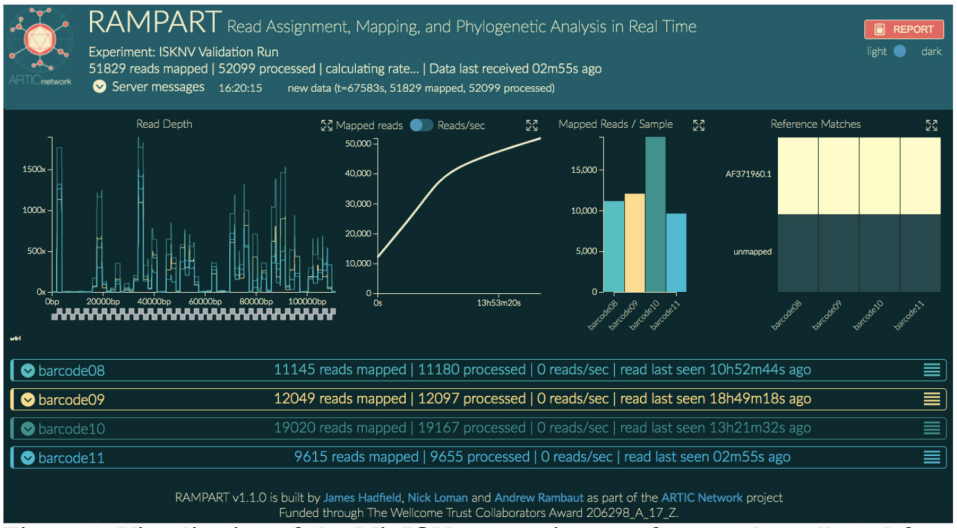


Figure 1. Visualization of the MinION sequencing run for samples collected from Lake Ghana. ISKNV genomes recovered by the multiplex PCR approach using nanopore sequencing.

References:

ARTIC Network. 2020. Read assignment, mapping and phylogenetic analysis in real time (RAMPART). https://artic.network/rampart.

FAO. *2018*. The State of World Fisheries and Aquaculture 2018 – Meeting the Sustainable Development Goals. *Food & Agriculture Org*. Rome. 224 pp. Licence: CC BY-NC-SA 3.0 IGO. http://www.fao.org/3/i9540en/ i9540en.pdf.

Gardy, Jennifer, Nicholas J. Loman, and Andrew Rambaut. 2015. "Real-Time Digital Pathogen Surveillance - the Time Is Now." *Genome Biology* 16 (1): 155.

Quick, Joshua, Nicholas J. Loman, Sophie Duraffour, Jared T. Simpson, Ettore Severi, Lauren Cowley, Joseph Akoi Bore, et al. 2016. "Real-Time, Portable Genome Sequencing for Ebola Surveillance." *Nature* 530 (7589): 228–32.

Ramírez-Paredes, José Gustavo, Richard K. Paley, William Hunt, Stephen W. Feist, David M. Stone, Terry Field, David J. Haydon, et al. 2019. "First Detection of Infectious Spleen and Kidney Necrosis Virus (ISKNV) Associated with Massive Mortalities in Farmed Tilapia in Africa." *Transboundary and Emerging Diseases*. https://onlinelibrary.wiley.com/doi/10.1111/tbed.13825/.

ARE YOU REALLY WHAT YOU EAT? – MANIPULATION OF PIGMENT PROFILES IN THE AMPHIPOD *Gammarus locusta*

Hilke Alberts-Hubatsch, Mareike Miriam Klug, Kai-Uwe Ludwichowski, Jan Beermann

Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Aquaculture Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
halberts@awi.de

Introduction

In aquaculture, marine specimens from lower trophic levels have the potential to serve as alternative food source for farmed fish and invertebrates, replacing traditional meal and fish oils. Marine amphipods are a natural food source for many marine fish and thus became of increasing interest for feeding fish in aquaculture (Alberts-Hubatsch *et al*. 2019).

In the amphipod *Gammarus locusta*, diet can heavily impact the nutritional composition such as fatty acid profiles. This low-trophic species also produces other compounds valuable for feeding in aquaculture such as pigments, i.e. astaxanthin. This study investigated the impact of feed type on the pigment profile and content of *G. locusta* in relation to changes in feed type.

Material and Methods

Freshly hatched juvenile *Gammarus locusta* were extracted from a steady culture and raised on *Fucus* spp. for two weeks to produce a baseline in feeding status. Four treatments in quadruplicates were designed to reflect successive changes in feeding between a marine control diet, consisting mainly of dried *Fucus serratus*, and a terrestrial diet consisting of dried leaves of the carrot *Daucus carota*. The specimens in the first treatment (A) were exclusively fed with dried algae for 28 days, the second treatment (21/7) was fed with algae for 21 days, followed by 7 days of feeding with carrot leaves, the third treatment (14/14) were fed algae for 14 days, followed by carrot leaves for 14 days and the fourth treatment (C) was exclusively fed with carrot leaves. Feeding was carried out in excess twice a week, remaining feed was removed from the containers and replaced with fresh feed.

At the end of the experiment three individuals of each replicate were pooled and stored at - 80°C for pigment analysis. Pooled samples as well as triplicate samples of the diets were then analyzed on a Hitachi La Chrome Elite™ RP-HPLC with diode array detection at 475 nm and astaxanthin as standard.

Results

We found varying contents of carotenoids in the four treatments with highest amounts of carotenoids in gammarids fed the carrot leaf diet (1.04 ± 0.12 mg/kg dry weight) and lowest content in the *Fucus* diet (0.47 ± 0.13). In total, six major different carotenoids were detected in the four treatments (Table 1). A shift in pigment profile in correlation to the diet could be observed, with the algae treatment resulting in five major carotenoids shifting to only three major pigments in the carrot treatment.

No differences were observed in the astaxanthin content between the treatments ($p \geq 0.05$, $F(3, 17) = 0.8996$; $p = 0.4659$), but distinct differences were observed in lutein content ($p \leq 0.05$, $F(3, 17) = 7.562$; $p = 0.003$), with the gammarids fed carrot leaves showing highest levels of lutein (0.73 ± 0.10) and specimens from the *Fucus* diet having lowest amounts.

Table 1 Total amount (mg/kg) of major carotenoids of *Gammarus locusta* in relation to type of feeding. C indicates feeding with carrot leaves, F indicates feeding with *Fucus* spp., 14/14 and 21/7 indicate intermediate feeding regimes (see text).

Carotinoid	C	F	14/14	21/7
Fucoxanthin	---	1.11 ± 1.63	---	0.10 ± 0.10
Violaxanthin	---	0.13 ± 0.04	---	0.05 ± 0.05
Astaxanthin	0.11 ± 0.01	0.18 ± 0.07	0.23 ± 0.06	0.21 ± 0.02
Zeaxanthin	---	0,04 ± 0,01	---	---
n.n.	0.19 ± 0.02	---	0.18 ± 0.05	0.10 ± 0.01
Lutein	0.73 ± 0.10	0.12 ± 0.04	0.57 ± 0.12	0.47 ± 0.10
Σ	1.04 ± 0.12	0.47 ± 0.13	0.98 ± 0,17	0.78 ± 0.13

(Continued on next page)

Discussion

The pigment profile of *Gammarus locusta* was strongly affected by the pigment profile of the feed type. The pigment profile completely changed within 28 days, with intermediate profiles reflecting both diets. Astaxanthin content was surprisingly highest in the intermediate treatment, whereas pure *Fucus* as well as pure carrot leaves diet did not show differences. The most apparent difference was observed in lutein content with the highest content in the carrot leave treatment and lowest in pure *Fucus*. Since no cantaxanthin was detected in any samples, we suggest the biosynthetic pathway through zeaxanthin (Gaillard et al. 2004). Both feeds contain similar amounts of β-carotin, which - as a precursor in the biosynthetic pathway - may result in similar amounts of astaxanthin in the gammarid. Even though astaxanthin content did not change with the diet, amount of lutein was six-fold higher than in the control diet.

Even though the astaxanthin content did not change between these diets, it is available in biologically significant amounts. In relation to dry mass, the astaxanthin content in our study varied from 20 to 100 mg/kg dry weight. Aquaculture species already benefit from an astaxanthin content as low as 50 mg/kg in their diet (Olsen & Baker, 2006; Wade *et al*. 2017).

The effect of lutein for aquaculture species has been hardly studied, but it is already known, that is has positive effects in the coloration of yellow croaker (Yi *et al*., 2015. However, effect of lutein on the metabolism of other aquaculture species needs to be investigated. Besides their valuable nutritional composition, marine gammarids can harbour pigment profiles that can benefit aquaculture species

References

Alberts-Hubatsch, H., Slater, M. J., Beermann, J. (2019): *Effect of Diet on Growth, Survival and Fatty Acid Profile of Marine Amphipods – Implications for Utilisation as a Feed Ingredient for Sustainable Aquaculture*. Aquaculture Environment Interactions, 11, pp. 481-491

Gaillard, M. *et al*. (2004): *Carotenoids of two freshwater amphipod species (Gammarus pulex and G. roeseli) and their common acanthocephalan parasite Polymorphus minutus*, Comparative Biochemistry and Physiology, Part B, 139, pp. 129–136.

Olsen, R. E. and Baker, R. T. M. (2006): *Lutein does not influence flesh astaxanthin pigmentation in the Atlantic salmon (Salmo salar L.)*, Aquaculture, 258, pp. 558–564.

Wade, N. M., Gabaudan, J. and Glencross, B. D. (2017): *A review of carotenoid utilisation and function in crustacean aquaculture*, Reviews in Aquaculture, 9(2), pp. 141–156.

Yi, X, Li, J., Xu, W., Zhang, W., Mai, K. (2016) Effects of dietary lutein/canthaxanthin ratio on the growth and pigmentation of large yellow croaker *Larimichthys croceus*, Aquaculture Nutrition, 22, pp. 683-690

KRILL HYDROLYSATE ADDED BY TOP-COATING ON EXTRUDED PELLETS INCREASE FEED INTAKE AND GROWTH OF ATLANTIC SALMON (*Salmo salar* L.) MORE EFFICIENTLY THAN BY ADDING KRILL HYDROLYSATE IN THE DIET PRIOR TO EXTRUSION

Sissel Albrektsen*, Lene Sveen, Gert Timmerhaus, Odd Helge Romarheim

Nofima AS, Kjerreidviken 16, NO-5141 Fyllingsdalen
E-mail: sissel.albrektsen@nofima.no

Introduction

The smoltification process in combination with pumping and handling of salmon during seawater transfer may easily cause physical damage and stress in fish. This may result in poor appetite, and it can take weeks to get the smolts to eat properly. Increased susceptibility to pathogens and infectious diseases in salmon after transfer, accompanied by high annual production losses of smolt, have been reported. This has been associated to poor skin quality and impaired immunity of fish. The aim of this project was to study the effect of a krill hydrolysate (OlyPep, Rimfrost AS) as attractant in transfer feed for Atlantic salmon (*Salmo salar* L.), and to reveal impacts on feed intake, growth efficiency, skin quality and robustness of the smolt.

Materials and methods

A practical formulated diet with 15 % fishmeal (FM) was used as control diet (D1), while all other diets were added variable levels of krill hydrolysate (KH) or krill meal (KM). The KH was added at two dietary levels (2.5 %, 5.0 %), exchanging respectively 1.08 % and 2.15 % of the FM protein in D1, and it was added either prior to extrusion (D2, D3) or by top-coating on the extruded and final coated pellets (D4, D5). A KM with low content of water-soluble compounds exchanged 4.3 % of the dietary FM protein in another diet (D6). Dietary plant ingredients were similar in all diets, except wheat that was used to balance the diets. The diets were iso-caloric and with mean energy contents of 22.9 ± 0.2 MJ kg^{-1}. Dietary protein and lipid levels were average 446 ± 6 g kg^{-1} protein and 263 ± 5 g kg^{-1} lipid. Atlantic salmon (mean weight 91.9 ± 0.3 g) were starved for one day and randomly distributed to 18 fiberglass tanks (1m^2), each with 80 fish. The fish were fed one of six diets in triplicate tanks for a feeding period of 84 days, starting out from the first day of feeding after seawater transfer. The fish were fed in excesses of appetite (10 – 15 %) by automatic feeders and exposed to continuous light (24h) and ambient water temperature (mean temperature 11.7 ± 1.6 °C) during the trial.

Results

In the first feeding period (Week 0 – 6), fish fed diets added KH by top-coating (D4, D5) both showed significant higher feed intake (total FI (%), daily FI (%/mean BW/d)) and growth rate (SGR, %) as compared to all other groups ($P < 0.05$), Fig. 1. Fish fed diets added KH prior to extrusion (D2, D3) also showed higher FI and SGR compared to fish fed the control diet (D1), but significant differences in FI was only found in fish fed D3 ($P < 0.05$), Fig. 1. The KM diet (D6) did not promote increased FI in the first few days/weeks after transfer. However, within 6 weeks of feeding, the KM increased the FI in salmon to a level in between fish fed D2 and D3, followed by moderately increased growth similar to fish fed D2, Fig. 1. Results may suggest higher quality of KM relative to the substituted FM. In the last feeding period (Week 6 – 12), all dietary groups showed efficient FI and growth.

For the complete feeding period (Week 0 – 12), significant higher FI and SGR was found in fish fed the two KH top-coated diets ($P < 0.05$). Fish fed the highest level of KH added into the diet (D3) also showed significantly higher FI and growth as compared to the control diet and the KM diet ($P < 0.05$). A significant linear relation between FI and growth ($P < 0.05$), while no significant differences in feed conversion ratio (FCR) ($P > 0.05$) was observed. No or small differences in nutrient and total energy digestibility, and protein utilization efficiency (PER, PPV, BV) were found, while fish fed D3, D4 and D5 showed the best results. Apparent amino acid digestibility was significantly increased in fish fed all KH diets ($P < 0.05$) as compared to the control group, while fish fed the krill meal diet performed similar as control fish ($P > 0.05$). Higher plasma phospholipids were found for all krill products and levels relative to the control diet ($P < 0.05$). Twelve weeks post-transfer, the mean final weight of fish fed KH added by top-coating (D4, D5) was 130 g higher than in fish fed the control diet, equivalent to 32 % higher SGR (%). The results suggest that the amino acids and soluble N-compounds in KH that leaks into the water following top-coating of KH on extruded pellets induce a more efficient appetite triggering impact than by adding KH to the feed prior to extrusion.

(Continued on next page)

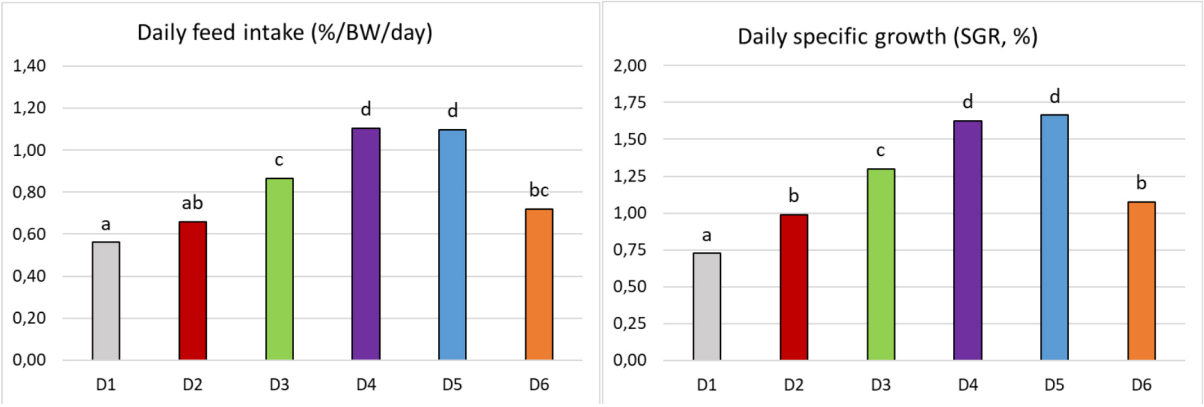


Fig. 1. Daily feed intake (%/BW/d) and growth (SGR, %) of A. salmon in the first feeding period (Week 0 – 6) after seawater transfer. A FM formulated diet served as control (D1), while KH was added to the feed at two dietary levels (2.5 %, 5.0 %) either prior to extrusion (D2, D3) or by top-coating to the extruded pellets (D4, D5). The KH exchanged respectively 1.08 and 2.15 % of the dietary FM protein. In addition, a krill meal exchanged 4.3 % of the dietary FM protein in another experimental diet (D6).

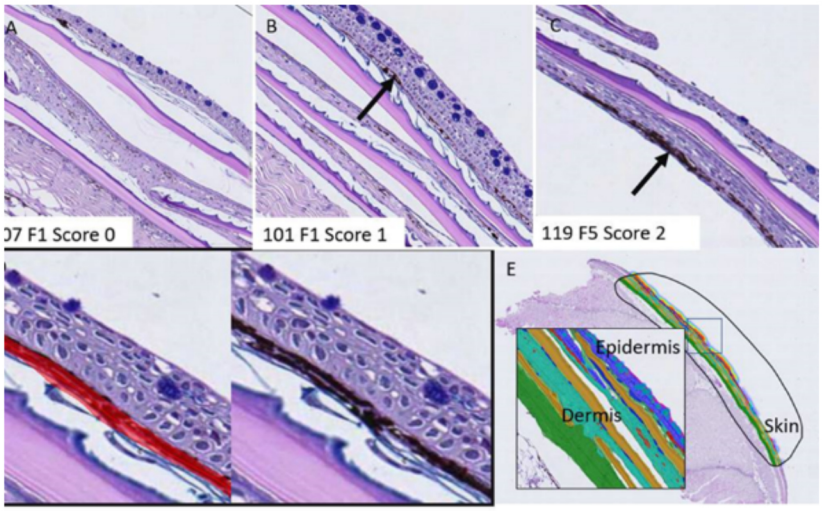


Fig. 2. Histological examination (Aiforia®) of the upper dorsal skin layers of experimental fish with OWI's skin score values of respectively 0 (A), 1 (B) and 2 (C), representative for Atlantic salmon with no, low or medium skin quality measured for scale loss.

Improved FI and growth of fish fed top-coated KH diets after seawater transfer was verified in another 6 week's feeding trial with A. salmon. Visual recorded differences in skin quality as observed in the first trial was followed up by visual (FishWell Operational Welfare Indicator scores (OWI's)) and histological (Aiforia®) examination of the dorsal skin quality of fish in the second trial. The visual skin quality, i.e. number of fish with scale loss, wounds or bleeding (n = 90 fish/diet) ranged on a scale (score values 0, 1, 2, 3) suggested better skin quality in the larger size fish fed the KH. The mean OWI scale loss score was lower in fish fed KH added by top-coating (mean score = 1.28) as compared to control fish (mean score = 1.70), but no significant differences were detected (Chi square P = 0.18, ANOVA p = 0.069). Significant higher values for dark pigmentation (melanin) in the upper dorsal skin layer were, however, found in fish with skin score = 2, as compared to fish with skin score = 1, Fig. 2. According to 2-way ANOVA, a significant dietary difference in skin quality was detected ($P < 0.05$). Dark pigmentation in the skin is consider a normal response to stress and skin damage in salmon.

Summary and conclusion

The positive FI and growth responses observed in fish fed the KH added by top-coating may be explained by efficient release of appetite triggering amino acids and N-compounds that leaks from the KH into the water and stimulate the FI of fish in the first period after seawater transfer. The increased FI was accompanied by equivalent increased growth. The FI stimulating effects of KH was less efficient when KH was added to the diet prior to extrusion. Visual and histological examination of the upper dorsal skin layer suggested improved quality with lower scale loss and less wounds in fish fed top-coated KH diets relative to fish fed the control diet. It is expected that the nutritional more well fed and larger fish fed KH will be able to grow a thicker and more robust skin. The observed improved quality of the dorsal skin is therefore considered a secondary response to the improved FI and growth of salmon. In conclusion, the KH significantly improved FI and growth of salmon in the first period after transfer and resulted in production of a more robust smolt with lower scale loss and more normal pigmented skin.

TRANSITIONING TO A CIRCULAR ECONOMY IN THE SEAFOOD SECTOR: BEST MANAGEMENT PRACTICES UNDER A NEXUS LIFE CYCLE NEXUS THINKING APPROACH

R. Aldaco*, I. Ruiz-Salmón, J. Laso, A. Quiñones, M.F. San Román, F.J. Amo-Setién, J. Cristobal, A. Fernández-Ríos, A. Irabien, M. Margallo

Department of Chemical and Biomolecular Engineering, University of Cantabria, Cantabria (Spain)
E-mail: aldaco@unican.es

Introduction

Transitioning to a circular economy in a seafood context requires "nexus thinking", which adopts a lifecycle approach to water-energy-food connections. This is essentially a transformative approach to governance, and also requires substantial changes in the way people behave. This work package focuses on how to govern such transformations, and the policy tools that will be required, including behavioral change interventions that go beyond mere education to influence how people make decisions about buying and consuming marine products.

Consequently, a new approach to assess seafood lifecycle is proposed based on the nexus of water-energy-food systems. The term "nexus" implies that the action in one of the systems has impacts on the others. Therefore, any strategy that focuses on one system without considering its connections with other systems may lead to acute unpremeditated consequences.

The water-energy-food nexus allows assessing the lifecycle of seafood products under a holistic manner considering the whole supply chain. Currently, there is no universally recognized methodology for nexus analysis. However, Life Cycle Assessment is particularly important for understanding the interconnections in the nexus, as it enables the consideration of entire supply chains. The definition of an integrated nexus eco-label facilitates the decision-making process to stakeholders and allows the development of strategies based on the circular economy concept (Ruiz-Salmón et al, 2021).

Materials and Methods

The work develops a methodology that combines water (WF), energy (EF), carbon (CF) and (NF) nutritional footprints to define an integrated nexus eco-label. The NEXUS Water-Energy-Food calculation comprises the following stages (Benini et al., 2014; He and Gu, 2016):

i. Selection of product environmental footprints: Establishment of representative environmental footprints to be included within the NEXUS eco-label. In this case, the WF, EF, CF and NF were selected to be included.

ii. Calculation: The assessment of the different environmental footprints is carried out following the guidelines and procedures based in the life cycle assessment tools.

iii. Normalization: Normalization is used to express the indicator data in a way that could be compared among all types of product environmental footprints. An internal normalization is considered in this methodology. In this way, the product with the lowest footprint in terms of WF, EF and CF is assigned a value of 100 and the rest of the products decrease in proportion. On the other hand, since the NF should be as good as possible, the product with the highest value will be assigned the value 100. In this way the value of the 4 variables for the different products are normalized on a scale of 1-100 (WFn, EFn, CFn and NFn), easily interpretable by the general population.

iv. Weighting: Assign weights to the different types of product environmental footprints based on their perceived importance to emphasize the most important potential impacts with the consideration of design requirements. It would obviously alter the results with different weights. As first assumption, all the indicators are given equal importance, so the weight by which they are multiplied would be 0.25, so that the final result of the NEXUS indicator for the product will be in the desired range between 1-100.

v. Aggregation: The resulting multi-criteria value of the NEXUS is obtained as follow:

$$NEXUS_i = w_1 \cdot WFn_i + w_2 \cdot EFn_i + w_3 \cdot CFn_i + w_4 \cdot NFn_i \quad [1]$$

Where WFn_i, EFn_i, CFn_i and NFn_i represent the value of the normalised footprints (water, energy, carbon and nutritional, respectively) for the analysed product (i). w_i is the correlative weight.

vi. Communication and dissemination of results: Once the NEXUS results are obtained, it is just as important to carry out good communication to disseminate the information to the interested audience in an effective way.

vii. NEXUS eco-label design

Once the NEXUS value has been obtained for each case study, it is necessary to design the ecolabel that will be used to communicate the results of the project to potential consumers and stakeholders.

(Continued on next page)

Results

This study will address the environmental analysis of the *Siberian sturgeon* (*Acipenser baerii*) from a fish farm in Teruel, Spain). The function was to evaluate the environmental impact of an aluminium can that sturgeon with sunflower oil. Based on the function, the selected functional unit (FU) was one can of 15.6 g containing the product. The production of the aluminium packaging, the electricity and fuel requirements and the production of sunflower oil are the main contributors in the Nexus footprint. The packaging represents between 50-70% of the total impact sturgeon, diesel production and consumption ranges from 21% to 40% and sunflower oil around 5%.

Conclusions

Measures to improve the environmental performance of canning products include the use of renewable energies, the eco-design of the packaging and the use of more sustainable materials, promoting circular economy approach. However, it should be also considered the preferences and behavior of consumers. These socials issues, as well as economic aspects, should be analysed for the packaging improvement.

The outcomes of the study will help to determine the environmental impacts and hotspots of canning products, proposing improvement measures regarding the process efficiency and the packaging. This will contribute to a more sustainable canning industry.

Nexus eco-label will help producers to introduce their seafood products in new green markets and consumers to identify more sustainable seafood products.

Acknowledgement

This work was supported by the EAPA_576/2018 NEPTUNUS project. The authors would like to acknowledge the financial support of Interreg Atlantic Area.

References

Ruiz-Salmón, I. et al., 2021.Life cycle assessment of fish and seafood processed products – A review of methodologies and new challenges, Science of The Total Environment, 761,144094,

Benini, L., Mancini, L., Sala, S., Manfredi, S., Schau, E.M., Pant, R., 2014. Normalisation method and data for envieonmental footprints. https://doi.org/10.2788/16415.

He, B., Gu, Z., 2016. Sustainable design synthesis for product environmental footprints. Des. Stud. 45, 159–186. https://doi.org/10.1016/j.destud.2016.04.001.

AQUACULTURE IN THE TIME OF COVID-19: CONSIDERATIONS FOR LOCAL AND GLOBAL OPPORTUNITIES AND RESPONSES

R. Aldaco*, I. Ruiz-Salmón, J. Laso, A. Quiñones, M.F. San Román, F.J. Amo-Setién, J. Cristobal, A. Fernández-Ríos, C. Campos, A. Irabien, M. Margallo

University of Cantabria, Cantabria (Spain).
E-mail: aldaco@unican.es

Introduction

Pollution, overfishing and the impacts of climate change are the main damages for oceans and, consequently, for fishing and the whole supply chain. To deal with these issues, the 14th Sustainable Development Goal (SDG) promotes the conservation and sustainable use of the oceans, seas and marine resources, which links to Zero Hunger (2nd SDG), Sustainable Consumption and Production (12th) or the urgent action to combat climate change and its impacts (13th), among others. The COVID-19 pandemic, unexpected target in the 3rd SDG (health), clearly damages the commitments proposed by the United Nations, adding an important handicap. Nevertheless, it also open the window to rethink the current status of the aquaculture, which are the challenges and opportunities and how to deal with them.

Some tools may improve the fish system (Aldaco et. Al, 2020). For instance, a robust inventory to improve the initial design of products, processes and services and to apply preventive and corrective actions would minimize the environmental impact associated to the life cycle phases, including some of the most relevant in each phase: food waste and discards in the process, distribution, packaging in the use, or final residue treatments. This approach is in progress on Neptunus Project (Neptunus Project, 2019), focused on the species farmed in European Atlantic region.

Year 2020: when the sector changed

The COVID-19 pandemic in 2020 has caused a global change. Particularly, in the fishing sector, several policies have been forced to be implemented to deal with health and environmental effects and socio-economic implications that have been triggered. In view of all these problems, it is necessary to adopt certain guidelines that will facilitate this situation in the near future.

Learnings and opportunities in the near future

Despite the clear negative consequences that COVID-19 has had on the fishing sector, the pandemic presents an opportunity to transform the food system to be greener, more inclusive, and resilient (Love et al., 2020).

1. The use of alternative networks (ANs), i.e. fish distribution models that serve local and regional food systems and deliver fish directly to consumers, present a segment of the food system that has not been fully taken advantage of it before. ANs have implications with respect to the organization of production and distribution of food, as well as for policy options for enhancing the systemic resilience of fish systems moving forward, allowing the sector to respond effectively.
2. The use of technologies can facilitate the adaptation of the commercial sector on the pandemics, allowing companies to promote their products and connect with consumers. Therefore, maintaining and building diversity and connectivity at the community, company, and country level are ways to build resilience and guard against bad outcomes.
3. With respect to the product, the sale of shelf-stable and frozen fish, instead of live-fresh fish, is an important option to consider since it guarantees less food loss and waste but may become unviable the sustainability for small aquaculture companies. Moreover, the promotion of shorter food supply chains, namely fresh meat and fish, under 'zero km' strategies, also minimize the environmental impact associated to the transport. Thus, the lifestyle during pandemic changed, making local purchasing gained importance in the collective thinking. Indeed, mobility restrictions, even in small towns, favor this. These local markets represent a more resilient and sustainable solution, reducing transportation, providing a better supply-demand balance, creating more transparency and tracking and contributing to waste reduction.
4. Finally, regarding the policies, COVID-19 provides an opportunity to integrate wider policies that are more coherent and make sense from an ecological perspective, considering the management and protecting the sector throughout their life cycle. The opportunity presented by the COVID-19 slowdown should be use to encourage inefficiencies within the system to reduce energy use, increasing profit and reducing environmental impacts.

(Continued on next page)

Conclusions

The COVID-19 pandemic has weakened the commitments proposed by the United Nations, making policies related to climate change and other environmental issues in aquaculture sector take a backseat and highlighting other social, health and economic aspects, hindering the improvement of the sector. Economic crisis and poorer material conditions for citizens; concern about COVID infections of aquaculture workers; closure of distribution channels or eventual plastic pollution in oceans from extra personal protective equipment delivery are few examples of what this pandemic has caused in short and long terms. This requires that life and economic derivatives maintain the sustainability and, consequently, it is necessary that the way in which it is produced and consumed is more efficient, reducing raw material and long-distance distribution and assuring food sovereignty by supporting local economies.

Therefore, certain support policies for the aquaculture sector have had to be brought with immediate responses against the economic and social consequences of the pandemic crisis. In addition, in order to deal with the effects on health, fish oils have been used as a nutrient for the sick. Environmentally, the quality of coastal waters has greatly improved and certain socio-economic problems have appeared such as the closure of distribution channels and many fish farms. Despite the clear negative impact of COVID-19 on this sector, the pandemic presents an opportunity to transform the food system to be more environmentally friendly, inclusive and endurable.

Acknowledgements

This work was supported by the EAPA_576/2018 NEPTUNUS project. The authors would like to acknowledge the financial support of Interreg Atlantic Area.

References

NEPTUNUS Project: Water-Energy-Seafood Nexus Eco-innovation and Circular Economy Strategies in the Atlantic Area. https://neptunus-project.eu/

Love, D., Allison, E. H., Asche, F., Belton, B., Cottrell, R. S., Froehlich, H. E., … Zhang, W.:Emerging COVID-19 impacts, responses, and lessons for building resilience in the seafood system. SocArXiv 2020.

Aldaco, R., Hoehn, D., Laso, J., Margallo, M., Ruiz-Salmón, I., Cristobal, J., Kahhat, R., Villanueva-Rey, P., Bala, A., Batlle-Bayer, L., Fullana-i-Palmer, P., Irabien, A., Vazquez-Rowe, I.: Food waste management during the COVID-19 outbreak: a holistic climate, economic and nutritional approach. Sci. Total. Environ. 2020. 742:140524.

GENOMIC PREDICTION AND GENOTYPE-BY-TEMPERATURE INTERACTION OF SEX TENDENCY IN EUROPEAN SEA BASS

F. Allal*[a], M. Besson[b], B. Sadoul, F. Ruelle [a], M. Pegart [a], M.-O. Blanc [a], A. Vergnet[a], F. Clota[a,c], N. Sánchez-Baizán[d], F. Piferrer[d], M. Vandeputte[a,c], B. Geffroy[a]

[a] MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34250 Palavas-les-Flots, France
[b] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[c] INRAE, GABI, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[d] Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain
E-mail: francois.allal@ifremer.fr

Introduction

European sea bass (*Dicentrarchus labrax*) is an important European aquaculture species. Females, performing better than males with later maturation and larger size, are preferred. Unfortunately, in aquaculture conditions, the sex-ratio is typically biased towards males. Thus, deciphering the sex determinism system and finding routes for controlling the sex-ratio of this species has been a long-lasting challenge to support sea bass production and selective breeding programmes. A polygenic threshold sex determination system was demonstrated (Vandeputte et al. 2007), where the genetic sex tendency is influenced by larval rearing temperature to determine the phenotypic sex (Piferrer et al, 2005). In this study, we applied two thermal treatments during early larval stage, a low temperature protocol (16°C, LT), known to favour more balanced sex-ratios, and a high temperature masculinizing protocol (21°C, HT). We used genome-wide SNP genotypes to estimate the genetic parameters and genotype-by-temperature interaction of sex tendency in European sea bass.

Material and Methods

We produced 8 families by mating 8 males with a same female. The progenies were reared in common garden under two thermal treatment in triplicate: a LT (16°C) and a HT (21°C) temperature protocol. 1013 fish were sexed at one year and genotyped for 57k SNPs with the DLabCHIP array (Griot et al., 2021). Pedigree was obtained using a subset of 1,000 highly polymorphic SNPs, using the R package APIS (Griot et al., 2020). After filtering SNPs with a call rate >0.90, a sufficient minor allele frequency (MAF>0.05) and no mendelian transmission errors, 39,607 polymorphic SNPs were retained to estimate genomic-based heritability, estimated sex tendency (with temperature as fixed effect) and the genetic correlation between treatments, with a threshold model using THRGIBBS1F90 (Tsuruta and Misztal, 2006).

Results and Discussion

The proportion of females obtained was 53.4% at LT and 25.3% at HT. As expected, the high temperature induced a marked masculinization, producing half of the females than at LT treatment. Sex tendency at LT (sex_LT) and at HT (sex_HT) were estimated as highly heritable with $h^2_{sex\_LT} = 0.65 \pm 0.06$ and $h^2_{sex\_HT} = 0.51 \pm 0.08$. A strong genetic correlation between sex_LT and sex_HT was observed ($r_G = 0.91 \pm 0.09$). The genomic-based estimation of the sex tendency of the animals is shown in Figure 1. Regarding the estimated sex tendency, as the combination of individual genetic sex tendency and the fixed effect of temperature, we observed that at LT, the mean sex tendency (0.12) is close to the theoretical threshold of zero (above which an animal is more likely to become a female). On the contrary, the distribution of the sex tendency estimated for animals at HT is typically displaced toward negative values with a mean at -0.89.

The lower heritability estimates for HT and the skewing of the estimated sex tendency toward negative values could result from the temperature-mediated impact on the sex at HT. At HT some phenotypically male fish can be considered as “reversed-females”, also known as “neomales”, confirming the hypothesis of a polygenic threshold model of sex determination.

These results confirm the high heritability of sex tendency in European sea bass, show the effect of larval rearing temperature on sex tendency is mostly additive (high genetic correlation between LT and HT), and highlight the efficiency of genomic-based mixed animal models to estimate breeding values for sex tendency in sea bass, with potential applications to identify sex-reversed “neomales” which would be of high interest to increase proportions of females in farmed populations of sea bass.

(Continued on next page)

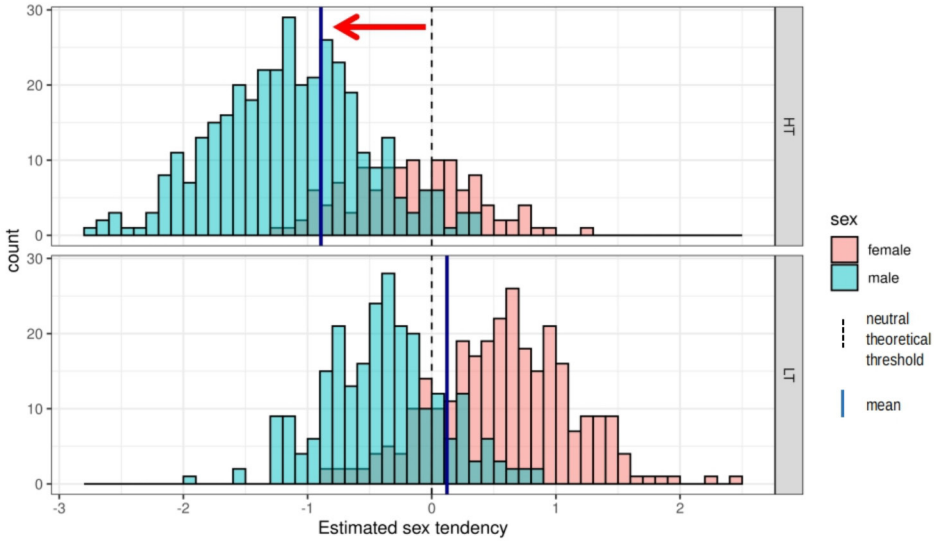


Figure 1. Distribution of the estimated sex tendency of phenotypic males and females reared at high temperature (HT) and at low temperature (LT). The red arrow shows the temperature-mediated shift of the mean sex tendency compared to the neutral theoretical threshold.

Acknowledgement

This work was partially supported by the 3S - Seabass Sex and Stress project (n° 4320175237) funded by the French Government and the European Union (EMFF, European Maritime and Fisheries Fund) at the "Appels à projets Innovants" managed by the France Agrimer Office, and the European Union's 7th Framework Programme under Grant Agreement 652831 (AQUAEXCEL2020, Transnational Access project AE040073)

References

Griot R., Allal F., Brard-Fudulea S., Morvezen R., Haffray P., Phocas F., Vandeputte M., 2020. APIS: an auto-adaptive parentage inference software that tolerates missing parents. *Mol. Ecol. Resour.*, 20, 579-590

Piferrer F., Blázquez M., Navarro L., González A., 2005. Genetic, Endocrine, and Environmental Components of Sex Determination and Differentiation in the European Sea Bass (*Dicentrarchus Labrax* L.). *Gen. Comp. Endocrinol.*, 142, 102-110

Tsuruta S., Misztal I., 2006. THRGIBBS1F90 for estimation of variance components with threshold and linear models. *Proc. 8th World Congr. Genet. Appl. Livest. Prod.* Belo Horiz. Minas Gerais Braz. 13-18 August 2006, 27–31.

Vandeputte M., Dupont-Nivet M., Chavanne H., Chatain B., 2007. A Polygenic Hypothesis for Sex Determination in the European Sea Bass *Dicentrarchus labrax*. *Genetics* 176, 1049–1057.

AQUACULTURE AND THE PRIMARY SECTOR: IMPACTS ON THE LOCAL ECONOMY

Gouveia, R. *, Machado, L, Almeida, A.

Faculty of Social Sciences, University of Madeira

Email:ricardogouveia@staff.uma.pt

While the idyllic image of islands is linked to a striving and successful tourism sector globally oriented, a sizeable share of the employment and GVA relates to traditional sectors such as the agriculture, fisheries and aquaculture. Such sectors are equally strategic in terms of external image conveyed abroad, cultural heritage and local identity. In this paper we compute based on a simple econometric model we estimate the impact of recent changes in the agriculture and fisheries sector to the regional GDP. The aim of this paper is to highlight the relevance of the primary sector as an alternative to tourism during the times of economic crisis.

MEDITERRANEAN MONK SEAL (*Monachus monachus*): PERCEPTION ABOUT FISH FARMING INTERACTIONS IN THE ARCHIPELAGO OF MADEIRA

A. Amaral[1]*, P. Almeida[1,2,3], R. Pires[4], C. Andrade[5,6,7]

[1]Universidade de Évora, Portugal
[2]MARE - Marine and Environmental Science Centre
[3]Fluviário de Mora
[4]Institute of Forests and Nature Conservation
[5]Mariculture Center of Calheta, Fisheries Directorate, Calheta, Portugal
[6]Oceanic Observatory of Madeira, Regional Agency for the Development of Research, Technology and Innovation, Funchal, Portugal
[7]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal
E-mail: anaamaral97@gmail.com

Introduction

The sub-population of monk seals from Madeira island, Portugal, is comprised of 21 individuals (aged over 1 year) in 2018, which is distributed across the island of Madeira and the Desertas Islands. It is an extremely vulnerable and endangered population classified under the IUCN red list of threatened species (Karamandilis and Dendrinos, 2015). The monk seals from Madeira may interact with several human activities at sea such is the case of fish farming. In Madeira island there are three aquaculture companies in the open sea (offshore): Marismar (Calheta), Aquailha (Ribeira Brava) and Ilha-Peixe (Machico). Aquaculture systems in an open environment are known for attracting fish-eating predators due to the large number of confined fish (Güçlüsoy, 2003). The aim of this study was to understand the perception from different maritime professional sectors about monk seal - fish farming interactions, in Madeira island.

Materials and methods

Interviews were conducted to fish farm workers (16), whale-watching's workers (14), diving club workers (10) and IFCN's (Institute of Forests and Nature Conservation) workers (6). The questionnaire surveys were conducted along the south coast of the island of Madeira at the workplace of each respondent. Interviews took place between February and May, 2021. Each interview lasted approximately 5 minutes. The questionnaire had question groups in order to understand the perception of the workers on the monk seal-fish farming interactions, the frequency of sighting of monk seals in each fish farm and assess whether the workers opinions were based on real observations or influenced by external factors. Data analysis was performed using *Microsoft Office Excel 2007*™ and *IBM SPSS Statistic 26* for Windows. Descriptive statistics were used and nonparametric tests.

Results

47 surveys were carried out, 74% of respondents didn't mention the monk seal as an animal that would cause any harm to fish farms. It was found that the perception of monk seal-fish farming interactions is independent of the career, the place where the respondents most often work and the respondent's place of residence. Most respondents claimed observing the monk seal close to fish farming rarely (54%) or never (26%), regardless of the frequency of their trips out to sea and the fish farm they passing by. It was considered that most of the respondents have their opinion influenced by external factors, with the main sources of influence being informal conversations, followed by Institute of Forests and Nature Conservation (IFCN) and media. Most respondents don't show animosity towards monk seals. In relation to aquaculture, it was found that there are significant differences between the degree of animosity shown depending on the maritime activity performed.

Discussion

Efforts to study these interactions have been carried out in Turkey, where negative interactions are particularly intense (Güçlüsoy, 2003). A similar sociological study about this monk seal sub-population from Madeira has been carried out by Hale (2011), but only covering the monk seal-fisheries interaction. The majority of respondents didn't mention the monk seal as an animal that would cause any harm to fish farms (74%), despite that this has been reported in Turkey (Güçlüsoy 2003). Nevertheless, in Madeira the majority of fishermen believe that the species doesn't damage fishing gear or catches (67%) (Hale, 2011). Less than 20% of respondents mentioned having seen the monk seal close to fish farming occasionally or always, likewise only 30% of the fishermen interviewed in the Hale's study (2011), claimed to have experienced monk seal – fishing gear interactions.

(Continued on next page)

Tourist/leisure activities and aquaculture are interactions identified as a threat to this species (Pires, 2020). Nevertheless, the perception of the inquiries was that these interactions on fish farms are rare, with no attacks. It should be noted that the presence of the monk seal in Madeira is a source of pride, which leads to greater awareness (Pires, 2020), reflected in some of influenced opinion's respondents who refer to the IFCN as a source of information for the knowledge of the species.

Further studies and awareness, through informal conversations, about this interaction aimed at each maritime activity are needed.

Bibliography

Güçlüsoy, H., & Savas, Y. (2003). Interaction between monk seals *Monachus monachus* (Hermann, 1779) and marine fish farms in the Turkish Aegean and management of the problem. *Aquaculture Research*, *34*(9), 777-783.

Karamanlidis, A. & Dendrinos, P. (2015). *Monachus monachus* (errata version published in 2017). *The IUCN Red List of Threatened Species* 2015: e.T13653A117647375.https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T13653A45227543.en. Downloaded on 17 May 2021.

Hale, R., Pires, R., Santos, P., & Karamanlidis, A. A. (2011). Mediterranean Monk Seal (Monachus monachus): Fishery Interactions in the Archipelago of Madeira. *Aquatic Mammals*, *37*(3).

Pires. R.; Aparicio, F. & Fernandez de Larrinoa, P. (2020). Estratégia para a Conservação do Lobo-marinho no Arquipélago da Madeira. Instituto das Florestas e Conservação da Natureza, IP-RAM Eds.

STRATEGIES FOR *EX SITU* CONSERVATION OF PORTUGUESE OYSTER *Crassostrea angulata* GENETIC RESOURCES

C. Anjos[1,2*], D. Duarte[1], P. Diogo[1], A.L Santos[1], S. Joaquim[2], D. Matias[2] and E. Cabrita[1]

1 Centre of Marine Sciences-CCMAR, University of Algarve, 8005-139 Faro, Portugal
2 Portuguese Institute for Sea and Atmosphere-IPMA, Av. 5 de Outubro, 8700-305 Olhão, Portugal
*e-mail: cmanjos@ualg.pt

Introduction

Portuguese oyster suffered a major decline on natural populations in the past decades in Europe and strategies for its restoration and management need to be developed. Cryopreservation is a helpful tool for the management of endangered genetic resources through the preservation of genetic material in ultralow temperatures (Martínez-Páramo et al. 2017). In invertebrates, such as oysters, it is possible to preserve the genetic resources of both parents, through sperm and oocytes cryopreservation. However, it is also possible to preserve the offspring by freezing embryos and larvae. The availability of cryopreserved sperm is useful when there is no synchrony between spawning of males and females. However, the cryopreservation of larvae presents several advantages, since it allows the availability of diploid animals upon thawing, which simplifies its application for aquaculture purposes (Labbé et al. 2018). Cryopreservation methodologies needs to be adapted according to the species and type of biologic material, being necessary to explore the type and toxicity levels of cryoprotectants and use of single or combined freezing solutions. The main goal is to achieve the conditions that produce the lowest levels of cryodamage, improving post-thaw quality. The objective of this work was to develop conservation strategies of Portuguese oyster through sperm and larvae cryopreservation to support this species management.

Materials and methods

Samples from 10 males of Portuguese oyster were collected by gonadal incisions following the protocol described by Riesco et al. (2017). Each sperm sample was cryopreserved with three cryoprotectant medium in a programed biofreezer at -6ºC/min. Cryoprotectant solutions were prepared in filtered seawater containing 20% dimethyl sulfoxide (DMSO) and 20% DMSO added with 0.9 M trehalose or sucrose. Post-thaw sperm motility was analyzed with CASA software. Flow cytometry was applied to determine the cell viability using PI and to detect levels of reactive oxygen species in live post-thaw sperm by combination of DHE and Sytox dyes. MDA quantification was used to assess lipid peroxidation.

Portuguese oyster larvae were produced by broodstock gonadal incisions to collect the gametes followed artificial fertilization and incubation until D stage. Pools of 60,000 D-larvae/mL (n=7-8) were divided in five aliquots, one used as control and the others exposed and cryopreserved to two cryoprotectant solutions. The solutions were composed by 20 % (v/v) ethylene glycol (EG) or DMSO, 2% (w/v) polyvinylpyrrolidone 40000 MW and 0.4 M sucrose in milli-Q water. Larvae samples were exposed for 3 minutes to cryoprotectant solutions in a 1:1 (v/v) proportion to evaluate the toxicity levels of cryoprotectants. For cryopreservation, samples were loaded into 0.5 mL straws and freeze in a programmable biofreezer. Larvae diluted in filtered seawater (FSW) was used as control. D-larvae quality was evaluated before and after cryoprotectant exposure and post-thawing, according to morphology, motility rate and velocity. Moreover, the whole transcriptome profile of fresh larvae, larvae exposed to DMSO solution, and post-thaw larvae were compared.

Results and discussion

Sperm cryopreserved with DMSO supplemented with sugars significantly improved post-thaw sperm viability and reduced levels of lipid peroxidation and ROS in comparison with DMSO treatment. This can be related with the fact that combination of permeable and non-permeable cryoprotectant interact as membrane fluidity regulator and increase the membrane hydrophobicity (Hassan et al. 2017). The supplementation with sugars was successfully applied in other oyster species such as Pacific oyster (Adams et al. 2004) and Australian flat oyster (Hassan et al. 2017).

(Continued on next page)

The toxicity tests showed that larvae exposed to cryoprotectant solutions containing DMSO had no significant differences in morphology and motility when compared to control. Larval movement (VAP) was significantly higher in DMSO treatment than EG treatment, suggesting that DMSO promoted lower toxicity. Cryopreservation reduced larval quality parameters when compared with control. This effect was also described for Pacific oyster by Labbé et al. (2018). Oyster larvae cryopreserved with DMSO solution showed a significant improvement of larval movement when compared to EG treatment. In this way, DMSO as internal cryoprotectant was beneficial in maintaining post-thaw larvae quality. The transcriptomic analysis of Portuguese oyster larvae did not show many differences in gene expression between fresh and cryoprotectant exposed larvae. Contrarily, after cryopreservation there was a significant number of genes in larvae up and down regulated compared with the ones in fresh larvae. This supports the results previously described, which suggested that quality reduction of D-larvae was related to cryopreservation or cold damage.

Conclusion

The current work provided methodological tools to set up a Portuguese oyster gene bank through the establishment of cryopreservation protocols for sperm and larvae to support the aquaculture and restoration programs.

Acknowledgements

Supported by 0139_VENUS_5_E (Interreg POCTEP), ASSEMBLE+ JRA2-H2020-INFRAIA-2016-2017 (No 730984), EBB-EAPA_501/2016 (Interreg Atlantic Area) and received national funds through FCT - Foundation for Science and Technology through project CCMAR/Multi/04326/2021. Catarina Anjos was supported by a FCT fellowship (SFRH/BD/130910/2017).

References

Adams S.L., Smith J.F., Roberts R.D., Janke A.R., Kaspar H.F., Tervit H.R., Pugh P.A., Webb S.C., King N.G. (2004) Cryopreservation of sperm of the Pacific oyster (Crassostrea gigas): development of a practical method for commercial spat production, Aquaculture 242, 271-282.

Labbé C., Haffray P., Mingant C., Quittet B., Diss B., Tervit H.R., Adams S.L., Rimond F. and Suquet M. (2018) Cryopreservation of Pacific oyster (*Crassostrea gigas*) larvae: Revisiting the practical limitations and scaling up the procedure for application to hatchery. Aquaculture 488, 227-234. doi: 10.1016/j.aquaculture.2018.01.023.

Martínez-Páramo S., Horváth A., Labbé C., Zhang T., Robles V., Herráez P., Suquet M., Adams S., Viveiros A., Tiersch T. and Cabrita E. (2017) Cryobanking of aquatic species. Aquaculture 472, 156-177. doi: 10.1016/j.aquaculture.2016.05.042.

Riesco M.F., Félix F., Matias D., Joaquim S., Suquet M. and Cabrita E. (2017) First study in cryopreserved sperm of Crassostrea angulata. General and Comparative Endocrinology 245, 108-115. doi: 10.1016/j.ygcen.2016.05.003.

Hassan M.M., Li X., Qin J.G. (2017) Improvement of post-thaw sperm survivals using liquid nitrogen vapor in a spermcasting oyster *Ostrea angasi*. Cryobiology 78, 1-7. doi: 10.1016/j.aquaculture.2004.08.034

THE ROLE OF STARTER DIETS ON THE DEVELOPMENT OF SKELETAL ABNORMALITIES IN ZEBRAFISH

A. Antinero[1*], S. Fragkoulis[1], Ch. Kourkouta[1], A. Printzi[1,2], D. Mazurais[2], J.L. Zambonino-Infante[2], G. Koumoundouros[1]

1, Biology Department, University of Crete, Heraklion, Greece
2, IFREMER, University of Brest, CNRS, IRD, LEMAR, F-29280, Plouzané, France
Email: ariel.021297@gmail.com

Introduction

The occurrence of skeletal abnormalities is a significant problem for aquaculture industry (Boglione *et al*. 2013), as well as for laboratory fishes (e.g. zebrafish, Martins *et al*. 2018; Printzi *et al*. 2021). Skeletal abnormalities have severe consequences not only on the production cost but also on the welfare of the fish (Boglione *et al*. 2013). Most of the skeletal abnormalities develop during larval rearing and early juvenile period (Koumoundouros 2010). Main factors that trigger the development of skeletal abnormalities are the adverse abiotic conditions, genetic factors and nutrition (Boglione *et al*. 2013).

In this study, we examined the effect of different starter diets on the development of skeletal abnormalities and survival rate in zebrafish *Danio rerio*.

Material and Methods

Four commercial (A-D) and one experimental (CTRL) starter diets were tested. Of the commercial, two are formulated specifically for zebrafish rearing (A and D), one for freshwater ornamental fish (B) and one for marine fish larvae (C). Larvae were initially fed with *Artemia* nauplii (4-7 days post fertilization, dpf), then with *Artemia* nauplii and dry diets (8-11 dpf), and finally with dry diets only (12-20 dpf). Larvae were fed five times per day. Trials were performed in triplicate, in a closed recirculation system, at 28^{o}C water temperature.

At 18-20 dpf, a random sample of fifty specimen were taken from each experimental population anaesthetized, formalin fixed and double-stained for the examination of skeletal abnormalities (Walker and Kimmel 2007). For the survival rate, the total number of fish in each diet was counted at the end of the larval rearing. The significance of the differences in the abnormality rates and survival rate among the dietary regimes was tested by G-test (Sokal and Rohlf 1981).

All diets were analyzed with respect to their proximate composition (Table 1).

Results & Discussion

Our results showed that starter diets had a significant effect on the development of caudal scoliosis ($p<0.05$, 19.3-54.7%, Fig. 1B, B') and gill-cover abnormalities of either light ($p<0.05$, 11.3-36.7%, Fig. 1C, C') or severe ($p<0.05$, 1.3-11.3%, Fig. 1D, D') intensity. Similarly, the survival rate was significantly affected by the nutritional regimes applied ($p<0.05$, 63.1-90.3%, Fig. 1A) in the different diets.

Existing literature demonstrate that larval diet has significant effects on the development of zebrafish skeleton (Martins *et al*. 2018, Printzi *et al*. 2021). Our results showed that the use of specialized zebrafish diets (A, D, CTRL) may comparatively decrease the incidence of skeletal abnormalities and increase fish survival rate. More research is required to fine-tune the nutritional composition of zebrafish starter diets whilst taking into account critical rearing parameters on zebrafish skeletal development.

Table 1. Proximate composition of each starter diet.

Feed	DM (%)	Ash (%)	Proteins (%)	TL (%)	NL (%)	PL (%)
Artemia	94.8	6.5	61.7	19.2	9.2	6.6
Control	94.0	12.0	54.7	16.7	4.7	11.7
A	93.9	13.9	66.8	18.5	4.0	8.8
B	93.9	12.0	63.5	12.6	5.7	4.6
C	93.2	14.0	67.3	16.8	6.8	5.5
D	91.0	12.7	67.7	13.0	4.0	6.3

DM, dry matter; TL, total lipids; NL, neutral lipids; PL, phospholipids.

(Continued on next page)

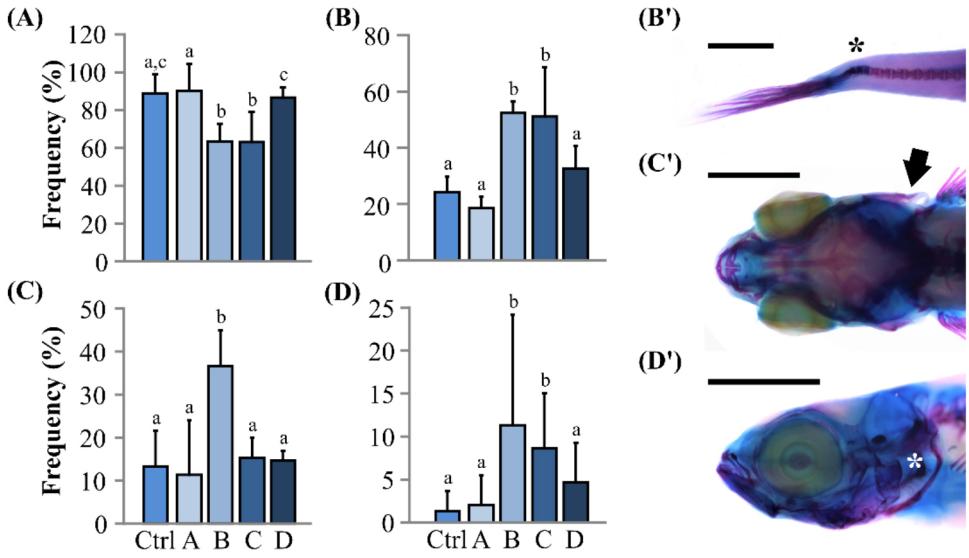


Fig. 1. Survival rate (A) and abnormalities frequency (B-D) in the five dietary regimes, at the end of the trials (18-20 dpf). (B-B') caudal scoliosis. (C-C') light gill-cover abnormalities. (D-D') severe gill-cover abnormalities. The absence of a common letter indicates statistically significant differences between diets (G-test, $p<0.05$). Error bars are equal to 1SD. Scale bars = 1.0 mm.

Acknowledgments

This study was made possible by the ERASMUS MUNDUS scholarship grant along with the people involved in the ACES+ program.

References

Boglione C, Gisbert E, Gavaia P, Witten PE, Moren M, Fontagné S, Koumoundouros G (2013) Skeletal anomalies in reared European fish larvae and juveniles. Part 2: Main typologies, occurrences and causative factors. Rev. Aquacult. 5: 121-167.

Koumoundouros G (2010) Morpho-anatomical abnormalities in Mediterranean marine aquaculture. Rec. Adv. Aquacult. Res. 661: 125-148.

Martins G, Diogo P, Pinto W, Gavaia P J (2018) Early transition to microdiets improves growth, reproductive performance and reduces skeletal anomalies in zebrafish (*Danio rerio*). Zebrafish 16: 300-307.

Printzi A, Kourkouta Ch, Fragkoulis S, Dimitriadi A, Geladakis G, Orfanakis M, Mazurais D, Zambonino-Infante J-L, Koumoundouros G (2021) Balancing between *Artemia* and microdiet usage for normal skeletal development in zebrafish (*Danio rerio*). J. Fish Dis., in press (https://doi.org/10.1111/jfd.13487).

Sokal R, Rohlf, F (1981) Biometry. San Francisco, California, USA: Second edition, WH Freeman.

Walker M, Kimmel C (2007) A two-color acid-free cartilage and bone stain for zebrafish larvae. *Biotech Histochem* 82: 23-28.

IMPACT OF STARVATION ON PLASMA CORTISOL IN GILTHEAD SEABREAM (*Sparus aurata* L.) FED WITH PHYTOGENIC FEED ADDITIVES

E. Antoniadou*, O. Ntantali, P. Panagiotaki, I.T. Karapanagiotidis, E. Golomazou

Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytokou str., 38446, Volos, Greece
E-mail: egolom@uth.gr

Introduction

In aquaculture a period of starvation is a common practice prior to handling, transportation and harvesting, in order to ensure good water quality and to reduce metabolic rate and stress (Waagbø *et al*. 2017). *Origanum vulgare* and *Cinnamomun zeylanicum* have proved their growth-promoting, genoprotective, antistress, antioxidant, antidiabetic, anti-inflammatory, antimicrobial, antiviral, antiparasitic, antineoplastic and immune modulatory effect (Khafaga *et al*. 2020). Very few studies have examined *Cannabis sativa* oil effects in fish as dietary additive, despite the fact that hempseed oil attracts the interest of scientific community. This study attempts to investigate the effect of *O. vulgare*, *C. zeylanicum* and *C. sativa* essential oils as phytogenic feed additives (PFAs), on plasma cortisol levels in *Sparus aurata* under starvation, in order to highlight the beneficial use of medicinal plants by the aquaculture industry.

Materials and methods

All the procedures involving fish were performed according to the EU guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). Seven isonitrogenous and isoenergetic diets were applied in gilthead seabreams (n=630, 5±0,12g) (*S. aurata* L.) in three replicates: two with cinnamon essential oil (*C. zeylanicum*) (CIN1%: 1% and CIN2%: 2% of the aquafeed), two with oregano essential oil (*O. vulgare*) (OR1%: 1% and OR2%: 2% of the aquafeed), two with hempseed oil (*C. sativa*) (CAN1%: 1% and CAN2%: 2% of the aquafeed) and one control diet (no supplementation). The feeding trial lasted 84 days and each diet was applied in six tanks. At the end of the feeding trial a 14 days starvation period was applied, in three of the six tanks for each feeding group. At the end of the starvation trial, fish were anesthetized and blood samples were obtained from the caudal vein. Plasma cortisol was measured using commercial Cortisol Elisa Kit, Cayman Chemical, USA (No. 500360) and the FLUOstar Omega microplate readers from BMG LABTECH.

Results

Significantly lower cortisol levels ($p<0,05$) were observed in normally feeding groups compared to groups that food deprivation was applied. Between groups that sustained the starvation challenge, significantly higher cortisol levels ($p<0,05$) were measured in OR1% and control groups, while OR2% and CIN1% were the groups with the statistically significant lower values ($p<0,05$). CIN2% and CAN2% supplemented groups presented higher cortisol levels compared to CIN1% and CAN1%, respectively. Group fed with OR1% incorporated diet showed significantly higher cortisol level than the group fed with OR2%.

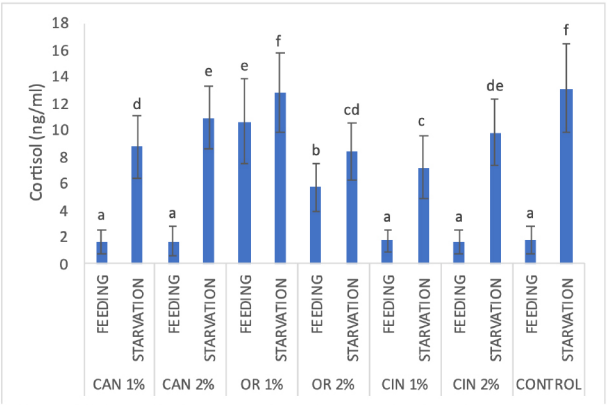


Fig. 1. Effect of *C. sativa O. vulgare* and *C. zeylanicum* incorporated diets on plasma of gilthead seabream (*S. aurata*) after feeding and starvation challenge. Letters a, b, c, d, e, f indicate significant differences between treatments (ANOVA; $P<0.05$).

(Continued on next page)

Discussion

Presently, starvation challenge proved to be a stress promoting factor compared to normally feeding process. All the experimental starvation groups supplemented with PFAs, except for the OR1%, revealed statistically significant lower cortisol levels, compared to control group, indicating that these additives maybe capable of restraining the elevated cortisol levels triggered by starvation stress. Fishfeed supplemented with 1% *C. zeylanicum*, proved to be the most effective, combining low cortisol level during feeding and reduced cortisol level under stress promoting practices. *O. vulgare* and *C. zeylanicum* incorporated diets consider to be welfare promoters under stress conditions reducing cortisol secretion (Santos *et al*. 2016, El-Hawarry *et al*. 2018). In conclusion, the current study revealed that PFAs reduced stress response in *S. aurata* exposed to starvation challenge, indicating that they maybe promising anti-stress agents used under stressful aquaculture practices.

Acknowledgements

«This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research – 2nd cycle" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)»

References

Dos Santos WM, de Brito TS, de A. Prado S, de Oliveira CG, de Paula AC, de M. Hoyos DC, Ribeiro PAP. (2016). Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress. Fish and Shellfish Immunology 54, 551–555.

El-Hawarry W.N., Mohamed R.A., Ibrahim S.A. (2018). Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*). The Egyptian Journal of Aquatic Research 44, 173-178.

Khafaga A.F., Naiel M.A.E., Dawood M.A.O., Abdel-Latif H.M.R. (2020). Dietary Origanum vulgare essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (*Cyprinus carpio*). Aquatic Toxicology 228, 105624.

Waagbø, R., Jørgensen, S. M., Timmerhaus, G., Breck, O., & Olsvik, P. A. (2017). Short-term starvation at low temperature prior to harvest does not impact the health and acute stress response of adult Atlantic salmon. PeerJ, 5, e3273.

PHYTOGENIC FEED ADDITIVES IN GILTHEAD SEABREAM (*Sparus aurata* L.): STRESS PARAMETERS EVALUATION UNDER HANDLING STRESS CONDITIONS

E. Antoniadou*, O. Ntantali, P. Panagiotaki, I.T. Karapanagiotidis, E. Golomazou

Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytokou str., 38446, Volos, Greece
Email: egolom@uth.gr

Introduction

Handling during intensive fish culture is an unavoidable and stressful practice that should be carried out only when necessary, as it elicits a maximal emergency physiological response. Essential oils of *O. vulgare* and *C. zeylanicum*, as phytogenic feed additives (PFA), have shown various beneficial effects in aquaculture, such as antistress, antipathogen, appetite-stimulating, growth-promoting, tonicity-enhancing and immunostimulatory activities (Abdel-Latif *et al*. 2020, Khafaga *et al*. 2020). *Cannabis sativa* oil is in the interest of scientific community during the last years, however few studies have examined hempseed oil effects on fish production as dietary additive (Pellati *et al*. 2018). The present study aimed to assess the impact of origano and cinnamon essential oils and hempseed oil, on plasma cortisol levels in *Sparus aurata*, after a handling challenge.

Materials and methods

All the procedures involving fish were performed according to the EU guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). 315 gilthead seabreams (5±0,72g) were grouped in 21 tanks. Two diets with cinnamon essential oil (*C. zeylanicum*) (CIN1%: 1% and CIN2%: 2% of the aquafeed), two diets with oregano essential oil (*O. vulgare*) (OR1%: 1% and OR2%: 2% of the aquafeed), two diets with hempseed oil (*C. sativa*) (CAN1%: 1% and CAN2%: 2% of the aquafeed) and one control diet (no supplementation), were applied in experimental fish in triplicates. At the end of the feeding trial, which lasted 84 days, three fish from each tank were randomly captured and kept out of water for 5 min using intense netting procedures. Fish were then anesthetized and blood samples were obtained from the caudal vein. Plasma cortisol was measured using commercial Cortisol Elisa Kit, Cayman Chemical, USA.

Results

Plasma cortisol values showed significant differences between experimental groups fed with diets supplemented with PFAs. Statistically significant lower cortisol levels (P<0,05) were observed in all groups fed with *C. sativa* and *C. zeylanicum*, compared to *O. vulgare* and control group. *O. vulgare* supplemented groups presented higher cortisol levels (P<0,05) compared to control group.

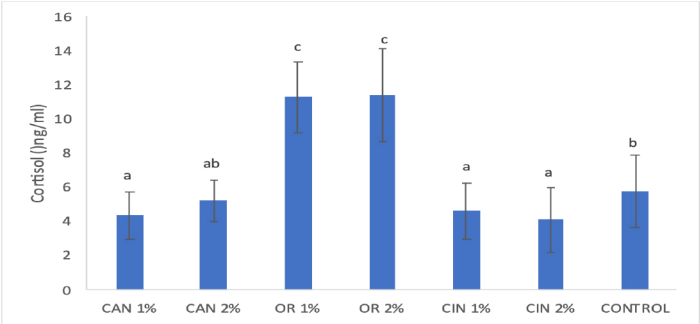


Fig. 1. Effect of *C. sativa*, *O. vulgare* and *C. zeylanicum* incorporated diets on plasma of gilthead seabream (*S. aurata*) after intense netting handling challenge. Letters a, b, c indicate significant differences between treatments (ANOVA; *P*<0.05).

(Continued on next page)

Discussion

PFAs have the ability to prevent increase of plasma cortisol after handling (Teixeira *et al*. 2017). In the present study, *C. sativa* and *C. zeylanicum* proved capable to restrain the elevated cortisol levels triggered by handling stress. Reduction of the cortisol level and insignificant mortalities (Santos *et al*. 2016) have been observed in the case of *C. zeylanicum* incorporated diets, under stressful conditions induced by hypoxia in fish.

Fish fed with *O. vulgare* incorporated diets showed statistically significant higher cortisol values compared to control group. According to Bodur et al. (2018) oregano essential oil, when used as an anaesthetic agent, enhances stress response since it does not block cortisol response. In conclusion, the current study has revealed that *C. zeylanicum* and *C. sativa* dietary supplementation reduces stress response in *S. aurata*, when exposed to handling stress, suggesting their potential use as natural anti-stress agents. More studies are necessary to elucidate the effects of *O. vulgare* essential oil on stress response.

Acknowledgements

«This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research – 2nd cycle" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)».

References

Abdel-Latif H. M. R., Abdel-Tawwab M., Khafaga A. F., Dawood M. A. O. (2020). Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture 526, 735432.

Bodur T., León-Bernabeu S., Navarro A., Tort L., Afonso J. M., Montero D. (2018). Effects of new plant based anesthetics *Origanum* sp. and *Eucalyptus* sp. oils on stress and welfare parameters in *Dicentrarchus labrax* and their comparison with clove oil. Aquaculture 495, 402-408.

Dos Santos W. M, de Brito T. S, de A. Prado S, de Oliveira C. G, de Paula A. C, de M. Hoyos D. C, Ribeiro P. A. P. (2016). Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress. Fish and Shellfish Immunology 54, 551–555.

Khafaga A. F., Naiel M. A. E., Dawood M. A. O., Abdel-Latif H. M. R. (2020). Dietary *Origanum vulgare* essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (*Cyprinus carpio*). Aquatic Toxicology 228, 105624.

Pellati F., Brighenti V., Sperlea J., Marchetti L., Bertelli D., Benvenuti S. (2018). New Methods for the Comprehensive Analysis of Bioactive Compounds in *Cannabis sativa* L. (hemp). Molecules 23, 2639.

Teixeira R. R., Souza R. C., Sena A. C., Baldisserotto B., Heinzmann B. M., Couto R. D., Copatti C.E. (2017). Essential oil of *Aloysia triphylla* in Nile tilapia: anaesthesia, stress parameters and sensory evaluation of fillets. Aquaculture Research 48, 3383–3392.

FISHERIES BY-PRODUCTS AS FUNCTIONAL INGREDIENTS FOR AQUACULTURE FEEDS

C. Aragão[1,2*], R. Colen[1], M. Cabano[1], J.A. Vázquez[3], L.T. Antelo[3] and S. Engrola[1]

[1]Centre of Marine Sciences (CCMAR), Faro, Portugal
[2]Universidade do Algarve, Faro, Portugal
[3]CSIC, Spanish Research Council, Institute of Marine Research, Vigo, Spain

*Presenting author: caragao@ualg.pt

Introduction

Aquaculture industry is increasingly incorporating principles of circular economy and zero waste to find alternative ingredients for aquafeeds. The reduction of fishmeal and fish oil inclusion is an established principle in contemporary fish feed formulations. Hence, by-products from other industries, such as rendered animal proteins, are progressively being used. Aquaculture production is seeking for functional ingredients that may enhance fish resilience and robustness, in face of the suboptimal conditions that may be imposed by the rearing procedures and new dietary formulations.

Fisheries and aquaculture activities, including the associated industrial processing, generate substantial side-streams. Valorization of these resources are of utmost importance. Dietary inclusion of fish hydrolysates in low-fishmeal diets have been shown to promote growth, antioxidant capacity and immune status in several fish species (*e.g.*, Khosravi et al., 2018; Siddik et al., 2020; Chaklader et al., 2021). Therefore, the objective of this work was to assess the benefits of inclusion of fish hydrolysates produced from by-products of the fisheries industry on the growth and physiological status of gilthead seabream (*Sparus aurata*) juveniles fed with low-fishmeal diets.

Materials and Methods

Four isonitrogenous and isolipidic diets (48% crude protein and 16% crude fat) were formulated with practical ingredients: a positive control, a negative control, and two experimental diets containing fish hydrolysates obtained from fisheries biomass previously discarded and nowadays landed due to obligation compliance in the legal framework of the Common Fisheries Policy of the EU. The fish hydrolysates were obtained by enzymatic hydrolysis of *Micromesistius poutassou* whole-body (**FHW**) or from *Trigla* spp. heads (**FHH**). Fish hydrolysates were analyzed and characterized before diet formulation. The positive control (**PC**) was a commercial-like diet, containing fishmeal, poultry meal and plant ingredients as protein sources, and including commercial fish protein hydrolysates. In the negative control (**NC**), fishmeal and soya products were completely removed from diet formulation, through an increase in poultry meal and plant ingredients, and feathermeal hydrolysate and hemoglobin were included instead of the commercial fish protein hydrolysate. The other two experimental diets were based on the NC diet, but feathermeal hydrolysate and hemoglobin were replaced by the experimental fish hydrolysates: **FHW** and **FHH**. Diets were manufactured at SPAROS Lda. (Olhão, Portugal).

Twelve homogenous groups of 90 juvenile gilthead seabream (± 8.0 g) were distributed into 500 L tanks at a density of 1.4 kg/m^3. Fish were supplied with flow-through aerated seawater (temperature: 21.7 ± 1.6 ºC; salinity: 37.3 ± 0.4 psu; dissolved oxygen in water above 85% of saturation) and subjected to natural photoperiod changes through summer conditions (mid-May to mid-July). Each diet was randomly assigned to triplicate tanks and tested over 8 weeks. Fish were fed to apparent satiety by hand, three times a day. Apparent feed intake was recorded daily, and utmost care was taken to avoid feed losses.

At the end of the trial, fish were weighed, as well as liver, viscera, and perivisceral fat from 12 fish per tank. Blood and liver samples were collected for posterior analysis of physiological indicators, antioxidant status and immune parameters. All samples were collected after a 24 h period of fasting. A digestibility trial is ongoing to determine the apparent digestibility coefficients of the dietary nutrients.

Results and Discussion

The fish hydrolysates from the fish whole-body or from fish heads presented differences in molecular weight distribution. In the latter (**FHH**), most of the peptides obtained had a molecular weight between 1-3 kDa, while for the former (**FHW**) a more equative distribution among fractions up to 3 kDa was found. Furthermore, the lipid content in hydrolysates from fish heads (**FHH**) was almost twice that from the whole-fish hydrolysates (**FHW**).

(Continued on next page)

Fish growth was affected by the replacement of dietary fishmeal. Fish fed with the **NC** diet ended the experiment with a significantly reduced final body weight when compared with the **PC** diet and presented the lowest growth rate (expressed as daily growth index or weight gain). The addition of fish hydrolysates (**FHW** and **FHH**) led to a significant increase in the average fish body weight when compared with the **NC** treatment, although still significantly lower than fish fed with the **PC** diet. However, growth rate was not significantly different in fish fed the **FHW** and the **PC** diets. These results indicate that experimental fish hydrolysates were able to partially mitigate the negative effects of fishmeal replacement.

Voluntary feed intake was similar among treatments, but dietary effects were found in feed efficiency. Fish fed the **NC** diet presented higher feed efficiency ratio (FCR) and lower protein efficiency ratio (PER) than fish fed the **PC diet**. The inclusion of fish hydrolysates had a positive effect on feed efficiency, resulting in FCR and PER values not significantly different from the **PC** treatment.

No significant differences were found in the hepatosomatic and viscerosomatic indexes among treatments. However, fish fed low-fishmeal diets (**NC**, **FHW** and **FHH**) ended the experiment with a significantly lower percentage of perivisceral fat when compared with fish fed with the **PC** diet. Fish survival (>96%) was high and not significantly affected by the dietary treatments.

Plasma and liver samples are still under analysis. These results will provide indications on the potential of fish hydrolysates as functional ingredients in diets for seabream juveniles. Up to this moment, it is clear that these hydrolysates were able to mitigate some negative effects of the total dietary fishmeal replacement by rendered animal and plant proteins. Thus, preliminary results suggest that these by-products from marine fishery activities are an interesting ingredient for aquafeeds, contributing to the sustainability of the fisheries and aquaculture industries through principles of circular economy.

References

Chaklader Md R., Fotedar R., Howieson J., Siddik M.A.B. and Foysal Md J., 2020. The ameliorative effects of various fish protein hydrolysates in poultry by-product meal based diets on muscle quality, serum biochemistry and immunity in juvenile barramundi, *Lates calcarifer*. Fish Shellfish Immunol 104: 567-578.

Khosravi S., Bui H.T.D., Herault M., Fournier V., Kim Y.-D., Lee B.-J., Kim K.-W. and Lee K.-J., 2018. Supplementation of protein hydrolysates to a low-fishmeal diet improves growth and health status of juvenile olive flounder, *Paralichthys olivaceus*. J World Aquac Soc 49: 897-911.

Siddik M.A.B., Chaklader Md R., Foysal Md J., Howieson J., Fotedar R. and Gupta S. K., 2020. Influence of fish protein hydrolysate produced from industrial residues on antioxidant activity, cytokine expression and gut microbial communities in juvenile barramundi *Lates calcarifer*. Fish Shellfish Immunol 97: 465-473.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 818173 and by the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education, Portugal) through project UIDB/04326/2020 to CCMAR and contract DL 57/2016/CP1361/CT0033 to CA. This abstract reflects the views only of the AquaVitae consortium, and the European Union cannot be held responsible for any use which may be made of the information it contains.

UNRAVELLING TRYPTOPHAN UTILIZATION FOLLOWING AN INFLAMMATORY INSULT

Cláudia Aragão[1,2*], Miguel Cabano[1], Rita Colen[1], Benjamín Costas[3] and Sofia Engrola[1]

[1]Centre of Marine Sciences (CCMAR), Faro, Portugal
[2]Universidade do Algarve, Faro, Portugal
[3]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal

*Presenting author: caragao@ualg.pt

Introduction

Nutrition has a pivotal role in aquaculture production, since it provides direct advantages on fish growth, health condition, and flesh quality. Functional amino acids regulate key metabolic pathways that may improve the animal's immune response and health. Therefore, functional amino acids are gaining *momentum* in aquaculture production, where few therapeutic possibilities are available against infectious episodes. Tryptophan is an essential amino acid with recognized roles in both neuro-endocrine and immune systems. Hence, it is crucial to understand how its availability will influence the metabolic changes associated with inflammation.

Tracer methodologies allow to understand how nutrients are metabolised by the animals under different situations. Through the labelling technique it is possible to assess in a time-course set-up changes in metabolism and *in vivo* nutrient utilization.

The objective of this study was to assess the absorption and metabolic utilisation of dietary tryptophan during the onset of inflammation in European seabass (*Dicentrarchus labrax*) juveniles.

Materials and Methods

European seabass juveniles (± 20 g) were reared in a recirculating aquaculture system following standard procedures. Fish were hand-fed one of the two experimental diets, formulated to contain tryptophan at the requirement (1xTrp) or at twice the requirement (2xTrp) level. Fish were fed the experimental diets for one or two weeks (referred thereafter as feeding periods).

After each feeding period, fish were transferred to the nutrient flux laboratory at CCMAR, after fasting overnight. Fish were then injected with Freund's Incomplete Adjuvant (FIA), a phlogistic agent widely known to boost the fish inflammatory response. After this procedure, fish were tube-fed with the corresponding diet (1xTrp or 2xTrp) labelled with ^{14}C-tryptophan and were then transferred to individual metabolic chambers, for 1, 2, or 4 h (n = 6-7 fish for each diet, incubation time, and feeding period). The tracer technique allowed to estimate the amount of tryptophan that was unabsorbed (water), catabolised (chemical trap) or retained in selected fish tissues (liver, head-kidney, posterior intestine, brain, and muscle), after the inflammatory insult. Furthermore, through chemical separation, the amount of free or bound tryptophan was assessed in each fish tissue, in order to obtain a more comprehensive indication of tryptophan's metabolism.

Results and Discussion

The preliminary results indicate that tryptophan is readily available for metabolic utilisation at the different tissues analysed. Furthermore, different patterns of tryptophan absorption were found at the diverse tissues and feeding periods. Tryptophan metabolism presented differences according to the tissue analysed and the differences observed at the different feeding periods indicate that metabolic adaptations occur with time.

Dietary tryptophan supplementation (2xTrp) resulted in higher tryptophan availability at important tissues involved in inflammatory and immune responses, which may suggest a potential benefit for the fish.

In conclusion, the *in vivo* tracer methodology was successful at unravelling the metabolic role of tryptophan during inflammation.

Acknowledgements

This project has received funding from the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education, Portugal) through projects INFLAMMAA (02/SAICT/2017/032349), UIDB/04326/2020 and contract DL 57/2016/CP1361/CT0033.

EFFECT OF MICROENCAPSULATED DIETS ON *Mytilus galloprovincialis* GUT MICROBIOTA

Ane del Rio-Lavín[1*], Sébastien Monchy[2], Camilla Campanati[3], Leire Arantzamendi[4], Izaskun Zorita[4], Urtzi Izagirre[5], David C. Aldridge[3] Miguel Ángel Pardo[1]

[1] AZTI, Food Research, Basque Research and Technology Alliance (BRTA). Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160 Derio - Bizkaia, Spain
[2] Univ. Littoral Côte d'Opale, CNRS, Univ. Lille, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[3] Department of Zoology, The David Attenborough Building, University of Cambridge, Pembroke Street, Cambridge, CB23QZ, UK
[4] AZTI, Marine Research, Basque Research and Technology Alliance (BRTA). Herrera Kaia, Portualdea z/g, 20110 Pasaia - Gipuzkoa, Spain
[5] Plentzia Marine, Station (PiE-UPV/EHU), Areatza Hiribidea, 47, 48620 Plentzia, Bizkaia, Spain
*Email: adelrio@azti.es

Introduction

Europe is a major contributor of mussels by supplying over a third of the world total production. It is known that inland hatchery cultures can support the extensive farming of this bivalve, by ensuring known quantity and quality of spats each year. However, these systems are still not economically viable, due to the high costs of conventional live microalgae feeds. For this reason, new alternative microencapsulated inert diets have been developed (Willer & Aldridge, 2019). The aim of the present study was to test microencapsulated feeds containing *Schizochytrium* sp. as alternatives or supplement to a live microalgal diet and evaluate their effect on *Mytilus galloprovincialis* spat and adult microbiota, with the subsequent link of the microbiota structure of mussel with growth rate, proximal composition and gametogenesis.

Material and Methods

Mytilus galloprovincialis spats and adults were cultured at hatchery semi-industrial scale for 8 and 6 weeks, respectively, with different feeding conditions: 1) NC (Negative Control: no food supplied); 2) A (commercial microalgae: 100% ShellfishReed); 3) B (BioBullets as alternative singular diet: 100% *Schyzochytrium*); 4) ABL (Alternative mixed diet: 40% A + 60% B; 5) ABM (Alternative mixed diet: 20%A + 80%B). To investigate their effect on gut microbiota, spat and adult mussel bacterial DNA from the digestive gland was sequenced (MiSeq Illumina) by targeting the V3-V4 16S rRNA gene. Sequences were processed with MOTHUR v1.44.0 (Schloss et al., 2009) and taxonomically affiliated by BLAST against SILVA database (Release 138.1). Alfa diversity estimators were calculated using Past 4.05 software (Hammer, Harper, & Ryan, 2001). Beta diversity analyses including hierarchical clustering analysis (based on Bray-Curtis dissimilarity coefficients), non-metric multidimensional scaling (NMDS) and permutational analysis of variance (PERMANOVA) were performed with R software using the "vegan" package (Oksanen et al., 2020) in order to investigate significant (p-value <0.05) differences of gut microbial community structure between different treatments and over time. Finally, indicator species and metabolic predictions of microbiota were investigated for each diet using LEfSe and PiCRUST programs.

Results and conclusions

Microbial diversity of adult and spat gut microbiota were composed of 11682 and 5083 OTUs (after quality control and data normalization), respectively. Overall, mussel gut microbiota was dominated by phyla Proteobacteria (representing 63% of the reads) and Bacteroidetes (25%), followed by other significant phyla such as Firmicutes (4 %), Campylobacteria (1.5 %), Planctomycetes (1.3 %) and Verrucomicrobia (1.1 %). As expected, differences were observed between adult and spat taxa composition, and different feeding conditions resulted in disparate proportions of bacterial community composition. Alpha diversity (Shannon, Simpson, Chao1 and Berger-Parker) for gut microbiota revealed no statistical significances (p-value > 0.05) between diet treatments. Hierarchical cluster analysis, based on Bray-Curtis dissimilarity, showed that samples were primarily clustered according to the diet and then according to time of exposure. However, mixed diets (ABL and AMB) were clustered together according to time. These results correlate with the NMDS, where significant differences (p =0.00099) were recorded across microbial communities from different feeding conditions and time exposure. Next steps will be to search for indicator species and perform metabolic predictions of microbiota using LEfSe and PiCRUST programs. The whole analysis would help to link of the microbiota structure of mussel with growth rate, proximal composition and gametogenesis.

(Continued on next page)

Acknowledgments

This project was supported by EIT Food Grant MIDSA (Grant number 20293). The authors want to acknowledge PIE-UPV/EHU staff to get access to their facilities to Camilla Campanati and AZTI's staff to their facilities through funding from the European Union's Horizon 2020 research and innovation program under grant agreement Nº 730984, ASSEMBLE Plus project. Ane del Rio is the recipient of a PhD grant from the Education Department of the Basque Government.

References

Hammer, O., Harper, D., & Ryan, P. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, 4, 1-9.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., . . . Wagner, H. (2020). Vegan: Community Ecology Package (v. 2.5-7).

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., . . . Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl Environ Microbiol, 75(23), 7537-7541. 10.1128/aem.01541-09

Willer, D. F., & Aldridge, D. C. (2019). Microencapsulated diets to improve growth and survivorship in juvenile European flat oysters (Ostrea edulis). Aquaculture, 505, 256-262. https://doi.org/10.1016/j.aquaculture.2019.02.072

EVALUATION OF MAIN DRIVERS FOR THE IMPLEMENTATION POTENTIAL OF BIOBASED AND BIODEGRADABLE ROPES IN MUSSEL AND SEAWEED AQUACULTURE SECTORS, TO BE MORE ECO-FRIENDLY AND CREATE CIRCULAR ECONOMY

L. Arantzamendi*, M. Andrés, J. Maher, O. C. Basurko, I. Zorita, Mª J. Suárez, A. Pocheville, M. González, M. Aguinaga, L. Van der Schueren

AZTI-Tecnalia; Marine Research Unit, Herrera Kaia. Portualdea z/g; 20110 Pasaia; Gipuzkoa, Spain
*Email: larantzamendi@azti.es

Introduction

BIOGEARS addresses the challenge of minimizing the use of plastics in the sea by developing prototypes of biobased and compostable ropes, **biogears**, and examines their use in culture of mussels and seaweed Integrated Multi-Trophic Aquaculture (IMTA) systems. The goal of the biopolymers used in the development of the biogears is to achieve competitive manufacturing of aquaculture ropes with adequate durability for mussels and seaweed offshore productions. At the end of life of biogears will enter in-land organic recycling circuits, hence greatly reducing carbon footprint along the whole value chain and generating new local biobased value chains. The aim of this study is to assess the acceptability and potential use of biogears (biobased ropes) by the European aquaculture sector to be more eco-friendly and create circular economy, by identifying the main drivers for this change.

Material and methods

To assess this, a preliminary **value chain description**, a **market study** and an **online survey** were conducted. In the market study, the need of ropes of the European mussel and seaweed sectors were estimated, and social, economic and technology drivers and current trends in aquaculture value chains were identified. To assess the impact of the main drivers identified, they have been categorized as: TECHNOLOGY, (TECH) SUSTAINABILITY (SUST), SOCIAL/POLICY (SOCP) and MARKET/ECONOMY (MARK). An online survey was elaborated and distributed at EU level. The results obtained in the questionnaire are used to complement the market analysis and to identify the pros and cons for the acceptability and implementation of biogears as solutions towards an eco-friendlier European aquaculture sector.

Results and discussion

The value chain has been analysed under circular economy umbrella identifying all stakeholders involved: raw material, bioplastic, biogears, aquaculture, wholesaler, retailers and consumers. The market study pointed out that the aquaculture rope demand in Europe for mussels and seaweed is estimated to be from 1 600 000 m to 3 300 000 meters, approximately. Seaweed market is a new market and is more oriented to sustainable and eco-friendly products. In this case the biobased rope is more in line with the philosophy of the seaweed business.

The preliminary results of the survey are shown in Figure 1. Respondents consider SUST, MARK, SOCP and TECH drivers, the order of driver impact on biogears acceptance and use. Regarding the intensity of agreement in the answers received, in SUST: respondents considered that aquaculture, as food producing sector, should be one of the first marine sectors to avoid plastic use, and that currently used plastic materials (100% petrol based) should be substituted by biogears made of more eco-friendly materials, that could potentially not affect as negatively on seabeds as those currently used if they are lost or abandoned. In MARK: stakeholders consider that they will buy or use biogears because they are compostable at the end of use, promoting also circular economy, and because they are made of biomaterials that come from natural resources that do not compete with food market, and will pay a competitive price for them. In SOCP: a remarkable awareness of current strategies for the development of aquaculture has been observed among respondents (Circular Economy Strategy and Farm to Fork Strategy) who consider that biogears can be drivers for the generation of new value chains, the production of healthy foods (minimizing use of plastic in productions and microplastics in food) and generation of new value chains and circular economy in the European aquaculture sector. In TECH: respondents consider that IMTA productions, can increase the positive perception and acceptability of aquaculture and can contribute to an environmentally sustainable development of aquaculture. Results will be further analyzed and discussed in the presentation.

(Continued on next page)

Fig. 1. Survey to **aquaculture stakeholders** to identify main drivers for the acceptability and potential use of **biogears** substituting currently used 100% petrol- based plastic gears.

BIOGEARS is supported by funding from the EU European Maritime and Fisheries Fund (EMFF).

USE OF PLASTICS IN EARTHEN POND AQUACULTURE (IMTA)

Ravi Luna Araújo[a], Laura Ribeiro[a], Hugo Quental-Ferreira[a], and Pedro Pousão-Ferreira[a*]

[a]IPMA - Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station; Av. Parque Natural da Ria Formosa, s/n, 8700-194 Olhão, Portugal
E-mail: ravi.araujo@ipma.pt

Introduction

The global production and the use of plastic materials have increased exponentially being the estimation to exceed 1 billion tons by 2050. Microplastics are usually defined as plastic items which measure less than 5 mm in their longest dimension. This material has been found in several habitats, including the sea surface, water column, benthos, estuaries, beaches and aquaculture facilities (FAO, 2017). One of the challenges that result from the growth of human population is the need to increase food production by better managing and sustainably exploiting biological and other resources from the ocean without threatening its quality. The potential of Integrated MultiTrophic Aquaculture (IMTA), a cultivation of aquatic animal species fed in combination with extractive species (organic and inorganic) in earthen ponds (Cunha et al., 2019) has been pointed out as a viable practice to contribute for this goal. Therefore, it is crucial to investigate the impact of microplastics in the aquaculture production. The aim of this study was to identify and quantify the plastics used in IMTA culture in earthen ponds.

Materials and methods

The IMTA procedure used in this study was undertaken in Portugal at the aquaculture research station in Olhão, Portugal (EPPO-IPMA), in earthen ponds with 2500 m^3 and 200% day^{-1} water renewal. The production system included: meagre (900kg), white seabream (240 kg) and zebra seabream (300kg). In addition, 425 kg of gigas oysters were distributed in 2 longlines, totalizing 85 bags of oysters, floats, ropes and other structures. The pond had a paddle aerator attached with the help of ropes and was entirely covered with a marine crow protection net measuring 58.6 x 37 m.

Results

Six types of plastics were found in the integrated cultivation ponds, polyethylene (PE); fibre-reinforced plastic (FRP); polyamide (PA); polystyrene (PS) and polyvinyl chloride (PVC). The total plastic weight per pond was 205.36 kg. The most abundant were PE (43%) and PA (36%), followed by PS (13%), FRP (6%) and PVC (2%). Polyurethane (PU) exists inside the aerator floats, but it was not accounted in this study.

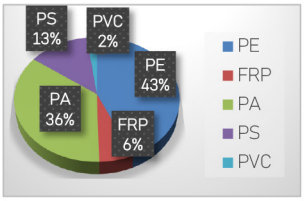


Figure 1 - Types of plastics in integrated aquaculture. **PE**: polyethylene; **FRP**: fibre-reinforced plastic; **PA**: polyamide; **PS**: polystyrene and **PVC**: polyvinyl chloride.

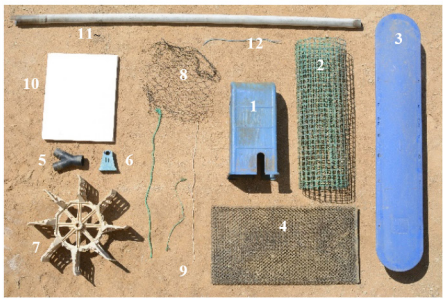


Figure 2 - Types of plastics by equipment. **PE**: 1- aerator cover, 2- output net, 3- aerator float and 4- oyster bag. **FRP**: 5- feeder part, 6- support aerator and 7- wheel aerator. **PA**: 8 - marine crow protection net and 9- ropes. **PS**: 10- oyster float plate. **PVC**: 11- feeder tube and 12- oyster bag holder.

(Continued on next page)

Discussion and conclusion

High levels of solar radiation and temperature are factors that contribute to the degradation of all types of plastics in the Mediterranean. Most of PA and PVC are not in contact with water, so they are degraded exclusively during sunlight. Although PS accounted for 13% of the total plastics in the pond, the degradation of this type of material is the fastest due to bream bites when trying to eat organisms encrusted in the oyster float plates. PE is mostly degraded by the encrustation of marine organisms and also by their removal through maintenance routines. The FRP, which is mostly present on the wheel aerator, wears out by water friction. It is possible to test solutions to reduce microplastics in aquaculture, such as: replacing feeder tubes (PVC) with aluminum tubes; use of sound systems and/or birds of prey to avoid the marine crow and thus reduce the plastics of the protection nets, the current ropes (PA) can be replaced by ropes of organic origin; oyster float plate (PS) can be covered with marine wood to avoid wear and tear and direct contact with water; PE and FRP materials must be studied to be replaced by alternative or biodegradable material. Further studies will be necessary on microplastics, such as degradation, quantification, bio-accumulation among others, to find ways to reduce their impact.

References

Cunha, M.E., Quental-Ferreira, H., Parejo, A., Gamito, S., Ribeiro, L., Moreira, M., Monteiro, I., Soares, F., Pousão-Ferreira, P. 2019. Understanding the individual role of fish, oyster, phytoplankton and macroalgae in the ecology of integrated production in earthen ponds. Aquaculture 512, 734297.

FAO. 2017. Microplastics in fisheries and aquaculture. Fisheries and aquaculture technical paper 615, 4-5. ISSN 2070-7010.

Acknowledgments

This work has been financed by the PLASTICSEA: IMPACT OF MICROPLASTICS IN THE OCEAN, SEA SALT AND AQUACULTURE (FA_06_2017_046) project.

EFFECT OF NEWLY ISOLATED FRESHWATER MICROALGAE ON THE ACTIVITY OF HEAD KIDNEY LEUKOCYTES ISOLATED FROM PIKEPERCH (*Sander lucioperca*) AND COMMON CARP (*Cyprinus carpio*)

László Ardó[1*], Bettina Ughy[2], Ágnes Dergez[3], Sai Divya Kanna[2], Ottilia Kóbori[3], Ákos Koós[3], Zsuzsanna J. Sándor[1]

[1] Research Centre for Aquaculture and Fisheries (HAKI), Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences – H-5540 Szarvas, Anna-liget u. 35, Hungary
[2] Institute of Plant Biology, Biological Research Centre (BRC), Eötvös Loránd Research Network, H-6726 Szeged, Temesvári krt.62, Hungary
[3] Department of Biomass Production and Valorisation, Division for Biotechnology (BAY-BIO), Bay Zoltán Nonprofit Ltd. for Applied Research, H-6726 Szeged, Derkovits fasor 2, Hungary
* E-mail: ardo.laszlo@uni-mate.hu

Introduction

Enhancing innate (non-specific) immune response known as immunostimulation has an emphasized importance in the intensification of aquaculture, as a promising method of preventing fish diseases (Sakai, 1999). Excessive use of antibiotics to prevent and treat bacterial diseases leads to the development of resistant bacteria and environmental pollution, therefore it is very important to develop new methods of disease prevention (Dawood et al., 2018). Immunostimulation by the application of microalgae can be such a new and innovative solution (Cerezuela et al., 2012; Messina et al., 2018). As a result, fish can be a safe, antibiotics-free food and this method can contribute to the reduction of antibiotic and chemical residues getting into the environment as well.

In this study we examined new microalgal strains isolated from Hungarian water courses. These strains were selected based on their growth capacity and content of potential immunostimulant substances (e. g. β-glucans, carotenoids or poly-unsaturated fatty acids (PUFA)).

Materials and Methods

Selected and cultured microalgal strains (named ST3i, ST4i, ST5i, ST6i, ST8i) were tested in vitro, on pikeperch (*Sander lucioperca*) and common carp (*Cyprinus carpio*). Leukocytes were isolated from the head kidney of the fish by gradient centrifugation (Secombes, 1990), and they were cultured in L-15 medium on microtiter plates at 18 °C. Suspensions of algae were diluted 10x, 50x, 250x and 1000x with L-15 (common carp) or the concentrations were set to 10; 20; 5 and 1 mg/l using L-15 (pikeperch). Leukocytes were incubated with these media for 48 (pikeperch) or 72 (common carp) hours. L-15 containing 50 μg/ml lipopolysaccharide (LPS) was used as a positive control. Respiratory burst activity (pikeperch) or nitric-oxide production (common carp) of the cells were then measured using photometric methods (Secombes, 1990; Green et al., 1992). Four fish were used from both species, and their average values were calculated for the statistical analysis (one way analysis of variance). Measurements were done with three replicates and each algal strains were tested on separate plates.

Results and Discussion

In the experiments with pikeperch leukocytes, all examined algae significantly ($p<0.05$) enhanced the respiratory burst activity of cells, when they were applied in lower (1 or 5 mg/l) concentrations (Table 1). However, leukocytes isolated from common carp had a significantly ($p<0.05$) higher nitric-oxide production when they were stimulated by the higher concentrations of algae (250x, 50x or 10x dilutions) (Table 2). Based on these *in vitro* results we can conclude that all five algal strains can be potential immunostimulants. However, the correct dose and time of application has to be determined by *in vivo* experiments.

(Continued on next page)

	Neg. control	Pos. control	1 mg/l	5 mg/l	20 mg/l	100 mg/l
ST3i	0.135±0.020	0.305±0.019*	0.399±0.043*	0.214±0.029	0.129±0.010	0.095±0.005
ST4i	0.123±0.020	0.252±0.016*	0.026±0.059*	0.338±0.052*	0.206±0.028	0.100 ± 0.053
ST5i	0.108±0.018	0.229±0.029*	0.319±0.040*	0.170±0,022	0.094±0.007	0.076±0.004
ST6i	0.116±0.062	0.278±0.056*	0.299±0.050*	0.310±0.031*	0.139±0.013	0.081±0.002
ST8i	0.142±0.021	0.265±0.039*	0.304±0.046*	0.306±0.045*	0.145±0.023	0.101±0.011

Table 1. Respiratory burst activity (presented as OD_{620}) of leukocytes isolated from the head kidneys of pikeperches. Asterisk (*) marks a significant ($p<0.05$) difference from the negative control. Values are averages of four fish ± standard error of method (SEM).

	Neg. control	Pos. control	1000x dilution	250x dilution	50x dilution	10x dilution
ST3i	0.011±0.001	0.122±0.009*	0.017±0.003	0.066±0.015*	0.116±0.079*	0.024±0.005
ST4i	0.010±0.001	0.110±0.005*	0.011±0.015	0.041±0.010*	0.147±0.011*	0.200±0.007*
ST5i	0.019±0.007	0.159±0.017*	0.026±0.013	0.061±0.013	0.154±0.011*	0.093±0.006*
ST8i	0.021±0.006	0.137±0.015*	0.016±0.002	0.069±0.013*	0.170±0.009*	0.045±0.004

Table 2. Nitric oxide production (presented as $OD_{540} - OD_{690}$) of leukocytes isolated from the head kidneys of common carps. Asterisk (*) marks a significant ($p<0.05$) difference from the negative control. Values are averages of four fish ± standard error of method (SEM).

Acknowledgement

The financial support of GINOP-2.3.2-15-2016-00058 project of Hungarian National Research, Development and Innovation Office is gratefully acknowledged.

References

Cerezuela R; Guardiola F. A.; Meseguer J; Esteban M. A. **2012.** Enrichment of gilthead seabream (Sparus aurata L.) diet with microalgae: effects on the immune system. Fish Physiology and Biochemistry 2012;38(6):1729-1739.

Dawood M. A. O.; Koshio S.; Esteban M. A. **2018.** Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Reviews in Aquaculture 2018;10(4):950-974.

Green L. C.; Wagner D. A.; Glogowski J.; Skipper P. L.; Wishnok J. S.; Tannenbaum S. R. **1982.** Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Analitical Biochemistry 1982; 126:131-138.

Messina M; Bulfon C; Beraldo P; Tibaldi E; Cardinaletti G. **2019.** Intestinal morpho-physiology and innate immune status of European sea bass (Dicentrarchus labrax) in response to diets including a blend of two marine microalgae, Tisochrysis luea and Tetraselmis suecica. Aquaculture 2019; 500:660-669.

Sakai M. **1999.** Current research status of fish immunostimulants. Aquaculture 1999; 172:63-92.

Secombes C. J. **1990.** Isolation of salmonid macrophages and analysis of their killing activity. In: Stolen J. S.; Fletcher, T. C.; Anderson, D. P.; Kattari S. L.; Rowley, A. F. (eds): Techniques in Fish Immunology 1990;1:137-154, SOS Publications, Fair Heaven, NJ, USA.

STRUCTURAL ENRICHMENT IN GILTHEAD SEABREAM AQUACULTURE: FUNDAMENTALS AND APPLICATIONS

P. Arechavala-Lopez*, A.R. Oliveira, C.M. Maia, M. Cabrera-Alvarez, J.L. Saraiva

Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal
*E-mail: pablo@fishethogroup.net

Introduction

Environmental enrichment is considered as a tool to guarantee or improve the welfare of captive fish. The deliberate addition of physical complexity to captive conditions allows the animals to have a greater control over their environment,and provides the opportunity to experience new situations while performing behaviours typical of their species in the wild. Well-designed structural EE may provide sensorial and motor stimulation that meet the animals' behavioural and psychological needs, while increasing the behavioural options and putatively reducing the stressors. We demonstrated experimentally that simple structural enrichment can be applied at different production stages of gilthead seabream (*Sparus aurata*) aquaculture, from broodstocks to on-growing sea-cages, not only improving the welfare conditions of captive seabream, but also providing benefits to the farmers. Here, we review the experimental and structures designs, results and conclusions from previous studies carried out on captive gilthead seabream at different life-stages.

Material and Methods

Four different experiments were carried out exposing gilthead seabream juveniles, adults and broodstocks, assessing the effects of structural enrichment of fish welfare with different tools and from different perspectives. However, structural enrichment was similar among experiments, which consisted on plant-fibre ropes, vertically positioned and equally distant, attached to the bottom or to the top of the rearing system and adapted to the appropriate size. First, we studied the effects of structural enrichment on 80 juvenile seabream (weight: 3.8 ± 0.1 g), randomly distributed in eight experimental floating cages within a larger tank. Half of the fish (four tanks) were exposed for 35 days to enrichment. Body condition, growth parameters, fins erosion and brain monoamines were assessed, together with the spatial distribution of the fish during the experiment (Arechavala-Lopez et al. 2019). Second, we explored the effects of structural enrichment on cognition, exploratory behaviour and brain physiological functions of 90 seabream juveniles (weight: 21.9 ± 0.8 g). Fish were distributed in six experimental tanks, half of them (three tanks) reared with the structures for 60 days. During last days, fish were moved into a new-designed experimental maze and video-recorded from above for 1 h, during four different days each group. Fish behaviour, brain monoamines and oxidative stress were assessed (Arechavala-Lopez et al. 2020). A third study was carried out on bigger seabream (weight: 217.58-55.96 g) in a floating sea-cage. Ten fish were tagged with "accel-tag" acoustic transmitters, and reared for one month together with 300 seabream in the same cage. Structural enrichment were deployed during the last two weeks of the experiment. Fine-scale daily activity and spatiotemporal distribution of tagged fish were assessed (Muñoz et al. 2020). A fourth experiment is being carried out on seabream broodstocks (weight ~1 kg), reared in six tanks of 2000 L at commercial densities. Three tanks were enriched with vertical structures (hanging ropes). The aim of this experiment is assessing the physiological and behavioural stress response of broodstock to common aquaculture procedures, such as handling, netting or crowding, as well as the potential effects on spawning and off-spring production (Oliveira, in process).

Results

Results from the first experiment showed that EE modified distribution of seabream inside the experimental cage, entailing a higher use of the inner area of the cage. In addition, a lower interaction with the net and lower aggressiveness among individuals was shown, which consequently, improved pectoral and caudal fins conditions. No effects of growth or brain monoamine levels were observed (Arechavala-Lopez et al. 2019). In the second experiment, we evidenced that deliberate addition of simple enrichment structures enhances welfare status of captive seabream juveniles, influencing positively on cognitive processes, behavioural responses such as exploratory behaviour, and brain-physiological functions. Improvements on spatial cognition and exploratory behaviour may be associated with changes in monoaminergic activity in telencephalon, and serotonergic activity in cerebellum, and are well reflected on antioxidant enzyme activities (Arechavala-Lopez et al. 2020). The third experiment exhibited differences in day vs. night patterns both on swimming activity and vertical distribution throughout the experiment mostly due to the presence of enrichment structures, enhancing the spatial use of fish in the sea-cage (Muñoz et al. 2020). The last experiment is in process, but we hypothesised that the presence of structural enrichment may help seabream broodstocks to cope with common aquaculture procedures, such as handling, netting or crowding, enhancing their physiological and behavioural stress response. It is also expected some positive effects of structural enrichment on spawning and off-spring production, due to the reduction of overall stress (Oliveira, in process).

(Continued on next page)

Discussion

We experimentally demonstrated that simple structures improve the welfare conditions of farmed seabream, and therefore, can be suggested as potential tools to be applied in seabream aquaculture. Moreover, results not only showed that structural enrichment promote positive welfare of seabream, but also that are not detrimental to the growth of the fish and might be used safely by the aquaculture industry. Together with environmental enrichment, innovative technological tools (e.g. acoustic telemetry and underwater cameras), may represent an advancement to monitor fish farming procedures and conditions, helping to promote fish welfare and product quality. Nevertheless, before implementing any environmental enrichment at commercial scale, structures must be appropriated designed and validated, to demonstrate on-farm the applicability and feasibility of these kind of strategies (Arechavala-Lopez et al. 2021).

References

Arechavala-Lopez, P., Diaz-Gil, C., Saraiva, J. L., Moranta, D., Castanheira, M. F., Nuñez-Velázquez, S., ... & Grau, A. (2019). Effects of structural environmental enrichment on welfare of juvenile seabream (*Sparus aurata*). *Aquaculture Reports*, 15, 100224. https://doi.org/10.1016/j.aqrep.2019.100224

Arechavala-Lopez, P., Caballero-Froilán, J. C., Jiménez-García, M., Capó, X., Tejada, S., Saraiva, J. L., ... & Moranta, D. (2020). Enriched environments enhance cognition, exploratory behaviour and brain physiological functions of *Sparus aurata*. *Scientific Reports*, 10, 11252. https://doi.org/10.1038/s41598-020-68306-6

Arechavala-Lopez, P., Cabrera-Alvarez, M.J., Maia, C.M., Saraiva, J.L. (2021). Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Reviews in Aquaculture* (submitted).

Muñoz, L., Aspillaga, E., Palmer, M., Saraiva, J. L., & Arechavala-Lopez, P. (2020). Acoustic telemetry: a tool to monitor fish swimming behavior in sea-cage aquaculture. *Frontiers in Marine Science*, 7, 645. doi: 10.3389/fmars.2020.00645

Oliveira, A.R. Assessing, monitoring and improving welfare of gilthead seabream throughout intensive aquaculture processes. PhD Thesis, in process.

SWIMMING ACTIVITY OF GILTHEAD SEABREAM IN SWIM-TUNNELS

P. Arechavala-Lopez [1,*], M.J. Lankheet [2], C. Díaz-Gil [3], W. Abbink[4], A.P. Palstra[4]

1 Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal
2 Experimental Zoology Group, Animal Sciences, Wageningen University & Research, Wageningen, Netherlands
3 Xelect Ltd., St. Andrews, United Kingdom
4 Wageningen University & Research Animal Breeding and Genomics, Wageningen Livestock Research, Wageningen, Netherlands
*E-mail: pablo@fishethogroup.net

Introduction

Understanding the basic biology of fish species is crucial for livestock production, and the integration of technological solutions can help to improve accuracy, precision and repeatability in farming operations, but also to improve the decision making in aquaculture management plans. In this sense, the use of accelerometer acoustic transmitters calibrated with oxygen consumption and body motion in swim-tunnels as a proxy of energy expenditure can be seen as a promising tool for production and welfare assessment in the aquaculture industry. Nevertheless, more studies are required to improve methodologies in this field of research, with issues arising from the complex interpretation of data acceleration, but also developing species- and life-stage-specific before acceleration transmitters can be extensively applied to reliably assess fish swimming activity. Therefore, we compared accelerometer acoustic transmitter outputs with swimming performance and body motion of gilthead seabream (*Sparus aurata* L.) in swim-tunnels at different flow speeds, which allowed us to characterize the swimming activity of this fish species of high aquaculture interest. In addition, we assessed the potential surgical/tagging effects through the comparison of oxygen consumption, cost of transport and body motion parameters between tagged and non-tagged gilthead seabream.

Material and Methods

A total of 50 seabream (20 cm in length and 200 g in weight, aprox.) were held in a 600L circular tank for two weeks before use in the swimming experiment. Swimming tests were performed in four Blâzka-type swim-tunnels. The flow in the swim tunnels was set at six different speeds during the experiment, from the lowest speed (flushing only, propellers not active) and increasing stepwise by 0.2 m s^{-1} per hour up to 1 m s^{-1} (Palstra et al. 2020) while accelerations, oxygen consumption and locomotion were assessed within each interval (Arechavala-Lopez et al. 2021). Acoustic transmitters equipped with accelerometer sensors (ThelmaBiotel Ltd., Trondheim, Norway; model A-LP7; AccelTag) were surgically implanted to 10 seabream individuals, which were allowed to recover for five days before the swimming experiment. A passive acoustic receiver (ThelmaBiotel Ltd.; modelTBR700) was positioned at the back end inside each tunnel, to record the accelerometer transmitters signals during the experiment. The accelerometers provided root mean square values of the three acceleration axes (ARMS, in m $cots^{-2}$), averaged overall samples in the sampling window, and were transformed into real accelerations (in m s^{-2}). The swim-tunnel system included a bypass with an oxygen probe in a four-channel respirometry system (DAQ-PAC-G4; Loligo Systems Aps, Tjele, Denmark) to measure total oxygen content of the water in percentage, which drops due to oxygen consumption of the fish (ΔO2%). Then, oxygen consumption rate (MO_2; in mg O_2 kg^{-1} h^{-1}) and cost of transport (COT; in mg kg^{-1} km^{-1}) were calculated for each individual and swimming speed. Additionally, critical speed (Ucrit), optimal speed (Uopt), and minimum cost of transport (COTmin) were estimated. Seabream locomotion was filmed with a camera positioned one meter below the center of the tunnel (Basler 2040-90um NIR USB3 camera; frame rate: 25 frames s^{-1}, exposure time: 15 ms). The following parameters were estimated: head orientation position (HO), head orientation frequency (HOF) and amplitude (HOA), tail beat frequency (TBF) and amplitude (TBA). Difference between tagged and non-tagged fish on respirometry and locomotion parameters were tested using a generalized linear mixed model (GLMM). In addition, the paired relationships between estimated parameters were also tested using a GLMM (for further details see Arechavala-Lopez et al. 2021).

Results

Tag implantation in the abdominal cavity had no significant effects on swimming performance and body motion parameters. Accelerations, cost of transport (COT) and variations on head orientation (angle with respect to flow direction) were negatively related to flow speed in the tunnel, whereas oxygen consumption (MO_2) and frequencies of tail-beat (TBF) and head movements (HOF) increased with flow speed (Figure 1).

(Continued on next page)

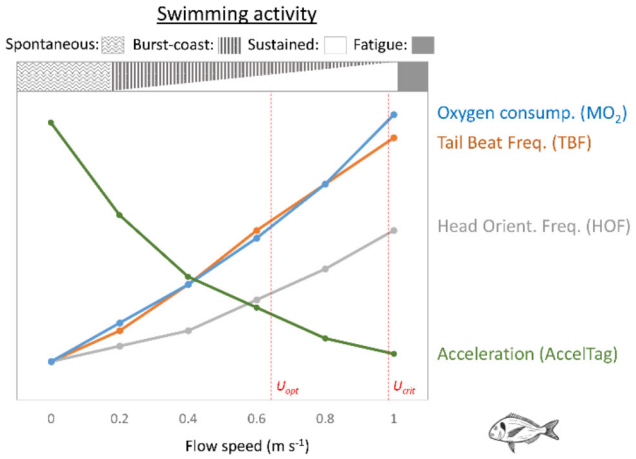


Fig. 1. Representation of the main results obtained in this study.

Discussion

These results show that accelerometer acoustic transmitters mainly recorded deviations from sustained swimming in the tunnel, due to spontaneous and explorative swimming at the lowest speeds or intermittent burst and coast actions to cope with water flow. In conclusion, accelerometer acoustic transmitters applied in this study provided a proxy for unsustained swimming activity, but did not contemplate the high-energy cost spent by gilthead seabream on sustained swimming, and therefore, it did not provide a proxy for general activity. Despite this limitation, accelerometer acoustic transmitters provide valuable insight in swim patterns and therefore may be a good strategy for advancing our understanding of fish swimming behaviour in aquaculture, allowing for rapid detection of changes in species-specific behavioural patterns considered indicators of fish welfare status, and assisting in the refinement of best management practices.

References

Arechavala-Lopez, P., Lankheet, M. J., Díaz-Gil, C., Abbink, W., & Palstra, A. P. (2021). Swimming activity of gilthead seabream (*Sparus aurata*) in swim-tunnels: acoustic accelerometry, oxygen consumption and body motion. *Frontiers in Animal Science*, 2: 679848. doi: 10.3389/fanim.2021.679848

Palstra, A. P., Kals, J., Böhm, T., Bastiaansen, J. W., & Komen, H. (2020). Swimming performance and oxygen consumption as non-lethal indicators of production traits in Atlantic salmon and Gilthead seabream. *Frontiers in Physiology* 11: 759. doi:10.3389/fphys.2020.00759

SUSPENDED STRUCTURAL ENRICHMENT IMPROVES GROWTH HOMOGENEITY OF JUVENILE SEABASS STOCKS

S. Nuñez-Velazquez [1,*], C. Díaz-Gil [2], G. Follana-Berná [1], J.L. Saraiva[3], P. Arechavala-Lopez [3]

1 Fish Ecology Group. Mediterranean Institute of Advanced Studies (IMEDEA-CSIC/UIB), Esporles, Spain
2 Xelect Ltd., St. Andrews, United Kingdom.
3 Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal.
*E-mail: samira.velazquez@gmail.com

Introduction

Structural enrichment is considered an useful tool to guarantee or improve the welfare conditions of captive fish (from a behavioural, physiological and psychological perspective) by increasing the heterogeneity and complexity of captivity environments (Arechavala-Lopez et al. 2021). However, the potential effects of structural enrichment to reduce growth variability among tanks has not been reported before. High growth heterogeneity (different individual growth) within the same stocking fish group leads to a greater investment and effort on the part of the fish-farming companies to try to reduce this variability, either through several grading or continuous feed adjustments, which consequently leads to higher stress to captive fish. Growth heterogeneity on European seabass (*Dicentrarchus labrax*) can be caused by sex differentiated growth (females growth faster than males) or due to indirect effects of social hierarchies and behavioural traits. In this study, we assessed the effects of suspended vertical structures (U-shaped ropes) on growth performance and tank-to-tank homogeneity of juvenile seabass.

Material and Methods

A total of 420 seabass (mean standard length ±SD: 9.8 ±1.1 cm; mean body mass ±SD: 16.5 ±5.6 g) were obtained from a commercial hatchery (Aqüicultura Balear S.A.- Culmarex; Mallorca, Spain) and acclimated to the laboratory conditions for one week at the Laboratory of Marine Research and Aquaculture (LIMIA) in Port d´Andratx, Mallorca, Spain. Then, seabass were randomly distributed in 6 circular tanks (water volume 150 L) in groups of 70 individuals. Three tanks were enriched with 3 plant-fiber ropes hanging from one edge of the tank to the other, two parallel (130 cm) and one perpendicular larger (170 cm), all of them at different depths and similar distances among them. The other three tanks did not present structural enrichment and were considered as the control or non-enriched (NE) treatment. They were daily fed by hand at 13:00 P.M. a commercial pelleted diet (sinking pellets; 2% of their body mass) specific for seabass (Skretting® 106 Perla MP). All tanks were thoroughly cleaned daily by siphoning faeces and uneaten pellets. The seabass juveniles were maintained under these experimental conditions for 30 days (11/03/2019 - 11/04/2019).

In order to assess the effect of structural EE on seabass body condition and growth performance, fish were anesthetized (Tricaine methanesulfonate, MS-222; 0.1 g L -1) at the beginning (Tinitial) and after a period of 30 days (Tfinal). Then, the standard body length (SL, cm) and total body weight (BW, g) were measured, and the Fulton´s condition factor (K = 100 x BW x SL-3) was calculated for each individual for both sampling times (initial and final). The heterogeneity of each parameter in each treatment (among tanks) was analysed by estimating Pearson's coefficient of variation in percentage (%CV), as the parameter standard deviation divided by the mean, and visually assessed for each treatment by plotting the distribution or frequency of each parameter.

Results

No statistical differences were found between EE and NE treatments at the beginning of the experiment regarding standard length, body weight and condition factor. Similarly, no significant differences were detected at the end of the enrichment experiment (30 days) on these fish condition parameters. Regarding distribution of data frequency, fish condition parameters differed visually between treatments. Frequency of final SL and final K of EE-fish group presented normal distribution curves narrower and less dispersed than the normal curve of NE-fish group. Similarly, distribution of final BW data also differed between treatments, but in this case EE-fish group showed a normal distribution, whereas NE-fish group showed a bimodal distribution (Figure 1). Additionally, group heterogeneity among tanks, assessed through the coefficient of variation (%CV), was higher in NE fish tanks compared to EE tanks for every estimated parameter.

(Continued on next page)

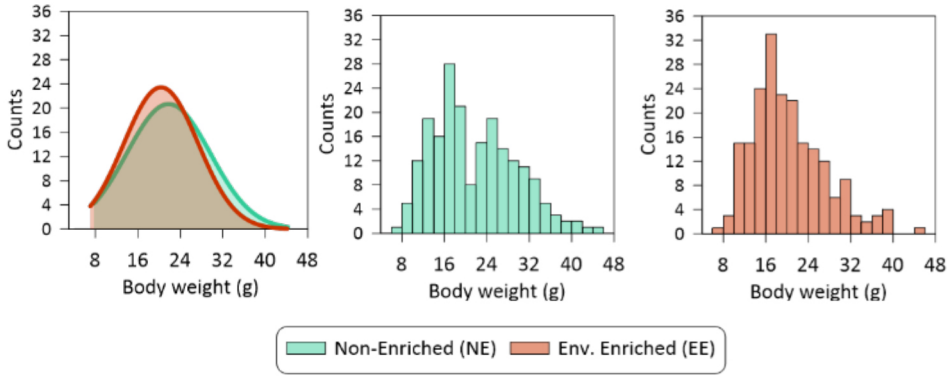


Fig. 1. Distribution of frequency of final body weight from European seabass individuals reared in enriched (EE, in red) and non-enriched (NE, in green) conditions under 30 days in experimental tanks.

Discussion

We demonstrated for the first time that suspended vertical structures (U-shaped ropes) can provide short-term benefits increasing the homogeneity (i.e. reducing CV) of body condition and growth performance of seabass juveniles reared in tanks. Indeed, one of the main effects of the suspended structures was the modification of BW distribution into a single curve, eliminating the undesirable "bimodal distribution" within a stocked fish group/tank. Coefficient of variation (CV) is widely used by aquaculture industry, as a relevant indicator of growth performance and development of farmed fish. High growth heterogeneity (different growth potential) within the same stocking fish group leads to a greater investment and effort on the part of the fish-farming companies to try to reduce this variability, either through several grading or continuous feed adjustments. Consequently, the fish handling and management procedures increase due to high heterogeneity, which can create more stressful situations, negatively affecting the welfare of the fish, with direct consequences to the companies. Therefore, suspended structural enrichment can positively affect the welfare of captive fish in aquaculture, providing not only ethical but economic benefits to fish farmers.

References

Arechavala-Lopez, P., Cabrera-Alvarez, M.J., Maia, C.M., Saraiva, J.L. (2021). Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Reviews in Aquaculture*, in press.

ANTIBACTERIAL ACTIVITY OF THE *Sea cucumber holothuria leucospilota* WHOLE BODY EXTRACT AGAINST *Staphylococcus aureus* STRAINS MRSA, SEA AND SEB

Noushin Arfatahery

Freie University Berlin. Institute Biology/Evolutionsbioloy

Correspondence to: arfa.n@fu-berlin.de (Noushin Arfatahery)

Introduction
Aquatic organisms are a source of organic compounds that hold various features such as medical and nutritional activities. 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 the whole body using chloroform and methanol. *Staphylococcus aureus* is one of the most common causes of seafood-borne diseases worldwide, attributable to food contamination by preformed enterotoxins. The extracts were evaluated for their antibacterial effects against methicillin-resistant (MRSA) and staphylococcal enterotoxins producing (SEA, SEB) *Staphylococcus aureus* strains.

Materials and Methods
Activities have been determined using three methods: disk diffusion tests, minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC). The results demonstrate that methanol and chloroform extracts have an inhibitory effect on the growth of all strains at MIC concentrations up to 100 mg/ml. Also, chloroform extracts demonstrate bactericidal activity against SEB in concentrations of about 100 mg/ml. The extracts also show bactericidal effects against MRSA and SEB below 100 mg/ml concentrations.

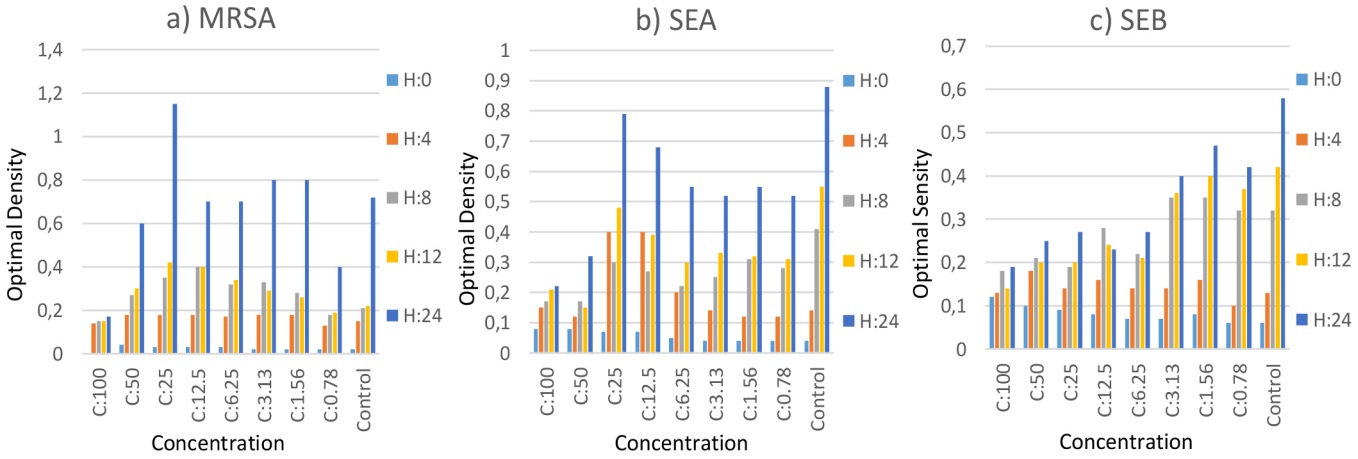
Results
The highest antibacterial activity was found in methanol extract. Therefore, sea cucumber extracts are good candidates for the identification of new antimicrobials. Yet, comprehensive investigations are needed to separate and identify the active components for *Holothuria leucospilota* from the Persian Gulf.

Table 1. Antimicrobial activity of whole body of *H. leucospilota* methanol and chloroform extracts

Staphylococcus aureus	extracts	MIC	MBC
MRSA	Methanol 40 µg/mL	100 mg/ml	100 mg/ml
SEA		100 mg/ml	-
SEB		50mg/ml	100 mg/ml
MRSA	Chloroform 40 µg/mL	50 mg/ml	-
SEA		50 mg/ml	-
SEB		50 mg/ml	100 mg/ml

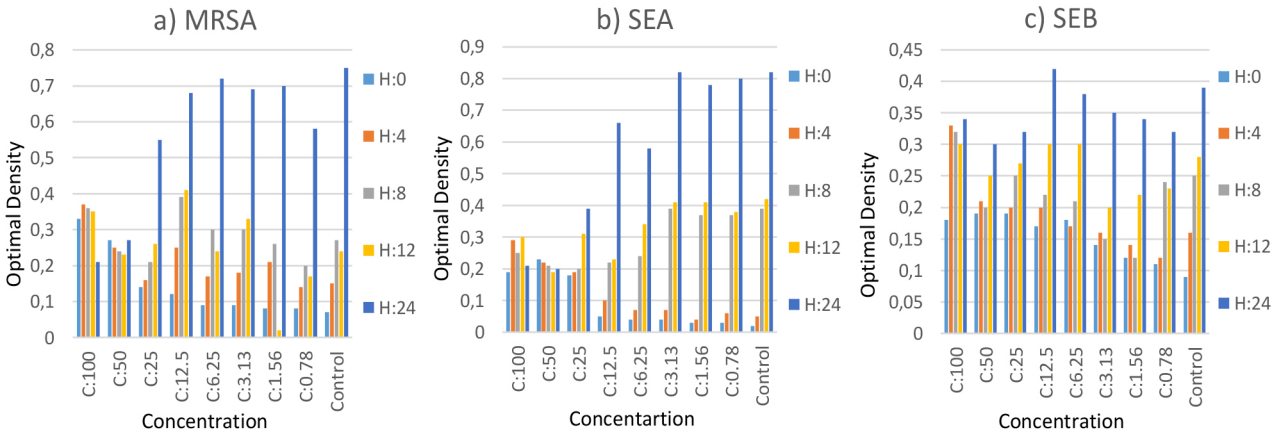
Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from the whole body of H. *leucospilota* methanol and chloroform extracts against *S. aureus* strains MRSA, SEA, and SEB.

(Continued on next page)



Concentration (mg/ml). Hours (H).

Figure 1. The effect of Methanol extract at different times (0,4,8,12,24) and concentrations. Optimal density of extract was read by the ELISA reader at 650 nm. concentrations of the chloroform extract showed Minimum Inhibitory towards the tested *S. aureus* strain MRSA with statisctical results ($p<0.05$; $F>13.3$) (a), *S. aureus* strain SEA with statisctical results ($p<0.05$; $F>14.1$) (b), and *S. aureus* strain SEB with statisctical results ($p<0.05$; $F>17.0$) (c), all with MICs of about 25 mg/ml. values.



Concentration (mg/ml). Hours (H).

CHARACTERIZATION OF TOXIN GENES AND ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS AUREUS ISOLATES IN FISHERY PRODUCTS IN IRAN

Noushin Arfatahery

Freie University Berlin, Institute Biology/Evolutionsbioloy

Correspondence to: arfa.n@fu-berlin.de (Noushin Arfatahery)

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.

Materials and 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. 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.

Studied genes of *Staphylococcus aureus*	Genes: positive		Genes: negative		Total	
Genes	**No.**	**Percent**	**No.**	**Percent**	**No.**	**Percent**
sea	95	45.2%	111	54.8%	206	100%
sab	38	18.5%	168	81.5%	206	100%
tst-1	8	3.9%	198	96.1%	206	100%
mec-A	49	23.8%	157	76.2%	206	100%
sea* + *seb	19	9.2%	187	90.8%	206	100%
sea* + *seb* + *mec-A	7	3.4%	199	96.6%	206	100%
sea* + *seb* + *mec-A* + *tst-1	2	0.97%	204	99%	206	100%

THE EFFECT OF TANK COVER ON WELFARE OF FARMED NILE TILAPIA

J. L. Saraiva [1], M. Nogueirinha [2], R. Teodósio [2,3], C. Aragão [2,3], S. Engrola [3], P. Arechavala-Lopez [1,*]

1 Fish Ethology and Welfare Group, Centro de Ciencias do Mar (CCMAR), Faro, Portugal.
2 University of Algarve, Faro, Portugal.
3 Aquaculture Research Group, Centro de Ciencias do Mar (CCMAR), Faro, Portugal.
*Corresponding author: pablo@fishethogroup.net

Introduction

The finfish aquaculture industry is presently the main supplier of fish for human consumption worldwide. With the increase in demand and the development of technologies that allow the intensification of fish rearing, welfare issues are deemed to increase. One of the main measures to improve the life of farmed fish is implementing environmental enrichment (Arechavala-Lopez et al. 2021). Within structural enrichment, the provision of tank covers is suggested to be a suitable option to provide shelter, with the advantage of being cheaper and easier to maintain than underwater structures, while also reducing external stressful stimuli. This would make tank covers a potentially suitable enrichment strategy for small scale fish farms. In this study we tested the welfare effects (using behavioural and physiological welfare indicators) of three levels of tank cover (fully covered, 50 % covered and uncovered) in an all-male population of Nile tilapia *Oreochromis niloticus*, a fish species with high farming interest world-wide.

Material and Methods

A total of 135 Nile tilapia males (Silver Natural Male Tilapia™ , Til-Aqua International
B.V., Netherlands) were distributed through 15 cylindroconical tanks of 100 L in a recirculating system. These fish were kept for seven days in the tanks prior to the experiment and exposed to different treatments (i.e. tank covers) during the following 21 days. Each tank held nine individuals of 35.27 ±0.54 g (mean ±SEM) at an initial density of approximately 3 kg/m^3. Tank covers were made of extruded polystyrene (Roofmate ™) and were disposed on the top of the tanks (approximately 10 cm above the water surface) and the cover effect on light intensity inside each tank was measured with a luxmeter. Three treatments were used: 100 % covered (corresponding to 18.8 ±1.5 lx), 50 % covered (94.2 ±2.6 lx) and uncovered (169.6 ±8.0 lx) (Fig. 1).

Five of the nine fish in each tank were tagged with individual Floy Tags ™ to allow visual discrimination and behavioural assessment. Each day, five tanks of different treatments were video recorded with a Go-Pro Hero 5 digital camera, slightly submerged, placed at the top centre of the tank aiming down and using the maximum wide angle possible for this model. The recording time for each tank was 25 min after 1-2 min for habituation. By the end of the experiment (21 days), each tank was observed three times, the interval between recordings was three to six days, and each marked fish within the tanks was observed for an accumulated total of 15 min. The Observer XT ™ (Noldus, the Netherlands) software was used to quantify behavioural states (i.e. freezing on the bottom, fast swimming, hovering) and events (chases and bites). At the end of the experiment, tagged animals were anaesthetised with a solution of MS-222 (Sigma) buffered with sodium bicarbonate at 1:2 and had a blood sample taken through punction of the caudal vein with a heparinised needle. Samples were centrifuged and supernatant plasma was analysed for cortisol through radioimmunoassay.

Results

The percentage of time that fish were in the freezing state was significantly higher in the 50 % covered tanks than in the open tanks. Tank coverage did not influence time spent in fast swimming. Fish reared in the open tanks spent a significantly higher percentage of time hovering compared with fish reared in the 50 % cover treatment. While the frequency of chases per min was significantly lower in the partially covered compared with the open tanks, the 100 % cover displayed intermediate values and did not differ significantly from any other treatment. Concerning frequency of bites per min, no significant differences were detected among treatments. Additionally, cortisol was significantly higher in 50 % than the 100 % cover while fish reared in the open tanks had intermediate levels and did not differ from the other treatments.

(Continued on next page)

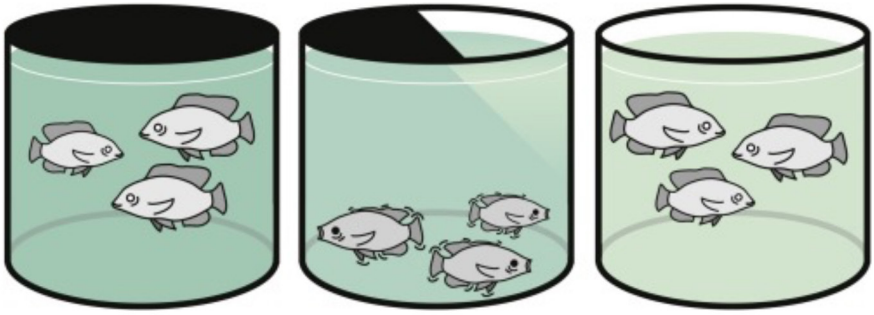


Fig. 1. Graphical summary of the experimental design and the effects of tanks covers (fully covered, 50 % covered and uncovered) on welfare of Nile tilapia (taken from Saraiva et al. 2021).

Discussion

Providing tank covers can improve welfare conditions of farmed fish in captivity, but poor environmental enrichment (EE) strategy or wrong designs can lead to fish welfare impairments. Contrary to our initial predictions, this work clearly demonstrated that fish under the 50 % cover treatment presented the worst welfare according to our chosen indicators. Fish under partially covered tanks spent the longest time frozen on the bottom, the least time hovering in the water column and had the highest cortisol levels. They also presented the least frequency of chases, which can be explained by the fact that if they are frozen on the bottom, they cannot be chasing conspecifics. In fact, freezing behaviour impairs the performance of many fundamental behaviours. We expected that there would be a progressive increase in good welfare indicators from the open to fully covered treatment, possibly due to protection from external disturbances, yet our data suggests otherwise. These results do not undermine the need to pursue better welfare solutions for fish, of which environmental enrichment and complexity is a promising avenue. Yet, they highlight the need to thoroughly test and conduct exhaustive trials when implementing EE measures, even as simple as tank covers, as well as the importance of species-specific knowledge when implementing environmental enrichment strategies.

References

Arechavala-Lopez, P., Cabrera-Alvarez, M.J., Maia, C.M., Saraiva, J.L. (2021). Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Reviews in Aquaculture*, in press.

Saraiva, J. L., Nogueirinha, M., Teodósio, R., Aragão, C., Engrola, S., & Arechavala-Lopez, P. (2021). The effect of tank cover on welfare of farmed Nile tilapia. *Applied Animal Behaviour Science*, 105396.

GUIDELINES ON CO-CREATING CLIMATE ADAPTATION PLANS FOR AQUACULTURE

Michaela Aschan*[1], Thuy T.T. Pham[1], Charlotte T. Weber[12], Ragnhildur Friðriksdóttir[3], Jónas R. Viðarsson[3], and Petter Olsen[4]

UiT The Arctic University of Norway, Norwegian College of Fishery Science, N-9037 Tromsø, Norway
[2]Akvaplan-niva AS, The Fram Centre, 9026 Tromsø, Norway
[3]Matis, 12 Vínlandsleið 113, Reykjavik, Iceland
[4]Nofima, Muninbakken 9, 9019 Tromsø, Norway

E-Mail: Michaela.Aschan@uit.no

Introduction

Climate change is having a significant impact on the biology and ecology of aquaculture species and will affect the productivity within seafood supply chains in the future. The challenges are further amplified when actors within the aquaculture sector have very different ideas and assumptions about climate change, and what risks and opportunities they entail. In order to address the challenges of climate change, several countries have developed national adaptation plans. However, aquaculture is rarely included in these plans, resulting in a general lack of documented adaptation strategies within the sector in most countries.

This study introduces guidelines for the development of climate adaptation plans (CAPs) for aquaculture, which are part of the recently published CEN[1] Workshop Agreement voluntary standard on "Good practice recommendations for making climate adaptation plans for fisheries and aquaculture" (CWA 17518:2020) and supported by the recent paper (Pham et al. 2021). The objective is to provide a stepwise process using a co-creation approach to facilitate stakeholders to plan strategies for climate adaptation. The three-step process is part of a cycle, including implementation, monitoring, and evaluation, generating iterative feedback loops over time. The guidelines are discussed in the light of other existing adaptation tools and highlight the advantages and challenges of developing CAPs within the aquaculture sector.

Framework for developing CAPs

The CAP guidelines were developed using two main approaches: the social-ecological approach and the co-creation approach. The former was employed to identify relevant key components of the sustainable aquaculture system. The latter was used to determine the level of stakeholder participation in the development of CAPs. After devising a prototype, the guidelines were applied to six aquaculture case studies within Europe including species from multiple trophic levels. The methodology and the CAP development process were modified and improved through iterative feedback loops. The CAP guidelines also followed the standardization process devised by the European Committee for Standardization (CEN).

Guidelines for developing CAPs for aquaculture

The guidelines consist of three main tasks: T1, assessment of risks and opportunities; T2, identification of adaptation measures; and T3, operationalization of the CAP. These tasks are conducted through seven steps (S1-S7) in conjunction with three stakeholder meetings. The tasks, steps, and stakeholder meetings are described below, in chronological order (Fig. 1).

Discussion and conclusion

Each CAP development process will be unique. A CAP for an aquaculture system may be developed and applied at local or at national level. To be effective, CAPs should be aligned with strategy and policy processes that are in development, under evaluation and/or revision. The "Strategic Guidelines for the sustainable development of EU aquaculture" are being reviewed by the Commission to support Member States and the sector in further developing aquaculture production. These revised Strategic Guidelines also suggest management measures as well as funding opportunities for climate adaptation in line with the CWA17518:2020.

(Continued on next page)

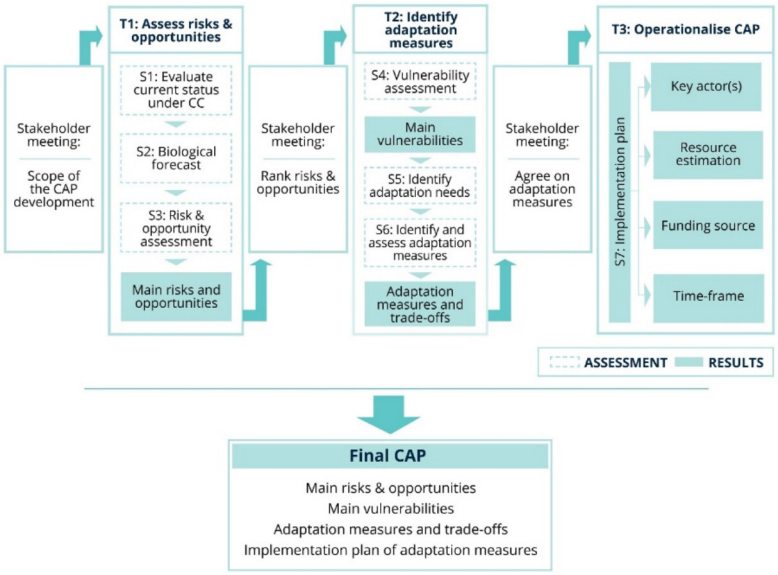


Figure 1: Flowchart of guidelines for developing a Climate Adaptation Plan (CAP). The CAP development consists of three tasks (T1-3) and seven steps (S1-7).

References

CWA. 17518:2020. *Good Practice Recommendations for Making Climate Adaptation Plans for Fisheries and Aquaculture*. (CEN Workshop Agreement European Committee for Standardisation).

Pham, Thuy Thi Thanh, Ragnhildur Friðriksdóttir, Charlotte T. Weber, Jónas R. Viðarsson, Nikos Papandroulakis, Alan R. Baudron, Petter Olsen, Juliana A. Hansen, Unn Laksá, Paul G. Fernandes, Tarub Bahri, Sigurður Ö Ragnarsson, and Michaela Aschan. 2021. "Guidelines for Co-Creating Climate Adaptation Plans for Fisheries and Aquaculture." *Climatic Change* 164 (3): 62. https://doi.org/10.1007/s10584-021-03041-z.

A CEN workshop for developing fisheries and aquaculture CAPs was established to ensure representability and involvement of a broad set of stakeholders. Throughout this process, the guidelines were evaluated, reviewed, and revised on several occasions based on the input and feedback of the CEN workshop participants. At the end, consensus was reached, and a CEN Workshop Agreement (CWA) was established, ensuring that the guidelines will be available through national standardization bodies for at least three to six years after they were published.

GWAS AND ACCURACY OF PREDICTIONS FOR RESISTANCE AGAINST OsHV-1 IN PACIFIC OYSTER (*Crassostrea gigas*)

M. L. Aslam [*a], Dagnachew [a], B. S., Heurtebise [b], S., Maurouard [b], E., Dégremont [b], L. and Lamy [b], J-B.

[a] Nofima, AS, *Ås*, Norway; [b] Laboratoire de Génétique et Pathologie des Mollusques Marins, Ifremer, France
E-mail address: luqman.aslam@nofima.no

Introduction

Pacific oyster (*Crassostrea gigas*) is a highly important shellfish species accounting ~98% of global oyster production. Infectious diseases present a significant threat to the sustainability of aquaculture production with high economic losses due to mortalities caused by diseases. Ostreid herpesvirus (OsHV-1-μvar) originates highly contagious viral disease to Pacific oysters which is serious concern for oyster farmers globally. OsHV-1 may cause massive mortalities (~100%) especially in susceptible phase (larvae and small size oysters). Selection and breeding for resistance against infectious diseases is highly valuable tool to help prevent or diminish disease outbreaks, and currently available advanced selection methods with the application of genomic information could pace up response to selection. Current study was designed to estimate genetic variation for resistance against OsHV-1, map quantitative trait loci (QTL), and explore potential of marker assisted and/or genomic selection [1].

Material and Methods

This study comprises of a resource population of subsided 1520 offspring belonging to ~8 F_2-families produced from a cross of highly resistant grand-parents and susceptible lines (kept free of OsHV-1 infection since 2010). These offspring were selected from a large size population of ~16,000 offspring which experienced natural field outbreak of OsHV-1 in summer 2017. F_2 individuals were recorded twice a day to check their status (alive vs. dead). Tissue sampling was performed for early dead individuals (i.e., mortalities in early phase of infection) and the late survivors (two months later). The tissue samples of F_1 parents and grand-parents were also collected for further genomic work.

Additionally, the whole genome re-sequencing (WGS) was performed on 67 individuals including grand-parents, F_1 parents and 5 individuals per F_2 family (4 survivors and 1 dead) using Illumina HiSeq 2500 platform. Genotyping calling using sequence data produced a quality genotypes for 1,684,660 SNPs. The genotype data from the sequenced individuals (grand-parents, F_1 parents and ~5 offspring per F_2 family) was used for the construction of linkage map. Linkage mapping was performed with Lep-MAP3 [2] using all the informative markers in VCF file.

The survival recorded F_2 individuals (n~1520) were genotyped using 40,625 SNP array (Thermo-Fisher, AXIOM), and 14,245 markers remained after quality filtering. Out of 14,245 array markers, 14,218 were in common with the markers discovered from sequence data which were allocated linkage positions using map developed from sequenced individuals.

Estimates of genetic variation and the breeding values were obtained using ASReml 4.0 [3] implementing genomic or pedigree-based relationship matrices (G-matrix and A-matrix, respectively) with the following mixed model,

$$y=\mu+Zu+e$$

where is a vector of 'n' phenotypic records, is an overall mean, is a vector of additive genetic effects distributed as or is the incidence matrix for additive effects, and is the vector of random residual effects with . Accuracy of prediction was computed using a cross validation scheme by masking the phenotypes of ~ 20% of the offspring. The cross validation process was repeated 20 times and the accuracy was computed as the correlation of the estimated breeding value (pedigree/genomic, PEBV/GEBV) with the phenotype scaled by the square root of heritability.

GWAS was performed by applying the same above model in GCTA program [4] with first 4 PCAs included as covariate and the SNP effects were also computed.

(Continued on next page)

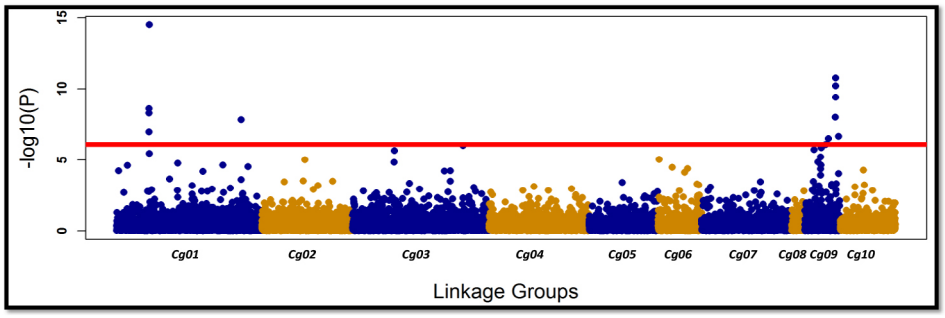


Figure. 1: Manhattan plot presenting association of SNPs with the trait.

Results and Discussion

We developed a linkage map ~1.18 million markers grouped into 10 linkage groups (*Cg*) which are consistent with the karyotype of this species. The estimated heritability for resistance to OsHV-1 (binary, dead/alive trait) using pedigree vs. genomic information was moderate to high (0.23 vs. 0.63, respectively). GWAS revealed strong signals of QTLs on two different linkage groups (*Cg01* and *Cg09*, including 6 and 10 SNPs respectively), presenting significant association to the trait with P-value crossing genome-wide Bonferroni corrected threshold (Figure 1). The proportion of total genetic variance explained by the top two SNPs (with single top-most SNP taken from each of the QTLs) was ~50% of the total genetic variance. The prediction accuracy for breeding values using genomic models exhibited 113% increase over the use of pedigree information.

In conclusion, results revealed moderate to high genetic variation for survival against OsHV-1 with two quantitative trait loci affecting resistance. The estimated breeding values using genomic information presented more than double accuracy compared to pedigree information, which should ultimately be reflected in overall genetic gain.

Acknowledgement

The research leading to the results of this study has received funding from EU-funded project VIVALDI (H2020 program, n°678589).

References

1. Meuwissen TH, Hayes BJ, Goddard ME: Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 2001, 157(4):1819-1829.
2. Rastas P: Lep-MAP3: robust linkage mapping even for low-coverage whole genome sequencing data. *Bioinformatics* 2017(33):3726–3732.
3. Gilmour AR, Gogel BJ, Cullis BR, Welham SJ, Thompson R: ASReml User Guide Release 4.1 Structural Specification. In. Hemel Hempstead, UK: VSN International Ltd; 2015.
4. Yang J, S.H. Lee, M.E. Goddard, P.M. Visscher: GCTA: A tool for genome-wide complex trait analysis. *Am J Hum Genet* 2011, **88**:76-82.

MARKER ASSISTED SELECTION FOR RESISTANCE AGAINST VIRAL NERVOUS NECROSIS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

M. L. Aslam [*a], S. Vela-Avitúa [b], V. Bakopoulos [c], K. Papanna [d], L. Kottaras [d], A. Dimitroglou [d], L. Papaharisis [d], C. S. Tsigenopoulos [e], I. Thorland [b]

[a] Nofima, AS, Ås, Norway; [b] Benchmark Genetics Norway AS (formerly Akvaforsk Genetics Center AS), Sunndalsøra, Norway; [c] University of The Aegean, Mytilene, Greece; [d] Nireus Aquaculture SA, Koropi, Greece; [e] Hellenic Centre for Marine Research, Crete, Greece.
E-mail addresses: luqman.aslam@nofima.no

Introduction

Contagious diseases are a major threat in aquaculture due to losses caused by high mortalities and the reduced growth of surviving fish. Viral nervous necrosis (VNN) is an infectious disease caused by nervous necrosis virus (NNV, red-spotted grouper nervous necrosis virus, RGNNV in European sea bass) which is considered a serious concern for European seabass producers, with fry and juveniles being highly susceptible. The outbreak of VNN may cause up to 100% mortalities at larval and around 20% mortalities at advanced juvenile stages[1, 2]. Moreover, the surviving fish present poor growth rate and ultimately high economic losses for the producers.

Selection and breeding for resistance against infectious diseases is highly effective tool to prevent and/or diminish disease outbreaks. Currently available advanced selection methods with the application of genomic/marker(s) information could pace up response to selection. The genetic variation for resistance against VNN obtained from the challenge tested population was presented previously [3]. The aim of current study was to further look into the genomic architecture of the trait and explore potential of marker assisted and/or genomic selection and obtain realized validation of QTL effects.

Material and Methods

This study comprises of a resource population belonging to multiple year classes (YC2016, YC2017, and YC2019) derived from the Nireus SA's breeding nucleus of European seabass. Families from each of these year classes were gone through the challenge test against VNN, and the subset of the families and individuals from year classes YC2016 and YC2017 including 30 (~27 individuals/family) and 92 (~8 individuals/family) families, respectively were genotyped. Additionally, 536 breeding candidates from multiple year-classes (YC2011-2016) pre-selected according to selection criteria defined in the commercial seabass nucleus work and linked to YC2016 and YC2017 through pedigree were genotyped. The tested individuals and the candidates from YC2016 and YC2017 were genotyped using SNPs based ~57K Affymetrix Axiom array (DlabCHIP) [4]. The detection of QTL was performed using survival phenotype and the genotype information on YC2016 and YC2017 whereas validation of QTL effects was performed using genotype information on candidates (YC2011-2016) and the phenotype of descendent year class, YC2019. For the purpose of validation, additional specific crosses were made in YC2019 using QTL information to produce resistant ("RR", homozygous for favorable allele), moderate ("Rr", heterozygous) and susceptible ("rr", homozygous for alternative allele) families.

Analyses: Genome wide association analysis was performed using following linear mixed animal model implemented in GCTA program with "--mlma-loco" function [5].

$$y=\mu+Xb+Ma+Zu+e$$

Where is the vector of phenotypes (0 dead or 1 alive); is the overall mean; is an incidence matrix for fixed effects and is a vector of fixed effect of year class; is the incidence matrix for SNP containing marker genotypes coded as , is the allele substitution effect of SNP, is a design matrix to relate the records to genetic values and is a vector of random additive genetic effects, it is assumed that , where is the genetic variance and is a genomic relationship matrix computed using VanRaden's method 1. The is the vector of random residual effects with .

Moreover, the accuracy of genomic prediction was evaluated using different models (GBLUP and Bayesian) to assess and compare the potential of genomic and/or marker assisted selection.

(Continued on next page)

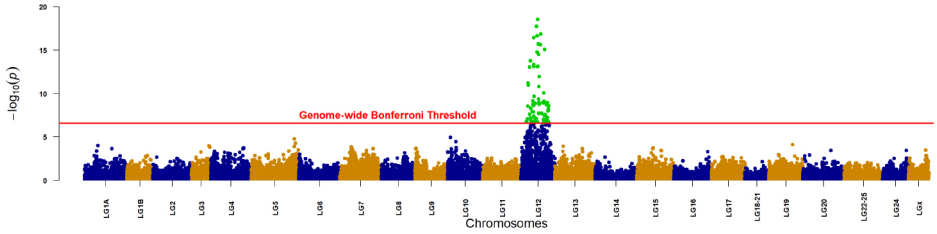


Figure. 1: Manhattan plot presenting association of SNPs with the trait.

Results and Discussion

The GWAS analysis revealed a strong signal of QTL at LG12 with 72 SNPs presenting significant association to the 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 ~33% of the total genetic variance. Multiple genes were identified within the QTL region with *REEP1* gene located immediately at the upstream of the highest significant SNP which seems to be more pronounced with functions involving nervous system. The mean accuracy of prediction for resistance against VNN obtained using different genomic models (GBLUP and Bayesian) was 0.72 with Bayesian models worked either better or equally well as GBLUP.

The validation results for survival of "RR", "Rr" and "rr" families which were specifically produced using QTL information on parents revealed >30% higher survival of "RR" over "rr" families and 7% higher survival over "Rr" families.

In conclusion, the tested realized effect of QTL showed >30% higher survival of families carrying homozygous favorable genotype over families containing homozygous unfavorable genotype. Hence, marker assisted selection using QTL information has a shown strong potential for improving resistance against VNN.

Acknowledgement

The results of produced in current study are a part of ROBUSTBASS (3rd Joint Transnational Call of the ERA-Net COFASP) and the FUTUREEUAQUA (EU - Horizon 2020) projects.

References

1. Le Breton A., *et. al.,*: **Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea-bass, *Dicentrarchus labrax (L.)***. *J Fish Dis* 1997, **20**:145-151**; 2.** Munday BL., *et. al.,*: **Betanodavirus infections of teleost fish: a review**. *J Fish Dis* 2002, **27**:127–142**; 3.** Vela-Aviatua S., *et.al.,*: **Advanced selective breeding for disease resistance in European Seabass (*Dicentrarchus labrax*)**. In: *Aquaculture Europe*. Berlin, Germany 2019: 1578-1579**; 4**. Griot R, *et al.,*: **Genome-wide association studies for resistance to viral nervous necrosis in three populations of European sea bass (*Dicentrarchus labrax*) using a novel 57k SNP array DlabChip**. *Aquaculture* 2021, **530**:735930**; 5.** Yang J., *et. al.,*: **GCTA: A tool for genome-wide complex trait analysis**. *Am J Hum Genet* 2011, **88**:76-82.

GENETIC PARAMETERS OF REPRODUCTIVE TRAITS FOR TILAPIA (*Oreochromis niloticus*) IN BRACKISH WATER AND FRESHWATER

Muhammad Hunaina Fariduddin Ath-thar[1,2*], Priadi Setyawan[1,3], John Bastiaansen[1], Mark Camara[1], nd Hans Komen[1]

[1]Animal Breeding and Genomics, Wageningen University and Research
[2]Research Institute for Freshwater Aquaculture and Fisheries Extension, Ministry of Marine Affairs and Fisheries Indonesia
[3]Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries Indonesia.
*Corresponding author. E-mail: farid.aththar@wur.nl

Introduction

A selective breeding program for growth rate of tilapia in brackish water is conducted in Indonesia. While focusing on growth trait, the reproductive performance of tilapia in brackish water is also considered important. To avoid the potential threat to the saline aquatic ecosystem, reduced or even repressed reproductive performance of tilapia in brackish water is desired. However, studies comparing reproductive performance between environments and estimates of genetic parameters for reproduction traits are very limited in any aquaculture species. Objective of this study were to investigate: 1) the reproductive performance of tilapia in brackish water and freshwater and 2) the genetic parameters of tilapia reproduction traits.

Materials and methods

Offspring of 91 full sib families were produced and per family 20 fingerlings were randomly chosen for communally grow-out in a brackish water (20-25ppt) and another 20 in freshwater. After the grow-out period of 120-147 days, fish were harvested and 6 fish per family were recorded for gonad weight and maturation score in each environment. Gonads were weighed and maturation stage was macroscopically determined according to Legendre & Ecoutin (1989). Gonadosomatic index was determined as GSI = gonad weight*100/BW. Gonad weight (GW) and GSI were analyzed separately for each sex. Genetic parameters were estimated from bivariate animal models in ASReml version 4.1 (Gilmour *et al*., 2015) accounting for fixed effects of pond and harvest weight. For genetic analysis maturation was reclassified as mature (1) or immature (0) (Legendre & Ecoutin, 1989) and analyzed for males and females together with sex as additional fixed effect.

Results

At harvest time, the mean weight was higher in brackish water (300.84±67.48) and significantly different ($P<0.05$) compared with the freshwater (238.13±56.65). Gonad weight for male and female and GSI for female in brackish water were higher and significantly different compared to freshwater ($P<0.05$). GSI of males showed no significant difference ($P>0.05$) between brackish water and freshwater (Table 1).

Most males were mature (~80% were stage 3) in both brackish water and freshwater (Table 2), while for the female, about half the fish were mature fish (stage 4-5) in both brackish water and freshwater. For males, heritabilities of GW and GSI were higher in freshwater, than brackish water and the genetic correlations were high. For females the opposite was found with higher heritabilities in brackish water and a moderate genetic correlation for GW (Table 3). The genetic correlation for GSI in females was high, but estimated with a very high standard error. Maturation was analyzed for both sexes combined and found to have a low heritability in brackish water and freshwater, 0.12 ±0.07 and 0.04 ± 0.07, respectively and a moderate genetic correlation, 0.47 ±0.74.

Table 1. Means ($\bar{x}$), standard deviations (σ), coefficients of variation, % (CV) of gonad weight and GSI male and female from brackish water and freshwater

Traits	N	Brackish water			n	Freshwater		
		$\bar{x}$	Σ	CV		$\bar{x}$	σ	CV
Gonad weight male	299	1.03	1.50	145.1	280	0.70	0.76	109.7
Gonad weight female	177	4.52	3.56	78.6	198	2.42	2.16	89.2
GSI male	299	0.31	0.50	159.7	280	0.28	0.31	111.1
GSI female	177	1.82	1.40	77.2	198	1.26	1.54	91.2

(Continued on next page)

Discussion

The final weight in brackish water was higher (P<0.05) compared with the freshwater and can explain the higher GW and GSI in brackish water. Males showed higher maturation stages compared to females, both in brackish water and freshwater. This was expected from comparing them at the same age. Heritability of maturation traits in brackish water was higher for males and lower for females in comparison to freshwater. The heritability of maturation in brackish water (0.12) was in good agreement with an earlier report from Egypt, 0.13 (Charo-Karisa *et al*., 2007). Our estimate from freshwater was however much lower at 0.04±0.07. Heritabilities for maturation were lower than the result from Thoa *et al*. (2015) who reported heritabilities of 0.40±0.06 in saline water and 0.32±0.06 in freshwater. Genetic correlations estimates had high s.e. because of the limited size dataset.

Conclusions

All reproductive traits showed higher values in brackish water compared to freshwater. Tilapia is a species with a high reproductive rate and their maturation was not compromised by the brackish water environment. To reduce the potential threat from escapees to the brackish water ecosystem, selection for reduced maturation in brackish water environment is most promising for the female reproductive traits that have the higher heritabilities and lower genetic correlations with reproductive traits in freshwater.

References

Charo-Karisa, H., Bovenhuis, H., Rezk, M.A., Ponzoni, R.W., van Arendonk, J.A.M., Komen, H., 2007. Phenotypic and genetic parameters for body measurements, reproductive traits and gut length of Nile tilapia (*Oreochromis niloticus*) selected for growth in low-input earthen ponds. *Aquaculture* 273, 15–23.

Gilmour, A.R., Gogel, G.J., Cullis, B.R., Welham, S.J., Thompson, R., 2015. ASReml User Guide Release 4.1 Functional Specification.

Legendre, M., Ecoutin, J.M., 1989. Suitability of brackish water tilapia species from the Ivory Coast for lagoon aquaculture. 1. Reproduction. *Aquatic Living Resources* 2, 71–79.

Thoa, N., Ninh, N., Knibb, W., Nguyen, N., 2016. Does selection in a challenging environment produce Nile tilapia genotypes that can thrive in a range of production systems?. *Scientific Report* 6, 21486. https://doi.org/10.1038/srep21486

CHARACTERIZATION OF MOLECULAR, CELLULAR AND HUMORAL IMMUNE MARKERS IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*) UNDER CHRONIC INFLAMMATORY CONDITIONS

R. Azeredo[1*], M. Machado[1], D. Peixoto[1,2], B. Costas[1,2]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Matosinhos, Portugal
E-mail: mleme@ciimar.up.pt
[2] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Porto, Portugal.

Introduction

Fish health and welfare are among the top priorities in what aquaculture research is concerned. Stress-inducing husbandry and rearing conditions (including alternative dietary formulations), bacterial infections or parasitic diseases may all lead to chronic inflammation (1, 2). Such an immune response channels energy away from growth, reproduction and other important physiological processes, to fuel immune-related metabolic responses. Despite a reasonable amount of studies on fish immune response, there is a lack of a solid inflammation model for fish and a battery of biological markers that can more accurately characterize chronic inflammatory responses. The present study aims to gather information on molecular, cellular and humoral parameters of European seabass (*Dicentrarchus labrax*) undergoing chronic inflammation that can be used as health indicators for applications in fish health management.

Material and methods

Juvenile European seabass (300 g ± 71.8) were acclimatized in a recirculating seawater system (temperature 16 ºC; Salinity: 35; Photoperiod: natural summer time; n = 12) for 30 days being fed a commercial diet throughout the whole experiment. At the end of this period, fish were intra-peritoneally injected with either Freund's Incomplete Adjuvant (FIA) to induce inflammation or Hanks Balanced Salt Solution (HBSS) to serve as sham, and distributed in duplicate tanks in the same system. Fish were sacrificed at 24 h, 7 days, 14 days and 21 days post-injection (n = 3 per tank). During sampling, blood was collected from the caudal vein and directly used for total peripheral cell counts, blood smears preparation and respiratoy burst. Plasma was then obtained for the assessment of humoral immune parameters. Peritoneal exudates were collected for total peritoneal leucocytes counts. The head-kidney was sampled for the evaluation of immune-related and opioid receptors gene expression measured by real-time quantitative PCR.

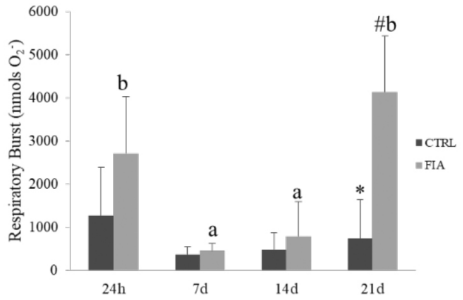


Fig. 1. Respiratory burst activity of circulating leucocytes of European sea bass intraperitoneally injected with HBSS (CTRL) or Freund's Incomplete Adjuvant (FIA). Values represent means ± SD (n = 6). Different letters stand for statistically significant differences between sampling times, within the same treatment. Different symbols stand for statistically significant differences between treatments (Two-way ANOVA; $P < 0.05$)

(Continued on next page)

Results

Though there were no significant differences in total peripheral cell counts between FIA and HBSS groups, FIA-injected fish presented higher total peritoneal leucocyte concentration than the HBSS group at 7, 14 and 21 days post-injection. Peripheral monocytes were also higher in FIA-injected fish than in the HBSS group regardless of sampling time, as well as both lymphocytes and neutrophils at 24h post injection. Total proteases activity and lysozyme activity were also induced in FIA-injected group, compared to the HBSS group, regardless of sampling time. Similarly, increased respiratory burst was observed in FIA-injected fish, though only in fish sampled at 21 days post-injection (Fig 1). Regarding head-kidney gene expression, interleukin 1β (*il1β*) and caspase 1 (*casp1*) were both up-regulated in FIA-injected fish, irrespective of sampling time. Moreover, interleukin 34 (*il34*) did not respond differently between groups or sampling times, but an increasing trend was observed within FIA-injected fish. In what opioid receptors are concerned, no differences were detected between FIA and HBSS groups but mu, kappa and nociceptin opioid receptor (*muor, kor1* and *nopr*, respectively) were found down-regulated in fish sampled at 7 d, 14 d and 21 d post-injection compared to levels measured at 24 h.

Discussion and conclusion

FIA is well-known to trigger and enhance inflammatory responses, thus serving as a very effective adjuvant during vaccination. Indeed, the results here reported are clear indicators of an inflamed peritoneal cavity and an ongoing systemic immune response that persist at least for 21 days. Locally, inflammation was characterized by an intense recruitment of immune cells that was still evident 21 days after injection thus illustrating the chronic character of the immune response. Cellular response was also noticed peripherally with neutrophils, lymphocytes and monocytes numbers rising in the blood of FIA-injected fish. Furthermore, cellular-mediated respiratory burst peaked at 21 days post FIA injection, suggesting that phagocytes were still actively fighting the phlogistic agent. Respiratory burst is a particularly interesting parameter to be seen enhanced at such a late time-point, as it is a key discriminant feature of active phagocytes (3), very much present at the onset of inflammation. Regarding the head-kidney molecular analysis, pro-inflammatory genes (*il1β*, *casp1*) were indeed higher in fish injected with FIA but such effect was regardless of sampling point, making these poor markers of a prolonged response. *il34* expression time-dependent increasing trend poses as good marker of chronic inflammation due to its importance as a second ligand of CSFR1, a pathway with high relevance in chronic inflammation settings (4). In conclusion, peripheral leucocyte counts and respiratory burst analysis seem to be reliable indicators of chronic inflammation easily obtained with a blood sample. Further investigation is nonetheless required to select more responsive and specific molecular markers.

Acknowledgements

This work was supported by project INFLAMMAA (PTDC/CVT-CVT/32349/2017), financed by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020, and through national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal). BC and DP were also supported by FCT (IF/00197/2015 and UI/BD/150900/2021, respectively).

References

1. Hedrera et al. 2013. DOI: 10.1371/journal.pone.0069983
2. Dezfuli et al. 2008. DOI: 10.1051/parasite/2008153426
3. Plouffe et al. 2005. DOI: 10.1111/j.1399-3089.2005.00227.x
4. Masteller and Wong. 2014 DOI: 10.1016/j.drudis.2014.05.016

TOWARDS A CIRCULAR AQUACULTURE: THE CONTRIBUTION OF H2020 GAIN PROJECT

Hallstein Baarset[1*], Christian Bruckner[2], Lars Svenningsson[3], Johan Johansen[4], Richard Newton[5], Wesley Malcorps[5], J.A. Vazquez[6], Ricardo I. Pérez-Martin[6], Carmen G. Sotelo[6], Martiña Ferreira[7], Leticia Regueiro[7], Roberto Pastres[8]

Affiliations:[1]Waister, Norway; [2]Salten Havbrukspark AS; Norway; [3]LS Optics AB, Sweden [4]NIBIO, Norway; [5]Institute of Aquaculture, University of Stirling. UK; [6]Instituto de Investigaciones Marinas (CSIC), Vigo, Spain [7]ANFACO-CECOPESCA,Vigo, Spain, 8 Ca' Foscari University of Venice, Italy
*hallstein.baarset@waister.eu

Introduction

One of the main problems associated with any food production value chain is valorisation of by-products and side-streams to reduce the environmental impact, while optimizing the use of resources (Newton et al., 2017). This is especially needed for the aquaculture sector, where circularity principles are key elements to meet sustainability requirements. In the H2020 project "GAIN" , we have used different strategies to address this issue by investigating innovative processes aimed at reusing: fish sludge, generated in Recirculating Aquaculture Systems (RAS), fish mortalities, by-products generated by fish processing (heads, frames and trimmings, skins, etc..), and shells from bivalves generated by the cannery industry. Main results and developments that will significantly contribute to the eco-intensification of this productive sector are presented.

Material and Methods

As a basis for investigating and developing technologies for valorizing aquaculture side-streams we analysed wastewater from land-based Atlantic salmon smolt production farms for its chemical characteristics with respect to its environmental footprint and the underlying valorisation potential. To recover particulate, flocculated matters, the S3 filterdryer system was developed, including a fine meshed filter cloth (mesh size 6 μm) capable to remove particles from aquaculture wastewater. In addition, the S3 filterdryer sanitizes and dries the resulting sludge (DM content 91-94%) without the use of added chemicals (polymers, coagulants, etc.). Besides, an alternative system, adding polymers and drying the sludge using the Waister superheated steam dryer to sanitise it, was also tested.

For the sanitation of fish mortalities, a new superheated steam dryer was developed, the Waister 15 generation.

In order to estimate the potential for valorisation of by-products, the yield of these by-products in salmon, trout, seabream, seabass, turbot and carp were determined. These by-products were used as raw material for performing enzymatic hydrolysis to obtain fish protein hydrolysates (FPH).

The suitability of bivalve shells for being used as filling material of a newly designed biofilter for nitrification and phosphate removal from RAS wastewater was investigated. 10L volume biofilters were used at lab-scale level for compative experiments between plastic material and crushed and whole mussel shells. For phosphate removal experiments, crushed shells biofilters were compared with calcite material, the latter simulating the abovementioned material calcined shells.

Results

The main challenge to purify aquaculture wastewaters lies with the very low levels of dry matter (DM) and the small particle size of solids present in wastewaters. The use of the newly designed S3 filtration system provides a 93 $\pm$ 2.8 % removal of suspended solids (SS) and a 80 $\pm$ 6.4 % removal of organic content determined as chemical oxygen demand (COD) in the treated wastewaters, confirming the utility of the S3 filter dryer as a technology meeting the regulatory demand of a 50% SS and a 20 % COD reduction as a standalone unit. The S3 system dries the sludge in parallel, achieving a DM content of 91-94%, without the use of chemicals and extremely low energy consumption: 300W per cubic meter wastewater for the entire process.

Sludge dried using conventional heating, as a superheated steam dryer, resulted in a product with excellent physicochemical properties and DM contents of 90-95 %, outperforming airdried fish sludge as a bioenergy source in cement production. Analysis of dried fish sludge shows that it is an excellent bio-fertiliser product with high content of N and P, while low level of K. The Zn content is currently the most limiting element for allowed spead of dried fish sludge on farmland.

(Continued on next page)

Waister implemented a novel drying technology of mechanical fluidisation and superheated steam for sludge of typically 10-50 % DM from polymer-based dewatering technologies. This technology can be combined with the S3 dewatering technology. Both systems stabilise the sludge and minimise shipping costs.

Regarding the treatment of fish mortalities, a single loop Waister 15 dryer was installed in Helgeland Smolt, finalising a well-functioning technology for mortalites disposal. The main challenges with the treatment of salmon mortalities were the high fat content and the presence of excess water mixed with the fish. The first problem was solved with the incorporation of additives, such as wood chips or dried spent grain, while the second was solved with manual feeding of the fish into the dryer. The dried product obtained can be used for bio-energy production, biofertiliser, or potential animal feed ingredient depending on the additive used prior to the drying.

The potential volume and economic value of by-products generated by the main aquaculture species in Europe was estimated.

Most by-products present a significant protein content: therefore, the use of proteolytic enzymes to breakdown these proteins and use the resulting peptides for different applications, i.e. as ingredient of aquafeeds was investigated, in order to determine optimal operational, e.g. temperature, pH and other parameters.

Mussel shells were tested as a replacement for plastic filling in a RAS biofilter. Results suggest that mussel shells could be effectively used, as they provide a suitable substrate for nitrifying bacteria and, due to their carbonate content, contribute to stabilize pH. Also, they showed interesting properties to remove dissolved phosphate from water, improving the quality of the effluent.

Acknowledgements
The research leading to these results has also received funding from the GAIN project, European Union's HORIZON 2020 Framework Programme under GRANT AGREEMENT NO. 773330.

References

Newton et al. 2014. Perspectives on the utilization of aquaculture coproduct in Europe and Asia: prospects for value addition and improved resources efficiency. Critical reviews in Food Science and Nutrition 54(4): 495-510

Morris, J. P., Backeljau, T., & Chapelle, G. (2019). Shells from aquaculture: a valuable biomaterial, not a nuisance waste product. Reviews in Aquaculture, 11(1), 42-57.

Newton et al. 2014. Perspectives on the utilization of aquaculture coproduct in Europe and Asia: prospects for value addition and improved resources efficiency. Critical reviews in Food Science and Nutrition 54(4): 495-510

FROM BLUE TO GREEN: TRANSFORMING FISH SLUDGE INTO A VALUABLE BIO-FERTILISER PRODUCT USING INNOVATIVE DRYING TECHNOLOGY

Hallstein Baarset[1]*, Christian Bruckner[2]

Affiliations: [1]Waister AS, Åslyveien 15, NO-3170 SEM, Norway, [2]Salten Havbrukspark AS, Sundsfjorden 22, NO-8120 NYGÅRDSJØEN, Norway
E-mail: hallstein.baarset@waister.eu

Introduction

The work on improved sustainability in the aquaculture industry by valorisation of side-streams being performed in the GAIN (Green Aquaculture INtensification in Europe) project has resulted in innovative machines in standardised sizes for processing of fish sludge at hatcheries. Initially, it proved challenging to achieve a stable process for fish sludge drying. However, through a number of improvements Waister has ensured a stable drying process with a predictable processing capacity.

GAIN is a Horizon 2020 project, delivering services and technologies to market within the project period to contribute to the ecointensification of European aquaculture production. Resource efficiency, reduced environmental impact, increased precision and valorisation throughout the production chain are all key elements in the approach to improve seafood self-proficiency and regional stability.

Achievements

Processed with the innovative technology, dried fish sludge from hatcheries has proved to be rich in nitrogen (N) and phosphorus (P), while relatively low on heavy metals. This allows the dried fish sludge, given the correct processing, to be utilised as a potentially valuable bio-fertiliser. By combining a mechanical fluidisation of the fish sludge in the drying chamber, inserting superheated steam with a relatively low temperature, Waister has achieved a significant decrease of N loss in the drying process. Through a number of innovations and improvements to the drying technology, Waister has achieved N levels as high as 7 % in the dried fish sludge.

There is no common EU classification standard of bio-fertiliser products from fish sludge. The Norwegian classification system defines four quality classes determined from the level of heavy metals in the bio-fertiliser product. The dried fish sludge from this innovative drying process produces a bio-fertiliser product in quality class I, which is the second best out of the four classes, allowing spread of up to 4 kg/m^2 over a period of ten years for use in agriculture, private gardens, and parks. Growth trials made on barley has shown up to 45 % better yield than the reference mineral fertiliser. The dried bio-fertiliser product is thereby proved as a well-functioning and safe product for application on lands for productions of both feedstock and crops for human consumption, thereby enabling a circular economy solution to an aquaculture challenge.
We will present the applicability of the dried fish sludge as a bio-fertiliser, as well as the standardised solutions for a stable technology on processing of transforming fish sludge into a valuable bio-fertiliser product.

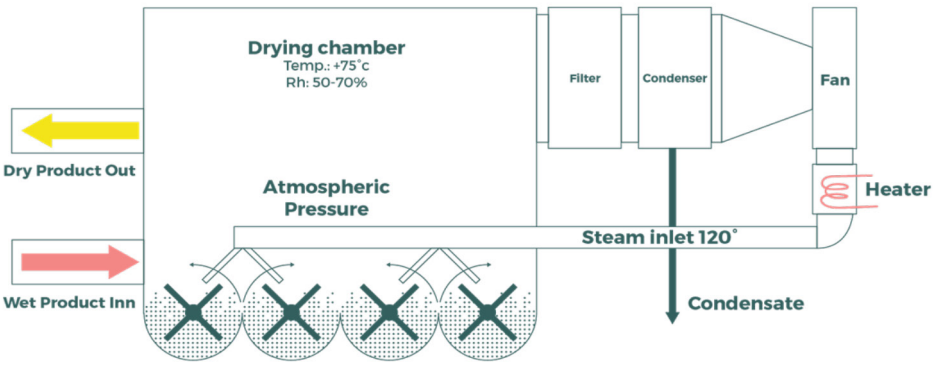


Figure 1: Schematic presentation of the innovative drying technology for enabling the transformation of fish sludge into a valuable bio-fertiliser product

Acknowledgements

The research leading to these results has also received funding from the GAIN project, European Union's HORIZON 2020 Framework Programme under GRANT AGREEMENT NO. 773330.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773330

A MODEL TO ASSESS THE SUSTAINABILITY OF AQUACULTURE SYSTEMS: FIRST RESULTS FROM THE SIMTAP PROJECT

J. Bacenetti[3]*, S. Le Féon[2], T Dubois[1], C. Jaeger[1], A. Wilfart[1], N.A. Corfini[1], G. Coppola[3], M Costantini[3], J. Aubin[1]

[1]UMR SAS, INRAE, Institut Agro, 35000 Rennes, France
[2]Independent Researcher in Environmental Assessment, Pépinière ESS, 23 rue des Chênes, 35630 Langouët, France
[3]Department of Environmental Science and Policy, Università degli Studi di Milano, via Giovanni Celoria 2, 20133, Milan, Italy.

jacopo.bacenetti@unimi.it

Introduction

Aquaculture is more and more considered as a major contributor to the growing demand in worldwide seafood production. Sustainability has becoming a key question for aquaculture systems.

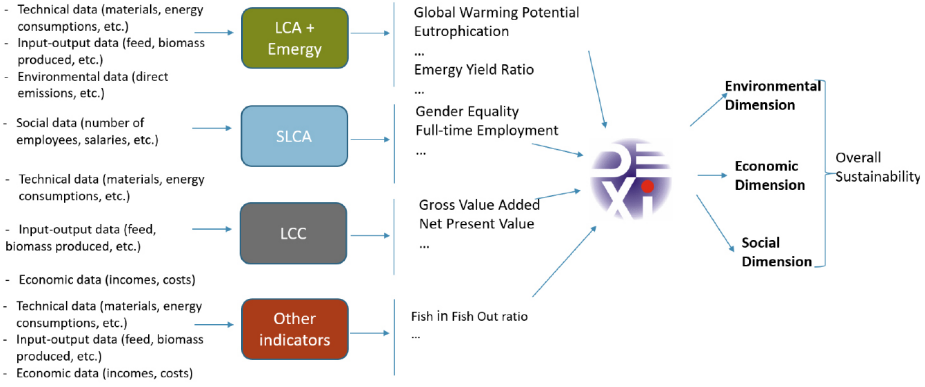
Among the different evolution strategies of aquaculture systems, Integrated Multitrophic Aquaculture (IMTA) goes further in associating complementary species in the same system of production.

The most common IMTA systems are aquaponic systems that use the nutrients present in the waste water from fish to support the growth of plants (Waller et al., 2015). More complex systems appear that, for example, combine polychaete-assisted sand filters and halophyte aquaponics for super-intensive marine fish farms. IMTA systems are designed as potential future solutions to decrease the impacts of fish production on ecosystems.

The SIMTAP project (EU PRIMA 2018) launched in June 2019 with the objective to develop self-sufficient IMTA systems in several Mediterranean countries (France, Italy, Malta and Turkey), with the aim to improve nutrient recycling. Beyond the objectives to develop effective systems of production, the project aims to assess their sustainability performance. Sustainability assessment of food systems needs indeed to merge multiple criteria and. Thus, in this context, environmental, social and economic impacts need to be evaluated together. The assessment of sustainability is also dependent of the context and to the stakes of the concerned people. Therefore, it is necessary to include the participation of different stakeholders from the concerned territories, as it has been shown previously in aquaculture sector. In this objective, the use of a multi-criteria decision analysis (MCDA) method is a relevant option.

Aiming at helping decision makers to make choices towards more sustainable options or scenarios, MCDA was chosen to gather the environmental, social and economic dimensions into a global sustainability assessment method. To simplify this complex and multidimensional issue, the DEXi method was selected as the MCDA method.

This paper presents the DEXi model developed with details on working group, meetings and discussions between stakeholders of the SIMTAP project, in order to obtain an operational model for sustainability assessment of aquaculture systems.



(Continued on next page)

The DEXiAqua model

The developed model is called DEXiAqua. The main steps were to (i) build a conceptual model based to describe the three pillars of sustainability in aquaculture system, according to technical and scientific literature leading to a tree of attributes, (ii) determine ponderation factors for the aggregation of the different attributes, (iii) determine thresholds to convert quantitative and/or qualitative values of indicators into scales for attributes (as for example low/medium/high). A template for data collection has been developed in order to collect the raw necessary data, and calculate first level of indicators.

DEXiAqua uses the DEX method to assess the sustainability of aquaculture systems through several indicators stemming from technical domain and reference methodologies (Life Cycle Assessment, Life Cycle Costing, Social Life Cycle Assessment, Emergy Accounting) selected and organized by the partners involved in the SIMTAP project. The DEX method consists in building a tree of attributes organized to characterize a complex problem. At the end of each branch of the tree, qualitative or quantitative indicators are measured. The value of each indicator is translated into a qualitative scale for the associate attribute by using thresholds values. Weightings – called utility functions – are used to build attributes from sub-attributes until the overall sustainability.

Conclusion

Despite collection of the data required for the model feeding can be money and time expensive, DEXiAqua is able to assess the overall sustainability of aquaculture systems and to help to define ways of improvement by identifying the hotspots. More case studies are required in order to confront DEXiAqua to a variety of systems with technical and contextual disparities, which could lead to changes in weighing of attributes for a better adaptation to contexts. In the second part of the SIMTAP project, the inland IMTA developed systems will be compared with commercial aquaculture systems.

Acknowledgements

This study was conducted within the framework of PRIMA S2 2018 project SIMTAP. SIMTAP (https://www.simtap.eu/) is part of the PRIMA Programme supported by the European Union

OPEN OCEAN CULTIVATION OF SEAWEED – SCALING UP PROVEN CONCEPTS

U. G. Bak*[1], F. Marsman[1], E. Berg[1], I. Menger[1], Ó. Gregersen[1]

[1] Ocean Rainforest Sp/F, Mjólkargøta 20, FO-180 Kaldbak, the Faroe Islands.

Introduction

We are facing a global crisis. Climate change and loss of biodiversity are a fact (Dasgupta, 2021). At the same time, the global human population is increasing, hence, sustainable, healthy, and tasty food is needed (WFS Webinar, 2021). Sustainably cultivated seaweeds in the open ocean are being mentioned as a blue biomass resource that could amend the negative impact of climate change and loss of biodiversity and add new healthy food and feed products to the market (Lange et al., 2021).

The large brown seaweeds (kelp) are among the fastest-growing crops on the planet. To grow, they only need sunlight, CO_2, and nutrients that are naturally available in the ocean. When this productivity becomes commercially exploited and transferred to the large surface of the open ocean, in sites with sufficient nutrients, large quantities of biomass can be produced. The CO_2 and nutrient mitigation provided by the kelp production improve ocean health and add new kelp-forest-habitats that provide a shelter, feeding chamber, and nursery for various low and high trophic animals.

The open ocean environment has previously been a major challenge for seaweed production due to the high wave exposure and deep waters (Bak et al. 2020). Nevertheless, the SME Ocean Rainforest has developed a MacroAlgal Cultivation Rig (MACR) that is suitable for these harsh ocean environments (Bak et al. 2018). The challenge is immediately to take this proven concept and bring it to a level of 1 Mio tons wet weight of annual production with a high economic, social, and environmental impact in 2030.

This work is part of the ongoing EU H2020-project AquaVitae www.aquavitaeproject.eu and the project SELBREED which is a project collaboration between Ocean Rainforest and Hortimare.

Methods

The main challenge is to reduce operational expenditures (OPEX) and capital expenditures (CAPEX) of production to make the business profitable. The methods investigated to reduce cost are:

- Upscaling operation through further rig development
- Testing re-use of aquaculture equipment
- Developing mechanized seeding, harvesting, and landing technics
- Increase yield through selective breeding
- Make use of multiple partial harvesting and optimize this method

Results

Upscaled macroalgal cultivation in the Faroe Islands was tested by improving the MACR further allowing the rig to hold a production of 40 km seeded grow line in an 8-ha area which has now been tested during winter storms and proven the survivability of the second MACR-version with a total of 100 km grow lines deployed and a productivity of 30 tonnes ww/ha/year.

Discarded anchors and buoys were re-used in the deployment of two new rigs, and the implementation of second-hand equipment was analyzed and found to reduce the CAPEX by 41% and lowered the CO_2-footprint by 46% in an optimal situation. However, the more the seaweed industry will scale up, the more limited the re-use opportunities will be.

Besides upscaling, the project has identified new harvesting methods that enable maximum yield and reduced OPEX. Yet there is a need to reduce OPEX even further and additional technological development to upscale the operation is needed.

The upscaling in seeding material for the 80 km of new grow lines was made without further investments in hatchery equipment, thus keeping a low cost of production. The seeding of ropes still needs to be automated and yield quantities should be improved by selective breeding. This is a challenging part of future upscaling.

(Continued on next page)

Figure 1 *The newly deployed MACR seeded with 40 km Saccharina latissima in the Faroe Islands at a site with >50 meters water depth (Ocean Rainforest Sp/f 2020).*

The yield is the main income driver. Today a vertical line of 10-meters yields 25 kg ww per harvest, being harvested twice a year, it yields 50 kg ww/grow line which occupies a sea surface area of one square meter. The aim is to make a crossbreed that can produce 30% more within the coming 2-3 years.

The company has discovered that sugar kelp (*Saccharina latissima)* can regrow after being harvested. The use of multiple partial harvesting of *S. latissima* (up to 6 harvests without re-seeding) has allowed making the highest known harvesting yield per seeded meter growth line and has reduced the production cost by 75% (Bak et al. 2018). A better understanding of the growth pattern and effect of partial harvesting will enable maximum yield and reduced OPEX.

The next step in the AquaVitae project will be to establish criteria for site selection and find suitable sites for large-scale production (>500 ha) in open-ocean environments in the Atlantic Ocean. Thus, the Faroese seaweed production adds crucial results for the future expansion of this underexploited aquaculture industry.

References

Dasgupta, P. (2021), The Economics of Biodiversity: The Dasgupta Review. London: HM Treasury.

World Food Summit Side-Event (2021), Short-cut to a healthy & sustainable diet and reduced use of antibiotics by microbial solutions and upcycling of food processing side-streams. Webinar.

Lange, L. et al. (2021), the 3F Marikat folder on Blue BioEconomy: Den blå bioøkonomi – værdiskabelse fra den blå biomasse.

STEROLS AND ANTIOXIDANT ACTIVITY IN SUPERCRITICAL CO_2 EXTRACTS OF *Cystoseira barbata*

S. Balbino*, I. Elez Garofulić, A. Dobrinčić, D. Cvitković, V. Dragović-Uzelac, R. Čož Rakovac

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb (Croatia)
E-mail: snedjer@pbf.hr

Introduction

Brown algae comprise a considerable number of the secondary bioactive metabolites, including sterols which are reported to be unique in terms of their structural and functional diversity (Hannan et al., 2020) and numerous health benefits (Plaza et al., 2008). Nowadays, in order to provide effective, reproducible and rapid extraction of lipophilic compounds, a supercritical CO_2 extraction emerges as an alternative technique with many benefits over conventionally used organic solvents (Patel et al., 2020). The current study aimed to explore the potential of supercritical CO_2 for the extraction of sterols from the Mediterranean brown alga *Cystoseira barbata*, whose lipophilic fraction is described as a rich source of sterols, mainly fucosterol (Cvitković et al., 2021).

Materials and methods

Cystoseira barbata, harvested from the Croatian coast in 2020, was lyophilised and extracted with supercritical CO_2 for 120 min at 400 bar, 40 °C and a flow rate of 20 mL/min. In addition, experiments with 10 and 20% (v/w) of ethanol (96%) added to extraction chamber as modifier were performed under the same conditions. A standard method (ISO 12228-1:2014) was used for the determination of sterols. Sterols were isolated by thin layer chromatography, silylated and analysed by gas chromatography. The antioxidant properties of algal extracts were determined as DPPH radical-scavenging activity by electron spin resonance (ESR) spectroscopy (Koprivnjak et al., 2008).

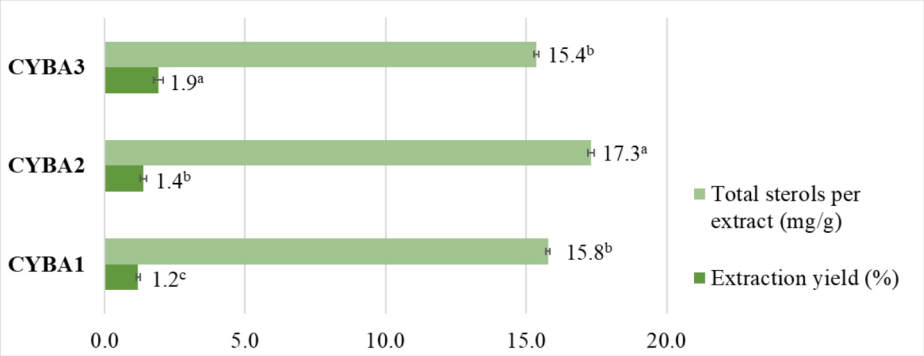


Fig. 1. Extraction yield and total sterols (means, n=3) in *Cystoseira barbata* supercritical CO_2 extracts (CYBA1 – no modifier; CYBA2 – 10% ethanol; CYBA3 – 20% ethanol). Values with different letters are statistically different at $p \leq 0.05$.

Sterols (%)	Cholesterol	Brassicasterol	Campesterol	24-Methylene-cholesterol	Campestanol	Stigmasterol	Sitosterol	Fucosterol	Isofucosterol	DPPH scavenging (%)
CYBA1	1.0^b	11.4^a	1.2^a	0.8^a	13.0^a	2.2^a	10.0^a	54.0^c	6.2^c	53.3^c
CYBA2	0.9^b	9.3^c	0.9^a	0.9^a	10.7^c	2.0^b	9.1^b	57.4^b	8.7^a	74.2^a
CYBA3	1.8^a	10.1^b	1.0^a	0.9^a	11.9^b	2.0^b	6.9^c	58.9^a	6.5^b	61.8^b

Table 1. Individual sterols (%) and DPPH radical-scavenging activity determined by ESR of *Cystoseira barbata* supercritical CO_2 extracts (CYBA1 – no modifier; CYBA2 – 10% ethanol; CYBA3 – 20% ethanol). Results are presented as means (n=3). Values with different letters are statistically different at $p \leq 0.05$.

(Continued on next page)

Results

Supercritical CO_2 extraction with 20% ethanol gave the highest yields of lipophilic *Cystoseira barbata* extracts, while significantly higher values were obtained by the addition of 10% ethanol when considering the results of total sterol per extract (Figure 1).

Considering individual sterols, fucosterol was dominant, followed by campestanol and brassicasterol, although their contents differed significantly between extracts. DPPH radical-scavenging activity was highest in extracts obtained by 10% ethanol (Table 1).

Discussion

Although the highest yields were obtained with the addition of 20% ethanol, the extracts obtained with 10% ethanol contained the highest levels of sterols. Due to its higher polarity, the addition of ethanol caused the greater extraction of other polar compounds such as chlorophylls and carotenoids (Cvitković et al., 2021), which therefore decreased the relative content of sterols in the extract. Nevertheless, considering the overall efficiency of sterol extraction results observed as total sterol content per algal dry weight, supercritical CO_2 extraction with the addition of 20% ethanol proved to be the most efficient, resulting in 0.29 mg/g algal dry weight. While fucosterol was dominant in all extracts, as expected (Hannan et al., 2020), its relative content was increased with ethanol addition. On the other hand, the levels of brassicasterol, campesterol and sitosterol were higher in the extracts without the addition of modifier. The antioxidant activity was highest in the 10% ethanol extract and correlated with the total sterol content (r=0.836).

References

Cvitković, D., Dragović-Uzelac, V., Dobrinčić, A., Čož-Rakovac, R., & Balbino, S. (2021). The effect of solvent and extraction method on the recovery of lipid fraction from Adriatic Sea macroalgae. *Algal Research*, *56*, 102291.

Hannan, M. A., Sohag, A. A. M., Dash, R., Haque, M. N., Mohibbullah, M., Oktaviani, D. F., Hossain, M. T., Choi, H. J., & Moon, I. S. (2020). Phytosterols of marine algae: Insights into the potential health benefits and molecular pharmacology. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *69*, 153201.

ISO 12228-1 (2014). *Determination of individual and total sterols contents — Gas chromatographic method — Part 1: Animal and vegetable fats and oils*.

Koprivnjak, O., Škevin, D., Valić, S., Majetić, V., Petričević, S., & Ljubenkov, I. (2008). The antioxidant capacity and oxidative stability of virgin olive oil enriched with phospholipids. *Food Chemistry*, *111*(1), 121–126.

Patel, A., Matsakas, L., Sartaj, K., & Chandra, R. (2020). Extraction of lipids from algae using supercritical carbon dioxide. In *Green Sustainable Process for Chemical and Environmental Engineering and Science* (pp. 17–39). Elsevier.

Plaza, M., Cifuentes, A., & Ibáñez, E. (2008). In the search of new functional food ingredients from algae. *Trends in Food Science & Technology*, *19*(1), 31–39.

Apocyclops panamensis AS LIVE FEED FOR *Sander lucioperca* LARVICULTURE

Laura Ballesteros-Redondo*, Harry W. Palm, Lukas Reiche and Adrian A. Bischoff

University of Rostock, Faculty of Agricultural and Environmental Sciences, Department of Aquaculture and Sea-Ranching, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany
*Correspondence: laura.redondo@uni-rostock.de

Introduction

Larviculture is one of the most important bottlenecks for rearing fish in aquaculture. Often high mortality rates and variability in larval quality and quantity result in unstable production, requiring larviculture improvement. Live feed use in larviculture has increased in recent years, demonstrating higher survival and better growth rates as seen in *Sander lucioperca* larviculture, with *Artemia* sp. and rotifers from the genus *Brachionus* most commonly in use (Policar et al. 2019). Rotifers seem to fulfil the nutritional requirements for pikeperch larvae better than *Artemia* sp. (Yanes-Roca et al. 2018, Imentai et al. 2019, Imentai et al. 2020) although other organisms like copepods, as live feed, are gaining attention (Ajiboye et al. 2010). More studies are needed in order to understand the early life cycles stages when the larvae start to feed and what they can ingest during the first days of exogenous feeding.

Material and methods

The experiment took place in June 2021 in order to investigate the stomach contents of pikeperch larvae after feeding them with *Brachionus plicatilis* (Bra) or *Apocyclops panamensis* (Apo) from dph 3 until dph 7. Both diets were divided in 2 further groups with different larval stocking densities, 50 (Bra-17 and Apo-17) and 100 larvae*l^{-1} (Bra-34 and Apo-34), each one with 3 replicates, resulting in 340 feed organisms per fish per day. From each replicate, 3 larvae were taken each day after 30 minutes from the first feeding (feeding regime was 3 times a day at 09:00, 12:00 and 15:00) for subsequent stomach content analyses. In addition, water parameters, survival (dead larvae), growth (length), and yolk sac consumption were monitored daily.

Results

At dph 1 larvae were very small and tiny, while complete yolk sac consumption occurred at dph 5. At day 7 post hatch, the highest survival rates were 56% in diet Bra-17 and Bra-34 and lower in Apo-17 (32%) and Apo-34 (50%) (Figure 1) although, there were no significant differences (ANOVA, p=0.07). The total body length did not show any significant difference between the diets (ANOVA, p=0.411), with the mean total body length for all measured larvae less than 4mm. From dph 7, we observed feed inside the stomach of the fish although we could not determine its amount.

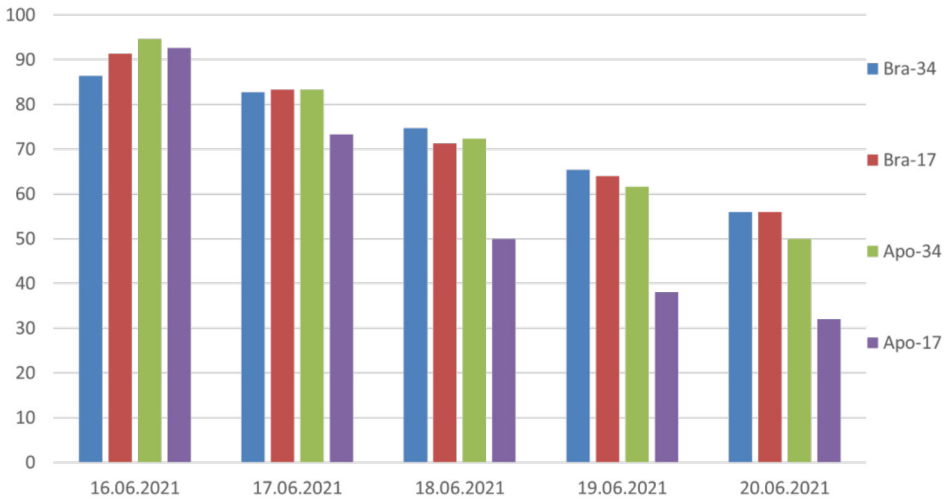


Figure 1. Survival rates from dph 3 until dph 7 for the 4 diets

(Continued on next page)

Discussion

Our study demonstrates for the first time that pikeperch larvae can ingest *A. panamensis* at dph 7 but that they are not able to digest them. The difficulty to count feed items inside the stomach of pikeperch larvae fed with *B. plicatilis* might indicate that the digestion was taking place, connected to the observed higher survival rates (even no significant differences) and confirming previous studies (Yanes-Roca et al. 2018, Imentai et al. 2019, Imentai et al. 2020). Our experiment also showed the importance of the larval quality for a successful pikeperch rearing (Policar et al. 2019) and the lack of an effect of the stocking density under application of the same amount of feed items per fish per day (Ballesteros-Redondo et al. in preparation).

References

Ajiboye, Olufemi & Yakubu, Adams & Adams, Temitope & Olaji, E.D. & Nwogu, N.A... A review of the use of copepods in marine fish larviculture. Reviews in Fish Biology and Fisheries. 21. 225-246 (2010). 10.1007/s11160-010-9169-3.

Ballesteros-Redondo, L., Palm, H.W., Bährs, H. and Bischoff, A. (in preparation). Quantity and concentration of *Brachionus plicatilis* as key variables for a successful larval rearing of *Sander lucioperca*

Imentai, A., Yanes-Roca, C., Steinbach, C., Policar, T., 2019. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. Aquacult Int 27, 1137–1149 https://doi.org/10.1007/s10499-019-00420-3

Imentai, A., Rašković, B., Steinbach, C., Rahimnejad, S., Yanes-Roca, C., Policar, T., 2020. Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca*) larvae. Aquaculture 529, 735636, ISSN 0044-8486, https://doi.org/10.1016/j.aquaculture.2020.735636.

Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Zarski, D. Recent progress in European percid fish culture production technology—tackling bottlenecks. Aquacult Int 27, 1151–1174 (2019). https://doi.org/10.1007/s10499-019-00433-y

Yanes-Roca, C., Mráz, J., Born-Torrijos, A., Holzer, A.S., Imentai, A., Policar, T., 2018. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. Aquaculture, Volume 497, Pages 260-268, ISSN 0044-8486, https://doi.org/10.1016/j.aquaculture.2018.08.004.

GENETIC ANALYSIS OF NINE HUNGARIAN COMMON CARP (*Cyprinus carpio*) STRAINS WITH A NOVEL MULTPLEX PCR SET FOR TETRANUCLEOTIDE MICROSATELLITES

Réka Enikő Balogh[*1], Dániel Péter[1], Adrienn Bíró[1], Szilvia Keszte[1], Béla Urbányi[1], István Lehoczky[2], Erika Edviné Meleg[2], Balázs Kovács[1]

[1] Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Gödöllő, Hungary, Páter Károly St. 1, 2100
[2] National Centre for Biodivertsity and Gene Conservation, Institute for Farm Animal Conservation, Gödöllő, Hungary, Isaszegi St. 200, 2100
E-mail: balogh.reka.eniko@uni-mate.hu

Introduction

Common carp (*Cyprinus carpio*) is a dominant fish species worldwide and in Hungary it is produced in the highest volume in aquaculture. The need for a comparable genetic analysis of different common carp stocks is increasingly important nowadays due to the high demand for genetic breeding. Multiplex PCR technique offers a cost-effective way for rapid screening of genetic markers, for instance microsatellites, which are versatile markers for numerous applications including population genetics, parental analysis or marker assisted selection for breeding programs. The aim of this study was to develop a novel multiplex set for tetranucleotide microsatellites that is suitable for a comparable genetic analysis of different common carp stocks. Subsequently, the genetic investigation of Hungarian strains was performed with this newly designed set.

Materials and methods

24 novel polymorphic tetranuceotid microsatellite markers were obtained by *in silico* analysis from the previously published common carp genome (Xu et al.,2014) and optimized individually on 8 samples originating from 5 different Hunagrian landraces (Tisza, Duna, Tata, Ráckeve, Velence). These markers were ordered in multiplex reactions and optimized on 15 samples, until three different multiplex sets were assembled from the most congruous markers designed with universal tailing (Glenn et al., 2005) and three different fluorescent primers (multiplex one: triplex PCR with PET, multiplex two: tetraplex PCR with FAM, multiplex three: triplex PCR with VIC) suitable for simultaneous fragment analysis. Subsequently, this set was utilized for population genetic analysis of nine Hungarian common carp strains (n=351): seven officially recognised landraces containing five native wild carp populations (*Cyprinus carpio carpio morpha hungaricus*) considered to be vulnerable to extinction by the International Union for Conservation of Nature (IUCN): Danube, Tisza, Velence, Ráckeve and Balaton, two mirror carp landraces (*Cyprinus carpio carpio morpha nobilis*) of the fish ponds Szeged and Nagyatád, the population of the gene bank in the National Centre for Biodivertsity and Gene Conservation and one endemic wild carp population from the thermal lake of Hévíz. Genotyping was performed on 3100 Genetic Analyzer (Applied Biosystems), alleles were identified by GeneMapper 4.0 software, and population genetic analysis was performed with MSToolkit and Genalex Microsoft Excel Add-ons, Micro-Checker, R ver 3.5.3, HP-Rare and Structure software.

Strains	n	MLG	Ar	pAr	H_{exp}	H_{obs}	F_{is}	dHW
Velence	36	28	2.26	0.25	0.36	0.17	0.53	7
Ráckeve	48	38	1.71	0.12	0.25	0.15	0.41	2
Gene bank	33	23	1.87	0.16	0.16	0.15	0.07	1
Danube	25	24	1.62	0.07	0.27	0.23	0.17	3
Tisza	23	20	2.15	0.30	0.20	0.17	0.19	3
Balaton	69	62	1.77	0.19	0.35	0.22	0.37	5
Szeged	30	21	2.21	0.25	0.17	0.13	0.23	5
Nagyatád	32	22	1.68	0.14	0.15	0.16	-0.07	0
Hévíz	43	28	1.58	0.07	0.22	0.10	0.54	5

Table 1: Measures of genetic variations within 9 Hungarian common carp strain using 9 tetranucleotide microsatellite loci (n: number of samples, MLG: multi-locus genotypes, Ar: average allelic richness, pAr: average private allelic richness, H_{exp}: expected heterozygosity, H_{obs}: observed heterozygosity, F_{is}: within strain inbreeding coefficient, dHW: number of loci deviating from Hardy-Weinberg equilibrium)

(Continued on next page)

Results

24 novel polymorphic markers were isolated with an average allele number of 5.2. The average PIC value (Polymorphic Information Content) was 0.59 and 15 of the isolated markers were highly polymorphic (PIC value > 0.5). The assembled multiplex set of 10 markers had an average allele number of 7 and an average PIC value of 0.5. One marker showed evidence of null alleles in 7 populations, therefore it was excluded from further analysis. All populations (except the one from Nagyatád) showed significant deviation from the Hardy-Weinberg Equilibrium with at least one marker (Table 1.). The mean expected heterozygosity (H_e) was 0.24 and the average observed heterozygosity (H_o) was 0.17. The DAPC-PCA analysis showed that most populations have very similar genetic background except the populations of Hévíz, Velence and Balaton. The pairwise F_{st} values and the Structure analysis supported the results of the DAPC-PCA.

Discussion and conclusion

A novel multiplex tetranucleotide microsatellite set was developed for common carp and utilized for population genetic analysis of 9 Hungarian strains. Based on our results, we can conclude that this multiplex set is sufficiently informative, therefore it could be a powerful tool for rapid and cost-effective analysis of this species. Most of the investigated Hungarian strains possess low expected heterozygosity values, suggesting low genetic variability. The observed heterozygosity values were slightly lower than the expected values. The within strain inbreeding coefficient shows that most of the strains are characterized by heterozygote deficiency, only the Nagyatád population showed a limited level of heterozygote surplus. All strains except the one of Nagyatád showed deviation from the Hardy-Weinberg Equilibrium (HWE) with at least one marker. The strains of Velence, Hévíz, Balaton and Szeged showed deviation with most of the markers, which can be the result of isolation. The STRUCTURE based analysis showed the highest delta K value with K=7. Among them, 3 clusters deviated markedly from the others on the dendrogram. The DAPC-PCA analysis and the pairwise F_{st} values indicated that the strains from the lakes of Hévíz, Velence and Balaton differed significantly from the others. Based on our results, the improvement of the genetic variability of the Hungarian wild carp populations is suggested with special emphasis on the strains of Hévíz, Velence and Balaton.

Acknowledgement

The work was supported by the GINOP-2.3.2-15-2016-00004 and the EFOP-3.6.3-VEKOP-16-2017-00008 projects and by the ÚNKP-20-3-I new national excellence program of the Ministry for Innovation and Technology from the source of the national research, development and innovation fund. The project is co-financed by the European Union and the European Social Fund.

References

Glenn TC, Schable NA (2005) Methods Enzymol 395:202–222.
Xu, P., Zhang, X., Wang, X. et al. (2014). Nat Genet 46, 1212–1219

DO CONSUMERS WANT TO EAT ALTERNATIVE PROTEINS FROM FISH WASTE? RAISING EUROPEAN CONSUMER AWARENESS THROUGH COMMUNICATION

Marija Banovic[1,a]

[1]MAPP Centre, Department of Management, Aarhus University, Aarhus, Denmark

[a]e-mail: maba@mgmt.au.dk

Acknowledgements
This study has received funding from EIT Food (https://www.eitfood.eu/) through the project "EcoD: Bringing new life to cod waste by turning it into food" (Grant number ID: 20432, EIT Food Business Plan 2020).

Introduction
The increased processing in fish production results in massive production of waste (20–80% of landed weight is wasted depending on the processing type) (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013). There are many efforts of using this waste in feed, food packaging, biofuels (Arvanitoyannis & Kassaveti, 2008; Jayasinghe & Hawboldt, 2012) and to recover more for use as human food, where this by-products can be transformed into high quality fish protein, which can be added to a wide range of food products and thereby contribute to both health and the circular economy (Alfio, Manzo, & Micillo, 2021). Thus, valorization and upcycling of by-products, such as those coming from fish waste, can have many potential applications through product development in the fish and food industry (Nawaz et al., 2020).

The next important step for European seafood industry is raising consumer awareness on the above issues, as well as on environmental and health potential of alternative proteins coming from fish waste. This is vital for managers and policy makers in order to be able to design and employ appropriate strategies around new alternative proteins coming from fish waste. Consequently, in this study, and in a cross-cultural context, it is examined how European consumers respond to new products with alternative proteins coming from fish waste while accounting for the different communication messages related to the health and environmental benefits.

Materials and methods
An online cross-cultural study has been conducted in three European countries, namely Denmark, France and Germany with a total sample of 2700 participants. Three experimental conditions have been considered: control and two message framing conditions (health vs. sustainability) to assess whether the consumers acceptance of alternative proteins coming from fish waste would be improved due to the health or sustainability messages or just plain preference (control condition) when focusing on possible food products. After reading and signing the informed consent, participants were randomly assigned to one of three above mentioned experimental conditions, where they answered questions regarding their attitudes towards fish protein, health and environmental consciousness, purchase behavior, and socio demographics, among others.

Results
The results show that the European consumers are open towards having alternative proteins from fish waste. They associate these new proteins with health benefits, and believe that they could add to a more diverse diet and more choice. Some differences have been found across European countries where French and German consumers seem to be more positive towards and more likely to buy food products containing fish protein than the Danish consumers. On the contrary, Danish consumers were found to be more reluctant towards these proteins and products containing them. In terms of communication, it seems that when health benefits are emphasized, consumers had more positive attitudes and more likely to buy food products with new fish protein, see Figure 1.

Conclusions
The valorization and upcycling of fish waste adds-value to the fish production and provides more choice for the consumers. This is significant as the European demand for fish products significantly surpasses the supply that fish sector can provide. In this context, fish industry and policy makers need to become more efficient in valorizing the fish waste and raising consumer awareness which is especially important now in the light of the green deal and farm-to-fork call for a fair, healthy and environmentally-friendly food system.

(Continued on next page)

References

Alfio, V. G., Manzo, C., & Micillo, R. (2021). From Fish Waste to Value: An Overview of the Sustainable Recovery of Omega-3 for Food Supplements. *Molecules, 26*(4), 1002.

Arvanitoyannis, I. S., & Kassaveti, A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International journal of food science & technology, 43*(4), 726-745.

Ghaly, A., Ramakrishnan, V., Brooks, M., Budge, S., & Dave, D. (2013). Fish processing wastes as a potential source of proteins. *Amino acids and oils: a critical review. J Microb Biochem Technol, 5*(4), 107-129.

Jayasinghe, P., & Hawboldt, K. (2012). A review of bio-oils from waste biomass: Focus on fish processing waste. *Renewable and sustainable energy reviews, 16*(1), 798-821.

Nawaz, A., Li, E., Irshad, S., Hammad, H., Liu, J., Shahbaz, H. M., . . . Regenstein, J. M. (2020). Improved effect of autoclave processing on size reduction, chemical structure, nutritional, mechanical and in vitro digestibility properties of fish bone powder. *Advanced Powder Technology, 31*(6), 2513-2520.

CONSUME IT, YET UNAWARE? THE INFLUENCE OF A HEALTH GOAL ON CHOICE OF AQUACULTURE PRODUCTS AMONG BRAZILIAN CONSUMERS

Marija Banovic[1,a], Jessica Aschemann-Witzel[1], Rosires Deliza[2]

[1]MAPP Centre, Department of Management, Aarhus University, Aarhus, Denmark
[2]EMBRAPA Agroindustria de Alimentos, Brazil

[a]e-mail: maba@mgmt.au.dk

Introduction

Brazil is one of the world's largest fish producer with an extensive maritime coast on the Atlantic Ocean and a great quantity of rivers, lakes and barrages. Fishing represents an important source of income for Brazil and the socio-economic characteristics of the fisheries are very much diverse, due to the coast geographic dimension as well as due to the different culture in the different geographical regions in terms of fishing and fish farming (FAO, 2020). Even though Brazil has a great potential in terms of seafood, Brazilians consume very little fish in general, accounting for only 9.02 kg/per capita/year where only 2.45 g/per capita/day of protein supply comes from fish and seafood (year 2018; FAOSTAT, 2021). This consumption differs depending on the region with northern states consuming more and southern ones consuming less fish. Underlying reasons of the low fish consumption mentioned are awareness, price, taste, and health issues (Baptista, Rodrigues, & Sant'Ana, 2020; Maciel et al., 2013; Mitterer-Daltoé, Carrillo, Queiroz, Fiszman, & Varela, 2013). To be able to develop suitable strategies to increase consumers' consumption of fish, a better understanding of consumers' fish choices across different Brazilian regions is of key importance. Therefore, in this study we account for the role of a health goal and awareness on the aquaculture product choice, and its relationship with taste and price in the congruent non-conflicting versus incongruent conflicting choice context.

Materials and methods

An experimental study was designed to examine the role of the health goal and consumer aquaculture product awareness, as well as its relationship with taste and price, in the context where all available choice-set options do not conflict with the health goal versus when all the options in the choice-set conflict with the health goal, taking into account different Brazilian geographical regions. The congruent actions were assumed (i.e., healthy - expensive) when the choice-set options do not conflict with the active health goal (i.e., congruent, healthy choice-set), and vice-versa when conflicting-choice set present. It is further assumed that this effect is moderated by consumers' aquaculture product awareness that increases (or decreases) the influence of the health goal contingent on the choice context (non-conflicting vs. conflicting). One thousand three hundred and three participants were randomly assigned to one of the four experimental conditions: 2 goals (health goal vs. control) x 2 choice-sets (non-conflicting vs. conflicting). The sample was representative of the Brazilian population.

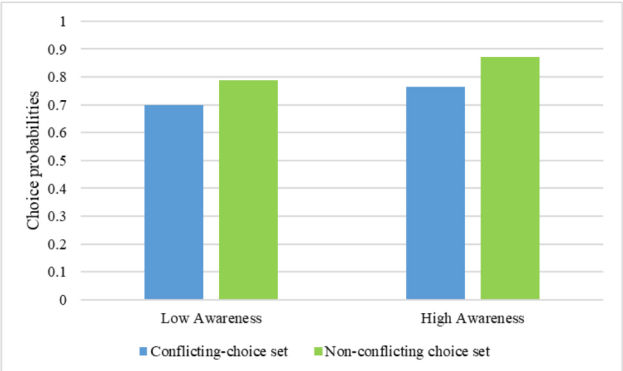


Figure 1. Impact of consumer awareness on choice of aquaculture products with health goal present.

(Continued on next page)

Results

The results from the binary logistic regression showed significant interaction effect between choice-set and consumer aquaculture product awareness when health goal present ($\beta = -0.867$, Wald = 6.416, $p = 0.011$), while region as a covariate was not significant ($p = 0.732$). This indicated that when health goal present with non-conflicting choice more congruent actions occurred (healthy - expensive) among those individuals who were high in awareness regarding Brazilian aquaculture then those individuals that were low in awareness, opting to choose more often higher-priced product options, see Figure 1.

Conclusions

The impact of health information on consumers' choice of aquaculture product should be carefully deliberated in both healthy and unhealthy choice contexts. This is particularly important as health information can be efficiently reinforced through taste establishing healthier food choices, and this is particularly true among consumers who possess higher levels of product awareness. On contrary, this can fail in the unhealthy choice contexts where this information could still increase consumers' product choice.

References

Baptista, R. C., Rodrigues, H., & Sant'Ana, A. S. (2020). Consumption, knowledge, and food safety practices of Brazilian seafood consumers. *Food Research International, 132*, 109084. doi:https://doi.org/10.1016/j.foodres.2020.109084

FAO. (2020). *The State of World Fisheries and Aquaculture 2020*. . Retrieved from

FAOSTAT. (2021). FBS: New Food Balances. Retrieved from http://www.fao.org/faostat/en/#data/FBS. http://www.fao.org/faostat/en/#data/FBS

Maciel, E. D. S., Savay-da-Silva, L. K., Vasconcelos, J. S., Sonati, J. G., Galvão, J. A., Lima, L. K. F. D., & Oetterer, M. (2013). Relationship between the price of fish and its quality attributes: a study within a community at the University of São Paulo, Brazil. *Food Science and Technology, 33*(3), 451-456.

Mitterer-Daltoé, M., Carrillo, E., Queiroz, M., Fiszman, S., & Varela, P. (2013). Structural equation modelling and word association as tools for a better understanding of low fish consumption. *Food Research International, 52*(1), 56-63.

Acknowledgements

This work has been funded by the Danish Agency for Science and Higher Education, Grant Nr. 8073-00029B, 2019-2020.

THE EFFECT OF BETANODAVIRUS (RGNNV GENOTYPE) ON IMMUNE AND OXIDATIVE STRESS PARAMETERS IN SEA BASS (*Dicentrarchus labrax*)

B. Carmo[1], R. Passos[1], D. Pires[1], M. Vaz[1], P. Santos[2] and T. Baptista[1*]

1- MARE – Marine and Environmental Sciences Centre, ESTM, Politécnico de Leiria, Av Porto de Pesca, 2520-620 Peniche, Portugal
2- CIIMAR: Interdisciplinary Centre of Marine and Environmental Research
E-mail: teresa.baptista@ipleiria.pt

Introduction

Betanodavirus is the aetiological agente of viral nervous necrosis (VNN), also known as viral encephalopathy and retinopathy (VER). This virus is of significant importance in aquaculture since betanodavirus infections are associated with high mortalities, that can be as high as 100% in some cases. Out of the four major genotypes, the red-spotted grouper nervous necrosis virus (RGNNV) is the most prevailing in warm-water fish species in the Mediterranean. This study aims to observe the effect of RGNNV on the immune and oxidative stress parameters of sea bass (*Dicentrarchus labrax*).

Materials and Methods

This time course study was conducted at the CETEMARES facilities (Leiria Polytechnic), in which sea bass individuals (33.99 ± 32.85 g) were infected with 10^6 $TCID_{50}$/fish with NNV (RGNNV genotype) through intramuscular injection (IM). For immune response analysis, blood was drawn with heparinized syringes from random fish (N = 6) before infection (0h), after infection (6h, 9h, 24h, 48h, 120h and 144h) and from moribund fish, and the blood was centrifuged for plasma. For oxidative stress, livers were collected at the same times, being stored at -80°C until further analysis. Lysozyme was determined according to Costas et al. (2011). Oxidative stress parameters, such as lipid peroxidation (LPO), catalase activity (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and total glutationse (tGSH) were determined by the methods described by Bird and Draper (1984), Clairborne (1985), McCord & Fridovich (1969), Habig et al. (1974) (adapted to microplate by (Frasco & Guilhermino, 2002)), and Baker et al. (1990), respectively.

Results and Discussion

Regarding lysozyme, values decreased over time since infection. Lysozyme is a defense molecule from the innate immune system with high importance in the response of the host to infections, mediating microbial invasions (Saurabh & Sahoo, 2008), which does not match the expected results, which would be an increase in the values after infection as it activated the complement system and phagocytes as a strategy of counteracting the infection (Magnadóttir, 2006).

For oxidative stress parameters, a significant increase in tGSH was observed at 48 hours, and in SOD values at 9h and 120h. CAT values decreased between 0h and 6h hours, and other sampling moments. Increased LPO values were observed at 120h, 144h and moribund fish and decreased GST values were observed at 144h compared to other sampling moments. It has been demonstrated that NNV induces ROS production (Krishnan & Kurcheti, 2021), which coincides with the increase and SOD values, which could be an attempt from the immune system of fish of trying to mantain the redox balance.

The excessive production of O^{2-} by SOD could inhibit the production of CAT (Bagnyukova et al., 2006), which could explain the decreased CAT levels observed after the first increase of SOD. GST participates in the removal and detoxification of aldehyde products from lipid peroxidation (Bagnyukova et al., 2006; Hermes Lima, 2004), and the lower values obtained point to a lack of action in the disposal of said products, which was observed in the high levels of LPO observed in the later sampling times. The increase of tGSH values is due to the damages induced on the organism by oxidative stress.

Conclusion

Oxidative stress is one of the many ways of assessing the response of aquatic organisms to infections with pathogens, and the consequent damages to the organism. In this study, *D. labrax* infected with red-spotted grouper nervous necrosis virus demonstrated a response to the infection through the production of various antioxidant compounds.

(Continued on next page)

References

Bagnyukova, T. V., Chahrak, O.I., Lushchak, V.I., 2006. Coordinated response of goldfish antioxidant defenses to environmental stress. *Aquat. Toxicol.* 78, 325–331. https://doi.org/10.1016/j.aquatox.2006.04.005

Baker, M.A., Cerniglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Anal. Biochem.* 190, 360–365. https://doi.org/10.1016/0003-2697(90)90208-Q

Bird, R.P., Draper, H.H., 1984. Comparative Studies on Different Methods of Malonaldehyde Determination. *Methods Enzymol.* 105, 299–305. https://doi.org/10.1016/S0076-6879(84)05038-2

Clairborne, A., 1985. Catalase activity, in: Grenwald, R.A. (Ed.), Handbook of Methods of Oxygen Radical Research. CRC Press, Boca Raton, FL, USA, pp. 283–284.

Frasco, M.F., Guilhermino, L., 2002. Effects of dimethoate and betaphthoflavone on selected biomarkers of *Poecilia reticulata*. *Fish Physiol. Biochem.* 26, 149–156.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249, 7130–7139.

Hermes Lima, M., 2004. Oxygen in biology and biochemistry. in: Storey, K. (Ed.), Functional Metabolism: Regulation and Adaptation. pp. 319–368.

Krishnan, R., Jeena, K., & Kurcheti, P.P., 2021. Nervous necrosis virus induced oxidative imbalance and host associated antioxidant response in Asian seabass brain. *Aquaculture*, *531* (January 2020), 735809. https://doi.org/10.1016/j.aquaculture.2020.735809

Magnadóttir, B., 2006. Innate immunity of fish (overview). *Fish & Shellfish Immunology*, *20*(2), 137–151. https://doi.org/10.1016/j.fsi.2004.09.006

McCord, J.M., Fridovich, I., 1969. Superoxide Dismutase. An Enzymic Function for Erythrocuprein (Hemocuprein). *J. Biol. Chem.* 244, 6049–6055.

Saurabh, S., & Sahoo, P. K., 2008. Lysozyme: An important defence molecule of fish innate immune system. *Aquaculture Research*, 39 (3), 223–239. https://doi.org/10.1111/j.1365-2109.2007.01883.x

Acknowledgements: FCT, Portugal, for financial support (UID/MAR/04292/2020), and Be4AquaHealth (MAR-02.05.01-FEAMP-0013).

ECO-INNOVATIVE FORTIFIED FARMED FISH: NUTRITIONAL BENEFITS ASSOCIATED WITH THE CONSUMPTION OF FORTIFIED GILTHEAD SEABREAM (*Sparus aurata*) AND COMMON CARP (*Cyprinus carpio*)

V. Barbosa[1,2,3,*], A.L. Maulvault[1,4,5], P. Anacleto[1,5,6], M. Santos[1], M. Mai[6], H. Oliveira[1,6], I. Delgado[7], M.Barata[1], L. Ribeiro[1], P. Eljasik[8], R. Panicz[8], J. Dias[9], P. Pousão-Ferreira[1], M.L. Carvalho[3], M. Martins[2], A. Marques[1,6]

[1]IPMA, I.P. - Division of Aquaculture and Seafood Upgrading, Portuguese Institute for the Sea and Atmosphere, 1495-165 Algés, Portugal
[2]MARE - Marine and Environmental Science Centre, Department of Environmental and Sciences Engineering (DCEA), NOVA School of Science and Technology (FCT NOVA), 2829-516 Caparica, Portugal
[3]LIBPhYs-UNL - Center of Atomic Physics, Physics Department, NOVA School of Science and Technology (FCT NOVA), 2829-516 Caparica, Portugal
[4]UCIBIO, REQUIMTE, NOVA School of Science and Technology (FCT NOVA), 2829-516 Caparica, Portugal
[5]MARE – Marine and Environmental Sciences Centre, Faculty of Sciences, University of Lisbon (FCUL), 1749-016 Lisbon, Portugal6
[6]CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto University, 4450-208 Matosinhos, Portugal
[7] INSA, I.P. - Food and Nutrition Departmen,. National Health Institute Doutor Ricardo Jorge, 1649-016 Lisbon, Portugal
[8] ZUT - Zachodniopomorski Uniwersytet Technologiczny w Szczecinie,. 70-310 Szczecin, Poland
[9] SPAROS - SPAROS, Lda., 8700-221 Olhão, Portugal
Email: vera.barbosa@ipma.pt

Introduction

A seafood-based diet has been widely recognized as beneficial to human health. Yet, most European consumers do not meet the dietary recommendations of eating at least two portions of fish (equivalent to 300 g) per week to ensure the provision of essential nutrients (EFSA, 2015). One third of global population has severe nutritional deficiencies, particularly iodine (I), selenium (Se) and iron (Fe), which results in impaired endocrine, neurophysiological and immunological functions (Pinkaew and Karrila, 2015). The development of eco-innovative farmed fish with adequate levels of essential nutrients through fortification with sustainable natural marine resources (e.g. iodine-rich macroalgae and Se-rich yeast) represents a potential strategy to overcome worldwide nutritional deficiencies, but also to increase consumer confidence in farmed seafood products (Tocher, 2015). The present study aimed to evaluate the potential nutritional benefits associated with the consumption of tailor-made farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*), fortified with macroalgae and Se-yeast enriched diets.

Material and methods

Three diets (1 control and 2 supplemented with different blends of I-rich macroalgae and Se-yeast) were tested for 3 months in each fish species, simulating a finishing diet period. At the end of the trial, fish were slaughtered following the typical commercial practices and fillets were collected without skin: one fillet was immediately stored ("raw sample") and the second fillet was steamed at 105 ºC for 15 minutes prior to storage ("cooked sample"). Iodine, Se and arsenic (As) contents were determined by inductively coupled plasma mass spectrometer, whereas macro and trace elements were quantified by micro-Energy Dispersive X-Ray Fluorescence (Barbosa et al., 2020).

Results and Conclusions

Results showed that biofortification strategies through the incorporation of I-rich seaweed and Se-yeast in seabream and carp diets can improve the nutritional quality, enhancing I, Se and Fe levels. Yet, whereas fortified carp fillets presented higher content of I (over 100% increase), fortified seabream fillets presented higher content of Se (over 90% increase) compared to non-fortified ones. Steaming increased I and Se contents in fortified seabream fish fillets and decreased Cl, Fe and Ca contents. Elements True Retention (TR) values ranged from 60% (Ca) to 134% (I) and from 65% (Ca) to 125% (I) in fortified and non-fortified seabream fillets, respectively. On the other hand, steaming increased Fe and Zn contents in fortified carp fillets but decreased K, Ca and As contents. TRs values ranged from 59% (As) to 128% (Fe) and from 64% (Ca) to 117% (Fe) in fortified and non-fortified carp fillets, respectively. Interestingly, higher TR was observed for As in non-fortified fillets (91% in seabream and 72% in carp), compared to fortified fillets (87-88% in seabream and 59-62% in carp). In terms of nutritional benefits, the consumption of 150 g of steamed fortified seabream provided higher

(Continued on next page)

contributions to the daily Adequate Intakes (AI) set for I (12%, 9% and 20% for adults, pregnant woman and children, respectively), Se (85%, 70% and over 100% for adults, pregnant woman and children, respectively) and Fe (over 100% for all groups). Similarly, steamed fortified carp provided increased contributions to the daily AI set for I (21%, 16% and 35% for adults, pregnant woman and children, respectively), Se (30%, 24% and over 100% for adults, pregnant woman and children, respectively), Fe (over 100% for all groups) and Zn (38%, 28% and 66% for adults, pregnant woman and children, respectively). It is worth mentioning that the consumption of fortified fillets still yielded Se and Fe intakes below the Tolerable Upper Intakes (Se: up to 99% UL in seabream and up to 34% UL in carp; Fe: up to 12% UL in seabream and 18% UL in carp). These findings highlight the benefits of developing eco-innovative fortified farmed fish, through sustainable, safe, and cost-effective feeds, as well as the benefits of steaming as a suitable cooking procedure for fish healthy human consumption.

Acknowledgements

This work was supported by the SEAFOODTOMORROW project from the European Union's Horizon 2020 research and innovation programme (GA no. 773400). V. Barbosa would like to acknowledge the financial support of NEPTUNUS project (EAPA_576/2018) under the Interreg Atlantic Area. This output reflects only the author's view, and the European Union cannot be held responsible for any use that may be made of the information contained therein.

References

Barbosa, V., Maulvault, A.L., Anacleto, P., et al., 2020. Enriched feeds with iodine and selenium from natural and sustainable sources to modulate farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) fillets elemental nutritional value. Food and Chem. Toxicology, 140, 111330. doi: 10.1016/j.fct.2020.111330

EFSA Scientific Committee, 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. EFSA Journal 2015:13(1):3982, 36 pp. doi:10.2903/j.efsa.2015.3982

Pinkaew, S., Karrila, T.T., 2015. Key properties of iodine-, iron- and zinc- fortified fish cracker: effects of ambient shelf storage on iodine retention and quality indicators. Int. J. Fam. Ther. 50, 1979–1987. doi:10.1111/ijfs.12871

Tocher, D.R., 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture, 449, 94-107. doi:10.1016/j.aquaculture.2015.01.010

A MULTI-USE OFFSHORE PLATFORM FOR CLEAN ENERGY AQUACULTURE

F. Robinson*[a], O.Georges[a], T. Bardócz[a], G.Dalton[b], K. Leyne[b]

[a] AquaBioTech Ltd, Central Complex, Naggar Street, Targa Gap, Mosta, Malta
Email: omg@aquabt.com
[b] University College Cork, Haulbowline Rd, Ringaskiddy, County Cork, Ireland

Multi-use platforms (MUP) combine offshore clean energy such as wind, wave, and solar energy conversion. It combines different activities within a specific marine area.

The MUSICA project demonstrates the viability of an MUP enabling infrastructure for multiple renewable energy, desalination, and Blue Growth aquaculture services for small islands, that can share the same space and work synergistically together, sharing supply chains. It reduces operating and maintenance costs and solves increasing demand for space.

Using part of the energy on-site, the MUP will provide green support services for the island's aquaculture production, provide up to 61% renewable energy power to Oinousses Island (Greece), and provide 1000m^3 desalinated water.

AquaBioTech is the consultancy partner in the project supporting design, installation, and testing of the aquaculture component of the MUP. Performance trials will be carried out to measure and evaluate structural performance of the cage and interactions with the MUP. The project will assess environmental data along with data gathered of the quantity and quality of the fish produced. This information will allow for the assessment of the economic benefit of the structure to the local aquaculture production potential and inform the development of offshore farming that can be integrated in to future MUPs.

The overall Aim of MUSICA is to accelerate the roadmap to commercialization of its Multi-Use Platform (MUP) and Multi-Use of Space (MUS) combination for the small island market, and de-risk for future operators and investors, by validation to TRL7 and providing real plans to move to mass market commercialization. The MUSICA solution will be a decarbonizing one-stop shop for small islands, including their marine initiatives and ecosystems.

Acknowledgement
The MUSICA project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 862252.

INNOVATIVE TECHNOLOGIES FOR REAL-TIME MONITORING AND HIGH-PRECISION DETECTION OF WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS - AQUADETECTOR PROJECT

Dannie O'Brien*[a], Steven Prescott[a], Tamás Bardócz[a], Freya Robinson[a], Joseph De Prisco[a], Yang Wang[b], Rhan Zhao[b], Wensheng Li[c].

[a] AquaBioTech Ltd, Central Complex, Naggar Street, Targa Gap, Mosta, Malta
Email: ddo@aquabt.com
[b] China Agricultural University, College of Information and Electrical Engineering, National Innovation Center for Digital Fishery
[c] Laizhou Mingbo Aquatic Products Co., Ltd., Sanshandao Street office, Laizhou City, Shandong Province, China

Aquaculture is the fastest growing food production sector and China has led this growth, accounting for 57.9 percent of the global share (FAO, 2020). European production, while smaller, is developing rapidly in areas of sustainability and automation innovation. There are significant skills and knowledge to be shared between the two markets, particularly in the growing sector of land-based recirculating aquaculture systems (RAS), which provide significant benefits including total control of the growth environment, low water requirement, mitigation of disease and parasite risk, and reduced distance to market, but which also pose unique technological challenges.

Water in RAS is continually reused and fish are entirely dependent on producer management for high water quality. Identifying the most important water quality indicators, their complex interactions, how to monitor them and mitigate dangerous conditions, is the priority of the AquaDetector China-Malta collaborative project. Optimising water quality for juvenile fish, which are sensitive to variations, will ensure the optimal survival, growth, and welfare of juvenile trout and grouper in RAS via visualisation of water quality distribution using validated CFD modelling.

The project is developing precise detection technologies to classify fish stress behaviours including hunger and cannibalism through video image detection and monitoring to ensure fish quality, productivity, and animal welfare in the RAS environment. Automation and precision observation will promote a shift in aquaculture production from highly labour dependent to highly technically oriented through enabling technologies including high-precision sensors and automated detection systems. These innovations will further progress aquaculture in both China and in Malta and promote collaboration between experts in the two countries for the improvement of smart and sustainable land-based fish cultivation.

Acknowledgement
Project AquaDetector funded by the Malta Council for Science and Technology through the Sino-Malta Fund 2019 (Science and Technology Cooperation). Grant agreement number: SINO-MALTA-2019-11 and financially supported by Science and Technology Cooperation – Sino-Malta Fund 2019: Research and Demonstration of Real-time Accurate Monitoring System for Juvenile Fish in Recirculating Aquaculture System (AquaDetector, Grant No. 2019YFE0103700), Ministry of Science and Technology, China.

SEA2LAND: MEDITERRANEAN INDUSTRY CASE STUDY ON TECHNOLOGY ADAPTATION FOR THE COLLECTION, CONCENTRATION, AND REUSE OF AQUACULTURE WASTE STREAMS FOR USE IN AGRICULTURE PRODUCTION

Tamás Bardócz, Freya M. Robinson, Joseph De Prisco, Dannie O'Brien, Michele Gallo

AquaBioTech Ltd (Trading as AquaBioTech Group), Central Complex, Naggar Street, Targa Gap, Mosta, Malta
Email: thb@aquabt.com

Based on the circular economy model, Horizon2020 SEA2LAND project promotes the production of large-scale fertilisers in the EU through value added raw materials derived from aquaculture and fish processing waste streams. This solution is expected to reduce the soil nutrient imbalance in Europe and reduce dependency on imported fertiliser products. The basis of the project is the regional production of biobased fertilisers, developing demonstration pilots that can be replicated throughout Europe, boosting local growth. Technologies ranging from well-known processes (bokashi, composting, etc.) to more sophisticated such as thermo-mechanical fractionation and enzymatic hydrolysis are being used to process challenging marine waste streams in to customized and locally specific fertilizer products. For the Mediterranean Case Study, Freeze Concentration (FC), the concentration of materials using freezing of water rather than evaporation and BioDrying, using metabolic heat to remove water from the waste matrix at the lowest possible residence time and minimal carbon biodegradation, are the main experimental methodologies to be tested.

Recirculating Aquaculture System (RAS) production, as an intensive and enclosed land-based method, provides a unique opportunity to collect and concentrate fish waste 'sludge- faeces and uneaten feed- for use in creating these new agricultural products, but high solids concentrations are required. For producers, technological adaptations to ease collection, processing, and concentration of these wastes are needed to facilitate their use as a value added product are the most important areas of research. Local waste valorisation networks will remove the burden on producers to dispose of their wastes and harness an underutilized source of nutrients.

Acknowledgement
The Sea2Land project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Gran Agreement NO 101000402.

This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

TIME-COURSE RNA-SEQ ANALYSIS OF BRAIN AND HEAD KIDNEY RESPONSE TO NERVOUS NECROSIS VIRUS INFECTION IN THE EUROPEAN SEA BASS

L. Peruzza[1], F. Pascoli[2], S. Ferraresso[1], R. Franch[1], M. Babbucci[1], G. Dalla Rovere[1], M. Freguglia[3], R. Menegatti[3], L. Biasini[2], A. Toffan[2], D. Bertotto[1], L. Bargelloni[1*]

[1] Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16 35020 Legnaro (PD) – Italy
[2] Division of Comparative Biomedical Sciences, OIE Reference Centre for viral encephalopathy and retinopathy, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padua, Italy
3 Valle Ca' Zuliani Soc. Agr. Srl, 45018 Porto Tolle, Italy
Email: luca.bargelloni@unipd.it

Introduction

Nervous Necrosis Virus (NNV) is one of the major viral pathogens in aquaculture, affecting a wide range of fish species and causing high mortality rates. The European sea bass (*Dicentrarchus labrax*) aquaculture production has long been affected, with significant economic losses, especially in the Mediterranean region. Selective breeding for increased resistance to NNV might help, together with other approaches (e.g. vaccination), to prevent such devastating disease. One of the major goals of the EU-funded project AQUAFAANG is the functional genomic characterization of important traits, which in turn might improve the accuracy of breeding value prediction. Here we present a detailed time-course RNA analysis on five time points and two tissues in juvenile sea bass, which is part of an integrated study to understand the genetic basis of response to NNV in this species.

Materials and methods

The experimental fish were generated at a commercial hatchery Valle Ca' Zuliani. Unselected juvenile fish (weight 6-20gr) were transferred to the IZSVe facility. After acclimation and quarantine, half of the animals were infected by intramuscular inoculum of 0,1ml RGNNV strain 283.2009 batch 5/19 at titre $10^{6.30}$ TCiD50/ml. The remaining fish were injected with DMEM. All fish were anesthetised before injection with MS222. Four fish per time point per group (NNV-infected, mock-infected) were sampled, euthanized, and brain and head kidney samples were dissected and stored for RNA analysis. Tissue sampling was carried out at 6h, 12h, 24h, 48h, and 72h post-inoculum. Total RNA was extracted from all samples, quality assess using Agilent 2100 Bioanalyzer, and only RNAs with RIN>7 were processed. cDNA libraries were constructed using QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina and sequenced on a HighSeq 4000 with a single 1 x 100 bp setup. High-quality trimmed reads were mapped on the European sea bass genome, (seabass_V1.0 GCA_000689215.1 in Ensembl). Normalisation was performed using the function RUVs (within the package RUVSeq/v1.18. Normalised counts were imported in edgeR/v3.26.8 to perform differential expression analysis in pairwise comparisons (time-matched mock-infected vs NNV-infected). Genes with an FDR adjusted p-value < 0.05 and FC>±1.5 were considered as differentially expressed (DEGs). Putative zebrafish orthologues to sea bass genes were obtained from Ensembl. Annotations from zebrafish orthologues were used for functional enrichment analysis in DAVID.

Results

Transcriptomic response to NNV in both tissues was significant already at 6h post-inoculum and became massive at 48h and 72h. The number of up- and down-regulated genes in each tissue is reported in Table 1.

While the transcriptome response to NNV was quite complex, functional enrichment analysis showed a few general trends. Gene Ontology (GO) terms and KEGG pathways related to immune response (e.g. GO:0051607~defense response to virus, dre04622:RIG-I-like receptor signaling pathway) and proteolysis/proteasome (e.g. GO:0006508~proteolysis, dre03050:Proteasome) were significantly enriched (FDR<0.05) in up-regulated genes at 24h, 48h, 72h in both tissues. Down-regulated genes in the brain were enriched in GO terms and KEGG pathways related to vision (e.g. GO:0007601~visual perception, dre04744:Phototransduction) as early as 12h after infection. In the head kidney, genes involved in glycolysis were significantly under-expressed in infected fish at 6h, while protein translation related genes (e.g. ribosomal genes) later on. The most relevant result was the significant involvement of different families of heat shock proteins (HSP40, HSP70, HSP90) in the host response to virus with up to 22 HSPs being differentially expressed. Of particular interest are HSP90ab1, HSP90b1, and HSP90aa1, which showed a complex, tissue-specific pattern being up- and down-regulated at different time-points.

(Continued on next page)

Table 1 DEGs in brain and head kidney (FC>±1.5, FDR<0.05)

	6h post-challenge	12h	24h	48h	72h
Brain up-regulated	99	37	19	773	1310
Brain down	2	25	32	479	1088
Head kidney up	29	26	64	228	401
Head kidney down	24	10	17	55	386

Discussion

Detailed time-course analysis of the brain and head kidney transcriptome revealed a vast, complex response, providing a large set of functionally relevant genes to inform genomic prediction of NNV-resistance in the European sea bass. Of particular interest are HSP90s as HSPs are known to have a key role in host-virus interactions (Wan et al. 2020) and HSP90ab1 has been reported to be essential for RGNNV entry into target cells in marine medaka (Zhang et al. 2020).

References

Wan Q, Song D, Li H, He ML. Stress proteins: the biological functions in virus infection, present and challenges for target-based antiviral drug development. Signal Transduct Target Ther. 2020 Jul 13;5(1):125.

Zhang W, Jia K, Jia P, Xiang Y, Lu X, Liu W, Yi M. Marine medaka heat shock protein 90ab1 is a receptor for red-spotted grouper nervous necrosis virus and promotes virus internalization through clathrin-mediated endocytosis. PLoS Pathog. 2020 Jul 8;16(7):e1008668.

Salicornia ramosissima BIOMASS INCORPORATION IN DIETS FOR JUVENILE WHITELEG SHRIMP (*Penaeus vannamei*): EFFECTS ON GROWTH PERFOMANCE, SURVIVAL AND NUTRIENT DIGESTIBILITY

A. Barreto*[1], A. Laranjeira[1], F. Cruz[2], A. Couto[2], J. Dias[3], B. Costas[2], R.J.M. Rocha[1]

[1]RIASEARCH Unipessoal Lda, Murtosa (Portugal)
[2]CIIMAR, Matosinhos (Portugal)
[3]SPAROS, Lda. Olhão (Portugal)

*Email: andrebarreto@riasearch.pt

Introduction

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 cultivation areas. Additionally, these are known for their ability to synthetize high concentrations of bioactive secondary metabolites which can have health promoting effects for the consumer. However, the green tips of Salicornia are the only part sold as food, while the remaining the plant is considered a residue, making the valorization of the latter crucial for a more sustainable and profitable production cycle. The principles of circular economy, where waste is recovered and utilized to create added value, are increasingly more important and being applied to economical relevant areas, including the aquaculture industry. The whiteleg shrimp (*Penaeus vannamei*) is the most produced crustacean species worldwide, making it a highly valued commercial product whose demand has substantially increased in recent years. However, feed associated costs are significant, making the creation 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 growth performance, survival and nutrient digestibility were assessed.

Methods

Five experimental diets were tested in quintuplicates: a commercial like diet (Control), two experimental diets containing *S. ramosissima* stems at 5% and 10% inclusion levels (SL5 and SL10, respectively) and two containing *S. ramosissima* leaves and seeds at 5% and 10% inclusion levels (SS5 and SS10, respectively). 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. Additionally, shrimp feces samples were collected after the feeding period and analyzed to determine the apparent digestibility coefficients of the experimental diets.

Table 1. 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 days.

	Control	SS5	SS10	SL5	SL10
Initial weight (g)			6.1 ± 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
RGR (% day^{-1})	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0
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}
Feed intake (% ABW day^{-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}
Survival (%)	96.7 ± 1.5	97.1 ± 2.4	98.2 ± 2.6	96.0 ± 2.7	97.8 ± 1.5

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.

Table 2. Apparent digestibility coefficients (ADC) of the experimental diets.

	Control	SS5	SS10	SL5	SL10
Dry matter[1]	58.2 ^{bc}	51.8 ^{b}	65.3 ^{bc}	51.0 ^{ab}	40.4 ^{a}
Protein[2]	81.5 ^{a}	81.3 ^{a}	87.0 ^{b}	81.5 ^{a}	82.6 ^{a}
Lipids[2]	83.6 ^{bc}	82.8 ^{bc}	86.2 ^{c}	81.1 ^{b}	74.9 ^{a}
Energy[2]	77.4 ^{bc}	73.5 ^{bc}	80.0 ^{c}	71.8 ^{b}	62.7 ^{a}

(Continued on next page)

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, 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 diet which produced significantly higher FCR and feed intake values than the remaining diets (Table 1). These results can be explained by the significantly lower apparent digestibility coefficients of dry matter, lipids and energy observed for SL10 when compared to the remaining diets (Table 2).

Conclusion

Data from this study indicate that *S. ramosissima* biomass can be included in diets for juvenile *P. vannamei* with no detrimental effects on growth performance or survival. The inclusion of Salicornia steams in whiteleg shrimp diets 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.

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No. 86283 (project AQUACOMBINE). This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein. BC was supported by FCT - Foundation for Science and Technology (IF/00197/2015).

MICRODIET FORMULATION IMPACTS ON *Penaeus vannamei* POST-LARVAE SURVIVAL AND FEED CONVERSION RATIO

D. Martins[1,3], A. Barreto[1]*, W. Pinto[2], M. Pacheco[3,4], R.J.M. Rocha[1,3,4], L. Conceição[2]

[1]RIASEARCH Unipessoal Lda, Murtosa (Portugal)
[2]SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-224, Olhão (Portugal)
[3]Departamento de Biologia, Universidade de Aveiro (Portugal)
[4]CESAM, Universidade de Aveiro (Portugal)
*Email: andrebarreto@riasearch.pt

Introduction

Shrimp aquaculture is considered one of the most profitable aquaculture industries. The increase in *Penaeus vannamei* production contributed to make this product available and accessible to a large number of consumers on a global scale. In order to respond to this increase in production, hatcheries need to intensify and improve post-larvae (PL) rearing. In the early stages of development, the use of diets with high protein levels are preferred and fishmeal and soybean have been used as the main source of this nutrient (Xie et al., 2016). However, these stages of production are often associated with non-optimal growth and low survival rates, which may be at least partially related to problems in nutrition. This indicates that there is room for improvement in this field and that new inert diets may play an important role in improving this production phase. In this context, innovative microdiets for *P. vannamei* PL were tested and their effects in shrimp survival and growth performance assessed.

Methods

Six experimental microdiets (52P, FISH, SQUID, KRILL, FISHHF and NBIND), using different marine ingredients as the main constituent, were tested in triplicates. The 52P, a commercial like diet with 52% protein was used as a control; FISH, SQUID and KRILL were formulated with fish, squid and krill meal as their main ingredients, respectively; FISHHF was formulated with fish meal as their main ingredient but with higher lipid levels; and NBIND differed from the commercial control by the type of binders used. White-leg shrimp PL (mean wet weight 13 mg) were kept at around 28 ºC and fed *ad libitum* for 21 days. At the end of the trial, shrimp PL growth performance, feed conversion ratio and survival were assessed.

Results and discussion

No significant differences between treatments were observed regarding final body weight (FBW) (Figure 1). The overall FCR values were low (≤ 1). The exception was FISHHF, which had significantly higher values than KRILL (FISHHF: 1.2 ± 0.4; KRILL: 0.6 ± 0.0). Survival was significantly higher in the 52P and KRILL treatments than in FISHHF, with no significant differences between the remaining treatments (Figure 2).

Clear benefits in shrimp PL growth performance and survival were observed when utilizing specific marine ingredients in the formulation of microdiets, namely, squid and krill proved to be good sources of protein for the development of shrimp. Increasing lipid levels did not benefit shrimp development (FISH vs FISHHF). Amongst all diets tested, the 52P, KRILL and SQUID seem to be the most adequate for this stage of shrimp development.

(Continued on next page)

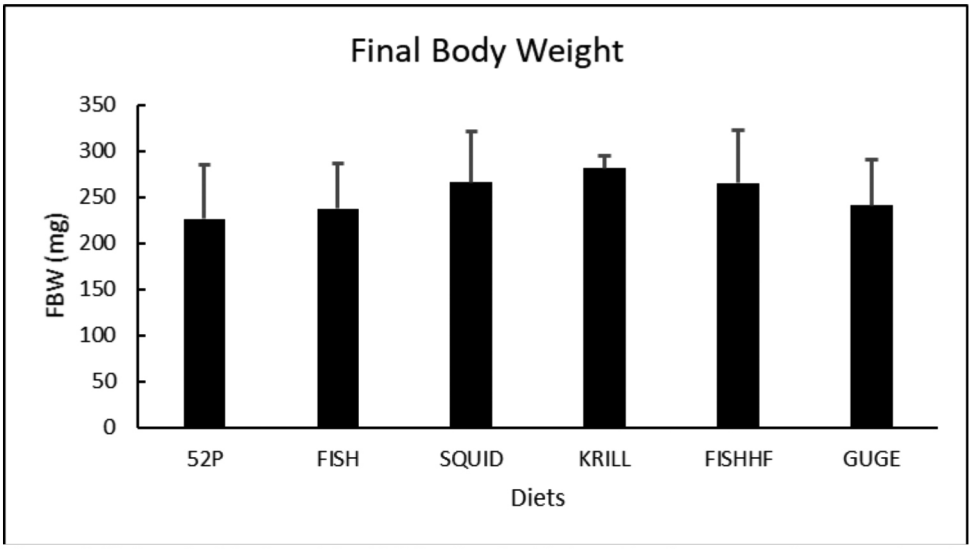


Figure 1 Shrimp final body weight (FBW) after the 21 days. Results expressed as mean (n = 3 experimental units).

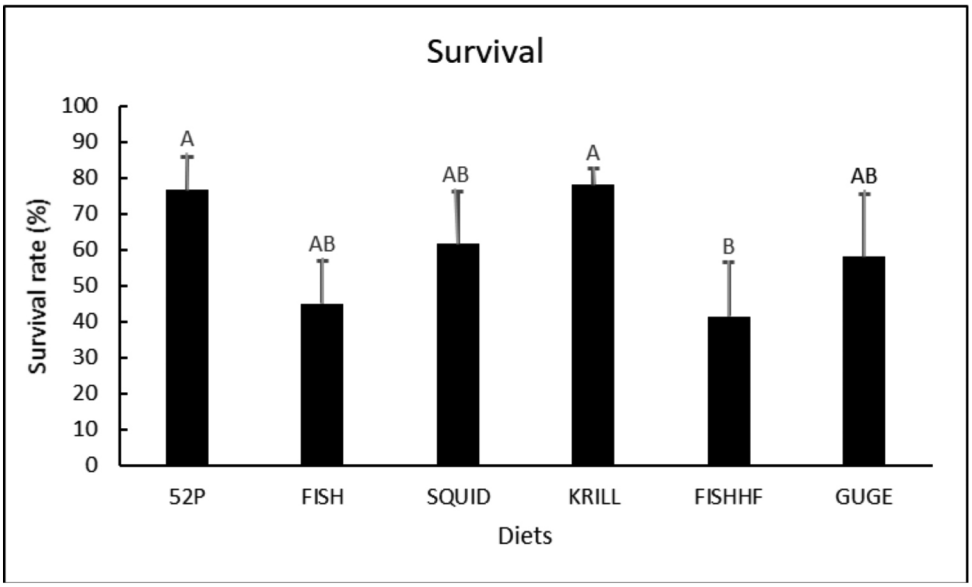


Figure 2 Shrimp survival after the 21 days. Results expressed as mean (n = 3 experimental units). Different letters indicate statistically significant differences between groups ($p < 0.05$).

References

Xie, S. wei, Liu, Y. jian, Zeng, S., Niu, J., & Tian, L. xia. (2016). Partial replacement of fish-meal by soy protein concentrate and soybean meal based protein blend for juvenile Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 464(July), 296– 302.

Acknowledgment

We would like to thank Adriana Laranjeira and Bruna Silva from RIASEARCH, and SPAROS pilot-plant for aquafeeds team.

This work is part of the project FA_05_2017_005 SHELLWIN, financed by the Blue Fund program of the Ministry of the Sea, Portuguese Republic.

QUANTIFICATION OF FEED INTAKE IN ATLANTIC SALMON (*Salmo salar*) JUVENILES WITH THE INRA'S AUTOMATED FECES COLLECTION SYSTEM

F. Barros*[1]; A. Santos[2], J. Couto[2], J. Dias[2], P. Rema[1]

[1] Universidade de Trás os Montes e Alto Douro, Vila Real, Portugal
[2] SPAROS Lda., Olhão, Portugal
Email: filipabarros0812@gmail.com

Introduction

Fish feeding behavior is a complex topic, since it is affected by several sensory systems comprising the detection of a feed item (vision, olfaction, acoustic, lateral line organ, and electroreception) up to its swallowing (taste, mechanoreception). Palatability and taste are terms often used interchangeably which are determined mostly by the chemical characteristics of the feed, although criteria such as the physical pellet properties can also affect the acceptability and final ingestion.

Over the last decades, the aquafeed industry has achieved a significant reduction on the incorporation levels of traditional marine-harvested resources (fishmeal and fish oil) and a concomitant increase on the use of oilseed-derived raw materials. In a scenario of low incorporation levels of fishmeal, marine protein hydrolysates or other phagostimulants have been shown to improve the palatability and feed intake, that often result in an enhancement of growth performance and overall status of fish and shrimp. Therefore, a reliable estimation of feed intake is a relevant criterion in fish nutrition.

Various methods are used to quantify the voluntary feed intake in fish. These are generally based on direct observation, the recording of feeding activity using on-demand feeders, the quantitative determination of gastrointestinal content using a non-invasive labelled feeds with isotopes or x-ray opaque particles and the measurement of waste feed. This last approach is relatively easy to implement provided that feed pellets present good physical stability after immersion. A study was undertaken to assess the use of INRA'S automated feces collection system, originally developed for digestibility studies as an experimental tool to quantify feed intake in Atlantic salmon (*Salmo salar*) juveniles.

Methods

The trial comprised 3 dietary treatments. To guarantee a high feed intake, a formulation containing high levels (30%) of fishmeal and krill meal, moderate levels of plant protein sources (wheat gluten, pea protein concentrate, soy protein concentrate) and wheat was used as positive control (diet PC). On the opposite, to attain a low feed intake scenario, a diet with moderate levels of fishmeal (5%), devoid of krill meal and with a very high level (41.5%) of soy protein concentrate, while maintaining the other ingredients constant served as the negative control (diet NC). The additional diet was based on the NC basal formula, but contained 5% of a marine protein hydrolysate (diet HYDRO). Diets were extruded and were isonitrogenous (45% CP), isolipidic (19.5% CF) and isoenergetic (21.6 MJ/kg).

Six homogenous groups of 20 fish with a mean body weight (BW) of 29 ± 3 g were randomly allotted to cylindroconical tanks (volume: 60 L). The tanks were equipped with the automated INRA feces collection system (Figure 1). In this open-flow system, the tank outlet water is sieved by moving grids, and all suspended residues (e.g. feces, pellets) are separated from the water and projected into a metallic tray. Although originally developed for feces collection, the system allows a full recovery of any uneaten pellets (Figure 1).

Experimental tanks were located outdoors, supplied with flow-through freshwater (flow rate: 4.2 L/min; temperature 10.6 ± 0.5°C, dissolved oxygen above 9.0 mg/L). Fish were fed, twice a day (10.00 and 15.00h) and the amount of feed distributed quantified on a daily basis. For approximately 20 minutes after each meal, the uneaten feed pellets were recovered with the automated INRA feces collection system. Every day, uneaten pellets from each tank were dried overnight in a convention oven and afterwards quantified in a dry basis. This procedure was repeated for a period of 15 consecutive days.

$$\text{Feed intake, \% biomass} = 100 \times \frac{\text{Distributed dry feed - Uneaten dry feed}}{\text{Fish biomass}}$$

(Continued on next page)

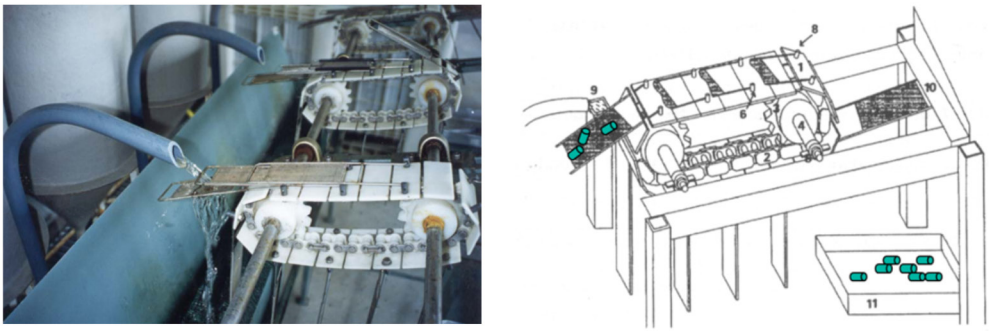


Figure 1. INRA feces collection system used for collection of uneaten pellets.

Fish fed the PC diet showed a significantly higher feed intake than those fed the NC and HYDRO diets ($P<0.05$). Moreover, fish fed the HYDRO diet showed a significantly higher feed intake than those fed the NC diet ($P<0.05$).

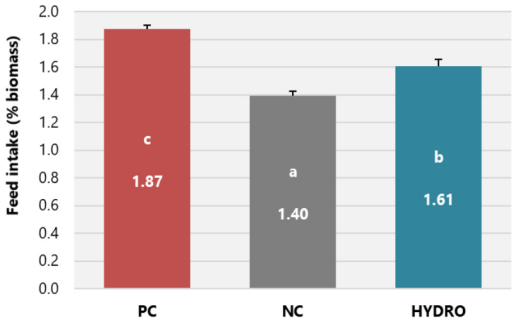


Figure 2. Feed intake (% biomass)

Results and discussion

The average feed intake measured throughout the experimental period varied between 1.40 and 1.87 % of biomass, with statistical differences among the various treatments (Figure 2).

Conclusions

Data from this study indicates that the INRA's automated feces collection system is a suitable methodological tool to recover excess feed and allows a precise measurement of feed intake in juvenile fish. The low intra-treatment variability allows the discrimination of small changes on feed intake between treatments. This methodology has also been successfully used to screen and identify various feed additives with a phagostimulatory potential.

Acknowledgements

This work is part of project 47175_FICA, supported by Portugal and the European Union through FEDER/ERDF, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

THE POTENTIAL OF SEA BASS *Dicentrarchus labrax* DERIVED HEPCIDINS IN THE TREATMENT OF IRON DISORDERS AND BACTERIAL INFECTIONS

C. Barroso[1,2,3*], P. Carvalho[4], J. F. M. Gonçalves[4,6], P. N. S. Rodrigues[1,2,4] and J. V. Neves[1,2,4]

[1] i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[2] Iron and Innate Immunity Group, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[3] Programa Doutoral em Biologia Molecular e Celular (MCBiology), ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[4] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[5] CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal
E-mail: carolina.barroso@i3s.up.pt

Introduction

Reducing the use of antibiotics in aquaculture is one of the biggest challenges of this sector. Antimicrobial peptides (AMPs) are considered promising alternatives to these drugs, but studies evaluating the potential use of AMPs in the production of sea bass are still missing, with only one report addressing the effects of synthetic hepcidins in infected sea bass (Álvarez et al, 2016). Most mammals present a single hepcidin gene, with limited antimicrobial activity and a major function in the regulation of iron metabolism, by inhibiting the iron exporter ferroportin (Nemeth et al, 2004). However, many fish, including the European sea bass (*Dicentrarchus labrax*), present two hepcidin types that have subfunctionalized, with the single type 1 hepcidin regulating systemic body iron, and the various type 2 hepcidins having an almost exclusively antimicrobial role (Neves et al, 2015). As such, in this study we evaluated the potential of bass derived hepcidins to treat or prevent iron disorders and infectious diseases, by administering them to animals subjected to various experimental models of infection or iron modulation.

Material and Methods

Several experimental models were performed to test if sea bass hepcidins can be effectively used to treat/prevent iron disorders or bacterial infections: 1) peptide administration - intraperitoneally (i.p) administered 100 µl of a 50 µM solution of hamp1 (QSHLSLCRWCCNCCRGNKGCGFCCKF) or hamp2 (HSSPGGCRFCCNCCPN-MSGCGVCCRF) to healthy sea bass; 2) iron overload – i.p. administered 100 µl (5 mg) of iron dextran; 3) iron overload+peptide – iron overload followed 24 hours later by peptide administration, as before; 4) infection – i.p. infected with 10^5 CFU of *Photobacterium damselae* spp *damselae* PP3; 5) infection+peptide – infection followed 24 hours later by peptide administration, as before; 6) peptide+infection – peptide administration followed 24 hours later by infection, as before. At pre-determined time points, animals were terminally sampled, and blood, serum and tissues collected, for determination of haematological and serological parameters, tissue iron content, CFU counts and gene expression analysis.

Results

We observed that administration of hamp1 can have a significant impact on several iron related parameters, when administered to healthy animals. This leads to a condition of anemia, which might be connected to the slight increase in mortality observed when hamp1 is also administered to infected animals, making them more susceptible. However, when administered to iron overloaded animals, it seems to limit the severity of its effects. Hamp2 on the other hand has shown no significant impact on iron metabolism, and had no discernible effects during iron overload. But when administered to infected animals, either before or after infection, reduced mortality very significantly (from around 55% to between 6-17%), decreased bacterial loads and reduced the impact on several iron related parameters, in particular limiting the development of anemia of inflammation.

(Continued on next page)

Discussion and conclusions

Our findings show that when hamp1 is administered to healthy animals interferes with the iron metabolism, leading to significant anemia. Administration during infection leads to an even more severe anemia of inflammation, likely contributing to the increased mortality. However, hamp1 seems to attenuate the effects of iron overload, and as such might have applications in the treatment of iron disorders. Hamp2 on the other hand presents itself as a viable alternative to the use of other prophylactic or therapeutic substances, in particular antibiotics, since it is a bioactive molecule that does not interfere with iron metabolism and is very effective in controlling bacterial infections. Additionally, the effects of hamp2 are likely not limited to a direct antimicrobial effect, but also by stimulation of the overall inflammatory response, but further studies are needed to address this. Nevertheless, costs of production of antimicrobial peptides still represent a significant barrier to their more generalized use.

Acknowledgments

This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), and by individual funding from the Portuguese Foundation for Science and Technology (FCT) through SFRH/BD/114899/2016 (CB).

References

Álvarez, C.A., Acosta, F., Montero, D., Guzmán, F., Torres, E., Vega, B., Mercado, L., 2016. Synthetic hepcidin from fish: Uptake and protection against *Vibrio anguillarum* in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol. 55, 662–670.

Neves, J. V, Caldas, C., Vieira, I., Ramos, M.F., S Rodrigues, P.N., 2015. Multiple Hepcidins in a Teleost Fish, *Dicentrarchus labrax*: Different Hepcidins for Different Roles. J. Immunol. 195, 2696–2709.

Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Kaplan, J., Ganz, T., 2004. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. Science 306, 2090–2093.

IDENTIFICATION AND CHARACTERZATION OF TWO BETA-DEFENSINS IN THE EUROPEAN SEA BASS *Dicentrarchus labrax*

C. Barroso[1,2,3*], P. Carvalho[4], J. F. M. Gonçalves[4], P. N. S. Rodrigues[1,2,4] and J. V. Neves[1,2,4]

[1] i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[2] Iron and Innate Immunity Group, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[3] Programa Doutoral em Biologia Molecular e Celular (MCBiology), ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[4] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
E-mail: carolina.barroso@i3s.up.pt

Introduction
Antimicrobial peptides (AMPs) are considered as promising alternatives to the use of antibiotics in aquaculture, due to their antimicrobial and immunomodulatory properties. In the European sea bass (*Dicentrarchus labrax*), a fish species with a high commercial value, only hepcidins and piscidins have been studied (Neves et al, 2015; Barroso et al, 2020). However, the characterization of other AMPs in sea bass is still missing. In this study, we identify two different beta-defensins in the European sea bass (Barroso et al, 2021). Defensin sequences were isolated from sea bass tissues and characterized, using phylogenetic and syntenic studies. The putative amino acid sequences were determined, as well as the tertiary structure of each defensin. Further studies are necessary to understand the functions of sea bass beta-defensins during infection.

Material and Methods
Healthy sea bass (*D. labrax*) with an average weight of 30 g, were kept at optimal conditions and used to collect samples for gene isolation and basal expression. Primers were designed according to conserved regions of beta-defensin mRNA sequences of other fish species and from sea bass expressed sequence tags (ESTs) and whole genome shotgun sequences (WGSS) available in the National Center for Biotechnology Information (NCBI) and Ensembl databases. The coding and genomic DNA products were sequenced and the putative amino acid sequences were determined. The tertiary structure of each sea bass beta-defensin was determined, using models available in the SWISS-MODEL server (https://swissmodel.expasy.org/). A phylogenetic and syntenic analysis of fish defensins was performed, as well as a comparison between sea bass defensins and other vertebrate peptides. The basal expression of each defensin was also evaluated, using several tissues collected from fish.

Results
We isolated in sea bass two different beta-defensins. Both genes consist in a three exon/two intron structure, similar to what is observed for other fish defensins. The tertiary structure consists in three antiparallel beta-sheets, with beta-defensin 1 presenting an extra alpha-helix at the N-terminal. Phylogenetic and syntenic analysis place sea bass defensins in the subfamilies 1 and 2, with other fish species, particularly Cyprinids and Salmonids, presenting additional defensins, separated in different branches. Both defensins are highly expressed in tissues including the spleen, head kidney or gill, with the lowest basal expression in the liver and brain.

Discussion and conclusions
Defensins are the most studied group of AMPs, with several fish and non-fish peptides being already characterized. However, an in depth study addressing the beta-defensin family in sea bass is still missing. Both sea bass defensins present similarities with other fish defensins, including their amino acid sequences and tertiary structure, and are included in the subfamilies 1 and 2. Other fishes, particularly Salmonid and Cyprinids, present several defensins, suggesting a diversification of this group, while in sea bass, hepcidins and piscidins become much more diversified. Further studies are necessary to understand their antimicrobial and immunomodulatory functions, particularly the chemotactic activity towards different cells. These peptides might be helpful in the development of novel prophylactic or therapeutic substances to be used in the production of sea bass.

(Continued on next page)

Acknowledgments
This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF), and by national Funds under the project UIDB/04293/2020 and individual funding SFRH/BD/114899/2016 (CB), from the Portuguese Foundation for Science and Technology (FCT, *Fundação para a Ciência e a Tecnologia*).

References

Neves J.V., Caldas C., Vieira I., Ramos M.F., Rodrigues P.N.S., 2015. Multiple Hepcidins in a Teleost Fish, *Dicentrarchus labrax*: Different Hepcidins for Different Roles. The Journal of Immunology 195, 2696–2709.

Barroso C., Carvalho P., Carvalho C., Santarém N., Gonçalves J.F.M., Rodrigues P.N.S., Neves J.V., 2020. The Diverse Piscidin Repertoire of the European Sea Bass (*Dicentrarchus labrax*): Molecular Characterization and Antimicrobial Activities. International Journal of Molecular Sciences 21 (13): 4613.

Barroso C., Carvalho P., Gonçalves J.F.M., Rodrigues P.N.S., Neves J.V., 2021. Antimicrobial Peptides: Identification of two Beta-Defensins in a Teleost Fish, the European Sea Bass (*Dicentrarchus labrax*). Pharmaceuticals 14 (6): 566.

CENTRAL REGULATION OF FOOD INTAKE IS NOT AFFECTED BY INCLUSION OF DEFATTED *Tenebrio molitor* LARVAE MEAL IN DIETS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*)

A. Basto [1, 2, 3 *], L.M.P. Valente [1, 2], M. Conde-Sieira [3], and J.L. Soengas [3]

[1] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2] ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Animal Physiology Laboratory, Department of Functional Biology and Health Sciences, Faculty of Biology and Marine Research Center, University of Vigo, E-36310 Vigo, Spain

[*] Presenting author: anafbasto@gmail.com

Introduction

Since the use of insect protein in aquafeeds was approved by European Union in 2017, its use as fishmeal (FM) alternative has been increasingly explored in several fish species. Despite several studies addressed the impact of insect meal-based diets in food intake of fish, there is no knowledge about their impact in mechanisms of food intake regulation. Understanding the underlying mechanisms responsible for feeding behavior in fish, namely stimulation (orexigenic) or inhibition (anorexigenic) of appetite, is pivotal for ensure the most adequate dietary formulations for aquaculture and consequently, 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). In this context, this study aimed to evaluate the impact of partial replacement of FM by defatted TM (*d*TM) on the expression of these neuropeptides in brain regions of European sea bass juveniles.

Material and methods

A FM-based diet with 48% crude protein on a dry matter basis was formulated and used as control (CTRL). Two other isonitrogenous and isoenergetic diets were formulated to replace 40 and 80% of FM by *d*TM (TM40 and TM80, respectively). Each diet was assigned to triplicate homogeneous groups of 25 fish (initial average body weight 55 ± 2 g) fed until apparent satiation for 10 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 24 ± 1 °C). Feed consumption was monitored during entire trial. At the end the trial, samples of plasma and different brain areas (hypothalamus and telencephalon) were collected at different post-prandial times (2, 6 and 24 hours after feeding) to evaluate circulating metabolites and mRNA relative abundance of neuropeptides involved in the regulation of food intake.

Results

All diets were equally accepted by fish, resulting in similar food intake and weight gain. Plasma cholesterol levels decreased 24 hours after feeding in fish fed TM40; fish fed *d*TM diets had higher cholesterol levels, 2 and 6 hours after feeding, than those fed CTRL diet. Increased non-esterified fatty acids (NEFA) levels occurred in plasma of fish fed TM80, regardless of the sampling time. At central level, no changes occurred in the mRNA abundance of neuropeptide Y (*npy*), agouti-related protein 2 (*agrp2*), pro-opio melanocortin a (*pomca*) or cocaine- and amphetamine-related transcript 2 (*cartpt2*).

Discussion and conclusion

The results obtained suggest that dietary replacement of FM by 50-80% of *d*TM for 10 weeks does not affect food intake and its homeostatic regulation in European sea bass at central level. Considering that the same experimental diets did not induce detrimental effects on nutrient digestibility, growth performance, and fillet nutritional value for human consumption (Basto et al., 2021), these results certainly will help to improve sustainability of diet formulation for this species using *d*TM meal in replacement of an important amount of FM. However, a short-term study is needed to fully characterize the response of mechanisms involved in food intake regulation immediately after exposure to diets rich in *d*TM.

Acknowledgements

This work was supported by research grants from the Spanish Agencia Estatal de Investigación and European Fund of Regional Development (PID2019-103969RB-C31) and Xunta de Galicia (Consolidación e estructuración de unidades de investigación competitivas do SUG, ED431B 2019/37) to JLS, and by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources, financed by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF) (NORTE-01-0145-FEDER-000040); the ANIMAL4AQUA Project, funded by Portugal 2020, financed by ERFD through COMPETE (POCI-01-0247-FEDER – 017610) to LMPV; AB was supported by Fundação para a Ciência e Tecnologia (FCT-Portugal) (SFRH/BD/138593/2018); Financial support from FCT provided to CIIMAR within the scope of UIDB/04423/2020 and UIDP/04423/2020 is also acknowledged.

References

Basto, A., Calduch, J., Oliveira, B., Petit, L., Sá, T., Maia, M.R.G., Cabral-Fonseca, S., Matos, E., Pérez-Sánchez, J., Valente, L.M.P., 2021. The use of defatted *Tenebrio molitor* larvae meal as a main protein source is supported in European sea bass (*Dicentrarchus labrax*) by data on growth performance, lipid metabolism and flesh quality. Frontiers in Physiology, 12, 659567.

Soengas, J.L., 2021. Integration of nutrient sensing in fish hypothalamus. Frontiers in Neuroscience 15, 653928.

Soengas, J.L., Cerda-Reverter, J.M., Delgado, M.J., 2018. Central regulation of food intake in fish: an evolutionary perspective. Journal of Molecular Endocrinology, 60:R171-R99.

DEFATTED *Tenebrio molitor* CAN FULLY REPLACE FISH MEAL IN DIETS FOR MARKET-SIZED EUROPEAN SEA BASS STILL ASSURING MUSCLE SENSORY ATTRIBUTES AND THE RECOMMENDED LEVEL OF EPA + DHA FOR HUMAN CONSUMPTION

A. Basto [1, 2 *], A. Marques[1], A. Silva[1, 2], T. Sá[1, 2], M.B.P.P. Oliveira[3], V. Sousa[1], E. Matos[4], L.M.P. Valente[1,2]

[1] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2] ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3] REQUIMTE, Chemical Science Department of Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[4] SORGAL, Sociedade de Óleos e Rações, S.A., Estrada Nacional, 109, Lugar da Pardala, 3880-728 S. João de Ovar, Portugal

[*] Presenting author: anafbasto@gmail.com

Introduction
Tenebrio molitor (TM) is one of the recently authorized species for use in aquafeeds. When defatted (*d*-) is particularly rich in highly digestible protein (up to 93% on a dry matter basis) and have a well-balanced amino acid profile able to meet European sea bass (*Dicentrarchus labrax*) requirements (Basto et al., 2020). However, *d*TM has limited long-chain polyunsaturated fatty acids (LC-PUFA), like eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3), with health benefits for consumers. When a new ingredient is tested in aquafeeds, the flesh nutritional value, sensorial properties and acceptance by consumers are important factors to consider. In this study, we have evaluated the impact of replacing fish meal (FM) with *d*TM in European sea bass growth performance, nutrient utilization, skin and muscle quality traits.

Material and methods
A FM-based diet with 47% of protein and 20% of fat was formulated and used as control (CTRL). Two other isoproteic and isolipidic diets were formulated to replace 50 and 100% of FM by *d*TM (TM50 and TM100, respectively). Each diet was assigned to quadruplicate homogeneous groups of 15 fish (69 ± 5 g) fed until apparent satiation for 16 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 22 ± 1 °C). By the end of the trial, feed consumption was determined and all fish were weighed and measured. Ten fish from the initial fish stock, and 4 fish per tank, by the end of the experiment were sampled for whole body (WB) composition, total lipid and FA profile analysis. Muscle from other 4 fish per tank were also collected for nutritional evaluation (lipid content and FA profile analysis), color analysis and textural properties (TPA and histology). Twenty fish per treatment were collected for sensory evaluation using a panel of 60 untrained panelists. After the growth trial, the apparent digestibility coefficients of the experimental diets were determined according to Cho & Slinger (1979), after including 1% Cr_2O_3, as inert marker, to the experimental diets.

Results
Fish fed TM50 had a significantly lower voluntary feed intake (VFI) and feed conversion ratio (FCR) compared to the CTRL, resulting in similar final body weight and condition factor among treatments. TM50 had the highest N and E retention efficiency values and reduced N and E overall losses. Total phosphorus (P) losses decreased with dietary *d*TM inclusion. The whole body lipids increased with dietary inclusion of *d*TM, mainly due to oleic acid (OA; 18:1n-9) gain and retention. Muscle total lipids levels remained similar among dietary treatments. The sum of saturated FA (SFA) increased in muscle of fish fed *d*TM, whereas monounsaturated FA (MUFA) didn't change. The dietary inclusion of *d*TM resulted in a concomitant increase of muscle LA level. Despite the relative percentage (% total FA) of muscle EPA and DHA decreased in fish fed *d*TM, when expressed in wet weight muscle EPA + DHA final contents were similar among all fish (0.30 g 100 g^{-1}).

Muscle cohesiveness and resilience were higher in fish fed TM50; this fish also had the largest-sized and lowest number of muscle fibers. Fish fed TM100 had a significantly lower number of smaller-sized muscle fibers (<25 µm) than those fed the CTRL. Skin and muscle lightness (L*), redness (a*), yellowness (b*) and Chroma (C*) values were similar among groups. The hue angle (Hº) was significantly lower in skin of fish fed TM100 compared to CTRL, but in muscle remained similar among all dietary treatments.

The panelist could not detect any significant differences in fish overall liking and acceptance that remained high for all fish, but *d*TM samples were strongly associated with a "pleasant taste" and "juicy texture".

(Continued on next page)

Discussion and conclusions

The results of the present study demonstrate that the substitution of 50% FM by *d*TM significantly improves FCR. Diets with *d*TM didn't impair European sea bass growth and still resulted in fair levels of EPA and DHA in muscle (0.30 g 100 g^{-1} of wet weight), which are above the recommended level for human consumption to decrease the risk of cardiovascular diseases (0.25 g 100 g^{-1} portion of fish; EFSA, 2010). Despite some differences observed in the muscle histomorphometric and instrumental texture measurements, all samples were characterized by their soft and pleasant texture. Altogether, these results evidence a great potential of *d*TM to fully replace FM in diets for European sea bass. However, the long term impact of feeding *d*TM diets still needs to be addressed.

Acknowledgements

Work supported by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF) and project ANIMAL4AQUA, funded by Portugal2020, financed by ERDF through the Operational Competitiveness Program (COMPETE) - POCI-01-0247-FEDER – 017610. AB was financially supported by FCT (SFRH/BD/138593/2018).

References

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 521, 735085.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout, Finfish Nutrition and Fishfeed Technology. Halver, J. E. Tiews, K., Berlin, Germany, pp. 239-247.

EFSA, 2010. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA 8, 1461-1566.

FEEDNETICS™ AS A TOOL TO PREDICT THE LONG-TERM EFFECTS OF FEEDING DEFATTED *Tenebrio molitor* LARVAE MEAL ON EUROPEAN SEA BASS GROWTH AND BODY COMPOSITION UNDER FARM-SCALE CONDITIONS

A. Basto [1, 2 *], A. Marques[1], M.B.P.P. Oliveira[3], L.M.P. Valente[1,2]

[1] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2] ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3] REQUIMTE, Chemical Science Department of Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

[*] Presenting author: anafbasto@gmail.com

Introduction
Since the insect protein from seven insect species was authorized by the European Union for use in aquafeeds, the interest in such ingredient has clearly emerged to both the scientific community and the aquaculture industry. In just a few years, the insect's production industry has risen in Europe, and the *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) are the main insect species produced. Consequently, they have been the most studied insect species as fishmeal (FM) substitute in diets for both freshwater and marine fish. Between both insect species, defatted (*d*-) TM seems more adequate to replace FM in European sea bass (*Dicentrarchus labrax*) (Basto et al., 2020), one of the most important fish species in Mediterranean aquaculture. However, long-term studies under farm-scale conditions are still not available. Thus, the main objective of this work was to predict the long-term effect of *d*TM as main protein source in diets for European sea bass reared under a farm-scale scenario, using the FEEDNETICS™ dynamic nutrient-based model smart-software.

Material and methods
A formulation with 40% (as feed basis) of FM was used as control (CTRL), and two other isoproteic, isolipidic and isoenergetic dietary formulations (47% protein, 20% fat and 24% energy), with 50% and 100% FM replacement by *d*TM (TM50 and TM100, respectively) were evaluated *in silico*. The FEEDNETICS™ was used to run predictions of a long-term trial with 300 000 European sea bass juveniles, stocked at 1/3/2021, with initial body weight of 5 g, considering a monthly mortality rate of 1% and a harvest weight of 500 g. Predictions were run considering the annual water temperature profile of Alvor (Portugal), obtained from www.seatemperature.org, which ranges between 13 °C and 24 °C. Feeding tables used were generated for each feed using FiT feeding tables™ (www.fitfeedingtables.com).

Results
The FEEDNETICS™ simulations indicate that feed efficiency should be slightly higher if fish were fed with *d*TM formulations (Figure 1). Consequently, those fish could reach the harvest weight of 500 g in approximately 12 months, while fish fed with CTRL would require 13 months. For the considered environmental conditions, the model suggests that formulations with *d*TM may result in slightly lower amounts of nitrogen and solids being released into the water. It is also expected that the increase of dietary inclusion of *d*TM may result in a concomitant decrease of total phosphorus wastes. Despite the model does not indicate differences in whole-body crude protein and crude fat among dietary formulations, it does in fact indicate some changes in amino acids and fatty acids (FA) profile. The formulations with *d*TM seem to increase total saturated FA, due to increased levels of palmitic acid; have no impact on total monounsaturated FA; reduce total polyunsaturated FA (LC-PUFA), due to decreased levels of some essential FA such as arachidonic, eicosapentaenoic and docosahexaenoic acids. The predictions also demonstrated that *d*TM may be responsible for a marked increment of whole-body linoleic acid. Despite simulations for TM50 and TM100 indicate a LC-PUFA reduction, a fair level of n-3/n-6 ratio (1.2) was still predicted for TM50 (Figure 2.).

Discussion and conclusions
In the long-term, TM50 seems to be the most promising dietary formulation for European sea bass reared under a practical farm-scale scenario. Overall, results of predictions based on this feed reflect a good balance between feed efficiency, growth performance, body composition and environmental outputs. The use of FEEDNETICS™ proved to be a useful tool to predict the outcome of nutritional trials. However, simulations still need to validated simulations through pilot scale trials.

(Continued on next page)

	CTRL - Alvor	TM50 - Alvor	TM100 - Alvor
PREDICTION DETAILS			
RESULTS SUMMARY			
Date for results summary	29/06/2023	31/05/2023	25/05/2023
Average body weight (g per fish)	502,1	502,8	501,0
Growth rate - cumulative (% BW per day)	1,2	1,3	1,3
FCR (cumulative g feed/g BW gain)	1,3	1,2	1,2
Feed cumulative (ton)	169,2	159,6	158,8
Total N waste (kg N/ton biomass gain)	62,4	56,7	56,1
Total solid waste (kg/ton biomass gain)	821,5	740,9	729,7

Figure 1. FEEDNETICS™ model results of feed efficiency and growth performance for the comparison of three dietary formulations under the temperature profile of Alvor (Portugal).

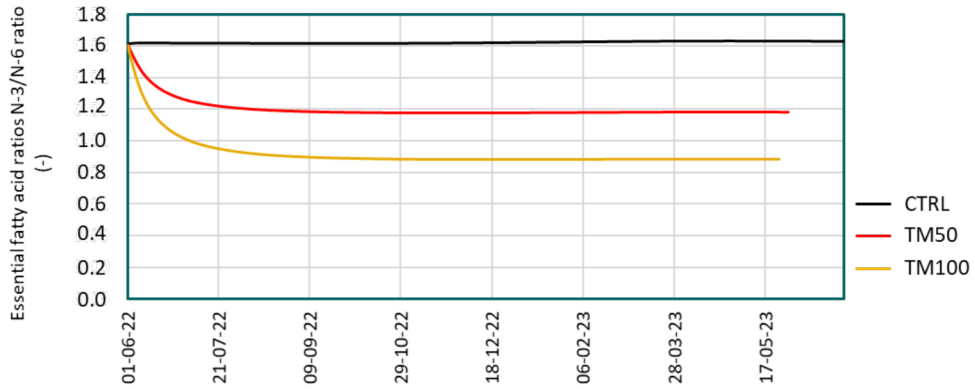


Figure 2. FEEDNETICS™ model results of LC-PUFA n-3/n-6 ratio for the comparison of three dietary formulations under the temperature profile of Alvor (Portugal).

Acknowledgements

The authors would like to extend their gratitude towards SPAROS Lda. (Portugal) for providing the FEEDNETICS™ academic licence used in this study. AB was financially supported by FCT (Portugal), through the grant SFRH/BD/138593/2018.

A STUDY OF THE DYNAMICS OF THE PROKARYOTIC COMMUNITY IN A RECIRCULATING AQUACULTURE SYSTEM (RAS) FROM A SOLE *Solea senegalensis* HATCHERY

Diana Bastos Almeida*[1,2,3], Catarina Magalhães[2,4,5], Zélia Sousa[4], Maria Teresa Borges[2,4], Eliane Silva[1], Isidro Blanquet[3], Ana Paula Mucha[2,4]

[1] ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto
[2] CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto
[3] SEA8 - Safiestela Sustainable Aquafarming Investments, Lda.
[4] FCUP – Faculty of Sciences, University of Porto
[5] School of Science, University of Waikato, Hamilton, New Zealand
* diana.almeida@ciimar.up.pt

Recirculating aquaculture systems (RAS) allow water reuse by managing waste and nutrient recycling, consequently making intensive fish production compatible with environmental sustainability, and improving fish welfare. A key aspect of these systems is the beneficial bacterial community of the biofilter[1]. In this study we aim to investigate the dynamics of this community in a sole (*Solea senegalensis*) hatchery RAS and its interactions with physical-chemical parameters.

Samples from different matrices (water, biofilter and tank wall biofilm) were collected from several compartments (Figure 1A) of a commercial hatchery (including the Pre-Ongrowing and Weaning working in RAS regime). Total DNA was isolated from the different matrices and the V4-V5 region of the 16S rRNA gene was sequenced using Illumina MiSeq® platform and analysed in the DADA2 pipeline using the SILVAngs database.

It was found that biofilm samples (both biofilter carriers and tank biofilm) were richer in terms of prokaryotic diversity than water samples. At beta diversity level, the Bray-Curtis dissimilarity index visualized through NMDS (Figure 1B) revealed high prokaryotic community dissimilarity between samples from the Pre-Ongrowing and the Weaning system. Overall, the prokaryotic communities were dominated by Proteobacteria (12-89%) and Bacteroidetes (8-86%) and a total of 58 genera contributed with more than 3% of the relative abundance across the different samples. The most abundant genera were *Tenacibaculum, Sulfitobacter, Leucothrix, Novosphingobium, Marinicella, Pseudoalteromonas, Polaribacter_2, Schleiferia* and *Algibacter*. Genera commonly associated with biofiltration activity in RAS[2], such as *Nitrospira* (nitrificaton), *Nitrosomonas* (nitrification) and *Thiothrix* (sulfide-dependent autotrophic denitrification) were found in the Pre-Ongrowing and Weaning biofilter, even though they were not the utmost representative genus found in these matrices. With the Adonis test, using the Bray-Curtis dissimilarity index for the distance matrix, it was found that prokaryotic community shifts were modelled by water parameters such as salinity and pH.

Our results demonstrated that the studied RAS sole hatchery was dominated by a highly dynamic prokaryotic community, sensitive to the physical-chemical changes within the different compartments of the same aquaculture unit, developing different community profiles. These are relevant findings for fine tuning when designing modulation protocols, without compromising fish welfare, since the community appears to be sensible to small variations. Future studies are fundamental to identify how the key players in maintaining a healthy RAS system can be used to achieve a healthy prokaryotic community when imbalances or fish disease outbreaks occur.

(Continued on next page)

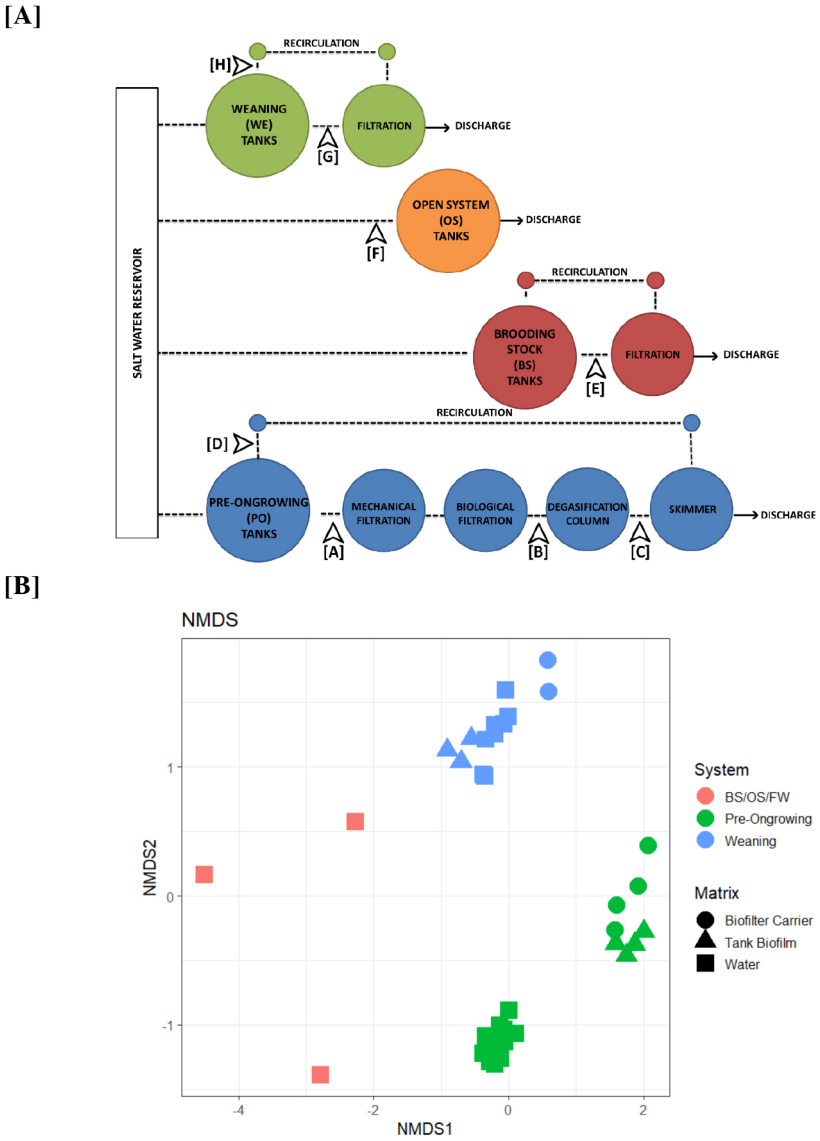


Figure 1: [A] Representation of the aquaculture unit studied. Water sampling points (A-H) are marked with arrows. **[B]** Unconstrained Ordination with Non-Metric Multidimensional Scaling (NMDS) between System-Matrix prokaryotic communities.

[1] Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.-P., d'Orbcastel, E.R., Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquacultural engineering. 43, 83-93.

[2] Espinal, C.A., Matulić, D., 2019. Recirculating Aquaculture Technologies, Aquaponics Food Production Systems. Springer, pp. 35-76.

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for the PhD fellowship ref. PD/BDE/135542/2018 and Safiestela Sustainable Aquafarming Investments, S. A. (SEA8). This research was supported by the project 39948_ FeedMi, supported by Portugal and the European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020; and by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

BIOFILM MODULATION OF RECIRCULATING AQUACULTURE SYSTEMS TO IMPROVE WATER QUALITY AND FISH WELFARE

Joana P. Fernandes*[2,4], Diana Bastos Almeida[1,2,3], Catarina Magalhães[2,4,5], Isidro Blanquet[3], Ana Paula Mucha[2,4]

[1] ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto
[2] CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto
[3] SEA8 - Safiestela Sustainable Aquafarming Investments, Lda.
[4] FCUP – Faculty of Sciences, University of Porto
[5] School of Science, University of Waikato, Hamilton, New Zealand
* jfernandes@ciimar.up.pt

Aquaculture has been rapidly growing as the increasing exponential population has imposed a higher demand for seafood supply. Recirculating aquaculture systems (RAS) can be used in intensive fish production, being considered a more sustainable and eco-friendly technology. In fact, with the RAS systems, it is possible to reuse water by managing the waste and nutrient recycling, and, at the same time, improve the fish welfare. One of the main advantages of RAS is the bacterial community that inhabits the biofilter [1]. They can improve the water quality of RAS systems as they can be involved in nitrification processes or prevent pathogen resistance. However, these communities are very sensitive to physical chemical fluctuations, which can result in pathogen outbreaks or inefficient removal of nutrients.

This work aimed for the modulation of the culturable bacterial community from a RAS system, in order to develop bacterial solutions for the maintenance of water quality and fish welfare. More specifically, this work was divided into main objectives: 1) isolate the nitrifying bacteria from the RAS system, to develop a formulation ready to use for application in case of unexpected fluctuation on nutrient levels or to reactivate the nitrifying bacteria in a new facility; 2) prevent diseases outbreaks caused by the pathogenic bacteria *Tenacibaculum maritimum*. Experiments using water and biofilter carriers were conducted aiming to isolate the culturable bacteria present in each matrix.

For the isolation of the nitrifying bacteria, different approaches were performed: a) direct isolation of nitrifying bacteria from the water and biofilter carriers, using selective AOB (ammonia oxidizing bacteria) and NOB (nitrite oxidizing bacteria) culture media and b) enrichment of nitrifying bacteria, using water and biofilter carriers from RAS system, and afterwards, isolate the culturable enriched community. For the direct isolation, samples of water and biofilter carriers were spread in ten-fold dilutions into 6 different culture media, rich in the respective nutrient (ammonia or nitrites). All the bacterial strains with different morphological features were selected and purified.

For the enrichment process, a set of microcosms were assembled: half only with water and the other half with water and biofilter carriers from the RAS system. The microcosms were supplemented with 335 μM of ammonium sulphate and 35 μM of sodium nitrite. The experiment was conducted in cycles of 14 days. After each cycle, part of the culture was diluted into a renewed media. Ammonium supplementation was performed in the beginning of each cycle (14 days) and supplementation of nitrites was performed at the beginning of each cycle and after 7 days. Each week, the cultures were transferred to new sterile flasks to avoid the oxygen depletion. The enriched cultures were incubated at room temperature, in dark and static conditions. After each cycle, a portion of the culture was spread in ten-fold dilutions, into 4 different selective culture media.

From the direct isolation, 20 bacterial strains were recovered and purified. All the strains will be cryopreserved at -80°C for further experiments. In addition, DNA extraction will be performed, and the extracted DNA will be used for the screening of nitrifying genes using qPCR and for further taxonomic identification of each bacterial isolate. Regarding the enrichment process, the enriched culture supplemented with sodium nitrite displayed removals higher than 95% of nitrite from the media. On the other hand, the enriched culture supplemented with ammonium displayed low removal of ammonia in all cycles. The isolation of the culturable bacteria of each enriched culture is still an ongoing work.

(Continued on next page)

For the second objective, related with prevention of outbreaks of *T. maritimum*, a direct isolation of the bacterial community present in the water and biofilter carriers was performed, using nonselective culture media, rich in different carbon sources and micronutrients. Previously, next generation sequencing data from the RAS system, more specifically, correlation and network analysis, was performed to assess the potential bacterial genera that can be negatively related with *T. maritimum*. After this analysis, different culture media were selected aiming the isolation of these particular bacterial genera. For that, samples of water and biofilter carriers were spread in ten-fold dilutions into 4 different culture media. Again, all the bacteria with different morphological aspects were isolated, purified and cryopreserved at -80°C, for further biotechnological applications. From this experiment, 256 bacterial isolates were retrieved and cryopreserved. For all isolates, a sample was collected for further DNA extraction and taxonomic identification. Assays targeting the inhibition of *T.maritimum* are being conducted. Two screening assays were developed, one based on streaking method and the other based on the disc diffusion method. Until now, 39 bacterial isolates were tested, in which 6 bacterial isolates presented potential inhibition capacity against *T.maritimum*. The bacterial isolates with the capacity to inhibit *T.maritimum* will be tested and validated in microcosms experiment, and effects driven by the addition of this bacterial isolates on the natural community as well as the impacts on the water quality will be assessed.

This work can contribute for the improvement and maintenance of water quality and fish welfare in aquaculture RAS systems, by developing new formulations for improvement and maintenance of the RAS systems.

[1] Almeida, D. B., Magalhães, C., Sousa, Z., Borges, M. T., Silva, E., Blanquet, I., Mucha, A. P**.,** 2021. Microbial community dynamics in a hatchery recirculating aquaculture system (RAS) of sole (*Solea senegalensis*). Aquaculture. 539, 736592. doi.org/10.1016/j.aquaculture.2021.736592.

This research was supported by the project 39948_FeedMi, supported by Portugal and the European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020; and by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. Diana Almeida acknowledge Fundação para a Ciência e Tecnologia (FCT) for the PhD fellowship ref. PD/BDE/135542/2018 and Safiestela Sustainable Aquafarming Invesments, S. A. (SEA8).

THE SALTBOX PROJECT: A CODED STRUCTURED LIGHT-BASED FISH BIOMASS ESTIMATOR FOR GILTHEAD SEABREAM AND EUROPEAN SEABASS

Alkisti Batzina [1,*], Dimitris Syvridis [2], Nafsika Karakatsouli [1], Christos Veinidis [2], Panos Tsotsis [2], Eva Iris Eleftheria Karellou [3]

1Laboratory of Applied Hydrobiology, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, 118 55 Athens (Greece)
2 Department of Informatics & Telecommunications, National and Kapodistrian University of Athens, Panepistimiopolis Ilisia 15784, Athens, Greece
3 Selonda Aquaculture SA, 19,3 km Markopoulou–Paiania Ave.,190 02, Paiania, Athens, Greece
E-mail: alkistibatzina@gmail.com

Introduction

The efficient management of intensive fish rearing requires the close monitoring of fish performance. The knowledge of fish sizes and biomass within sea cages is of utmost importance for the rational evaluation of the production process (e.g. growth, feed efficiency, drug administration, size variation, harvest). The estimation of fish biomass in the sea cage environment during intensive rearing has long been a research and technological challenge. Various equipment (mainly applied to salmon rearing) is available but none has yet been widely used for gilthead seabream and European seabass rearing for reasons of accuracy, complexity of use, time and cost efficiency.

Objectives

The main objective of the project is the design and manufacture of a fish size/biomass estimator adapted to the two major Mediterranean aquaculture species, gilthead seabream and European seabass. The ambition is to produce an underwater device that a) will not require time-consuming and invasive measurement procedures, b) will use high level automatization and minimize the need for expertise personnel, c) will be cost efficient.

Materials and Methods

The biomass estimator will be based on the reconstruction of the 3D scene where the fish live, using the coded structured light technology. This method requires a projector which projects a pattern onto a part of the area where the fish swim and a camera which records this area. The projected pattern contains coded information in order to find corresponding points between the pattern and the image which is acquired by the camera, in a similar manner to the one realized in stereoscopic vision and the way which the human eye operates. Finally, a method called triangulation can be used to extract the coordinates of the 3D points of the scene, exploiting the previously mentioned corresponding points. After the 3D reconstruction of the area where the fish live, it is feasible to isolate the fish whose bodies are entirely recorded by the camera. Using the resulting 3D representation of these fish, the extraction of geometric features related to their body is also feasible.

The estimator will be equipped with the necessary software which will be based on gilthead seabream and European seabass morphometrics and relationships with body weight. To achieve the latter a series of body measurements will take place in a large number of fish covering a body weight range from around 100 g up to commercial size. The equipment will be tested on the field and re-calibrated if necessary.

Expected outcomes

The successful completion of the project will provide a practical, economical, and reliable solution for estimating the size and biomass of farmed fish. In the short term it may lead to the development of a commercial product that will be affordable, even for small and medium-sized enterprises of intensive aquaculture. Overall, the project hopes to provide a vital tool to support an efficient fish farm management that will further promote the Greek aquaculture sector.

Aknowledgements

The project is co-funded by the European Maritime and Fisheries Fund and the Greek government.

EVALUATION OF ANTARCTIC KRILL (*Euphasia superba*) MEAL SUPPLEMENTATION IN DIETS FOR OLIVE FLOUNDER (*Paralichthys olivaceus*)

Tibiabin Benitez-Santana*, Kasun Tharaka, Buddhi E. Gunathilaka, Min-Gi Kim, Chorong Lee, Jaehyeong Shin and Kyeong-Jun Lee

Aker BioMarine Antartic AS
Oksenøyvn 10. PO Box 496NO-1327 Lysaker, Norway
tibiabin.benitez-santana@akerbiomarine.com

The supplemental effect of Antarctic krill meal (KM) into a low fish meal (FM) diet was evaluated for olive flounder (*Paralichthys olivaceus*). A 56% FM-based diet was regarded as a high FM inclusion diet (HFM), and a low-FM diet (LFM) was prepared by replacing 50% FM from the HFM. Four other diets were prepared by supplementing 3%, 6%, 9% and 12% KM into the LFM diet gradually replacing soy protein concentrate and tankage meal (designated as KM3, KM6, KM9 and KM12 respectively). Quadruplicate groups of fish were fed one of the diets for 12 weeks. The growth performance and feed utilization efficiency were improved by the dietary KM supplementation. Digestibility of dietary protein and dry matter was increased by the KM3-9 diets. Haematocrit and haemoglobin were increased by KM supplementation. The innate immunity and antioxidant capacity assessed by Ig, antiprotease, lysozyme, GPx and SOD and the condition factor of fish were significantly increased by KM3-9 diets. Moreover, goblet cell counts, villi length and fillet yield of fish were significantly improved by all the KM-containing diets (KM3-12). A 25-day-long challenge test with the *Edwardsiella tarda* pathogen showed that the cumulative mortality was higher in fish fed the LFM diet than in fish fed the HFM or KM-supplemented diets. The results indicate that dietary KM supplementation in a LFM diet can increase growth performance and feed utilization efficiency, diet digestibility, intestinal development and functions, innate immunity and disease resistance of olive flounder. The recommended level of KM inclusion in a LFM diet seems to be 6.6% according to quadratic regression analysis.

ETHOLOGICAL STUDIES ON AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822) UNDER DIATARY SUPPLEMENTATION OF THE CLAY MINERAL MONTMORILLONITE-ILLITE/MUSCOVITE (1G557) IN A COMMERCIAL RECIRCULATING SYSTEM

E. Berchtold, H. W. Palm, & B. Baßmann

University of Rostock, Faculty of Agricultural and Environmental Sciences, Professorship of Aquaculture and Sea-Ranching, Justus-von-Liebig-Weg 6, D-18059 Rostock, Germany

Clay minerals are considered to have positive effects on health and welfare of fish in aquaculture. These effects are caused by their molecular structure, which is able to adsorb harmful substances, thereby improving water quality. The clay mineral „Friedland Ton“ mined in Mecklenburg-Western Pomerania, NE Germany, contains montmorillonite-illite/muscovite and was approved as feed additive by the European Union under the abbreviation 1g557. Palm et al. (2015) described an increased survival rate, higher final weights, more efficient feed conversion and reduced size variance of White Leg Shrimp postlarvae (*Litopenaeus vannamei*) under application of feeds containing 2% 1g557, or a mixture of 2% 1g557 and 2% of the microalgae *Chlorella vulgaris*.

To analyse the behaviour of African catfish *in situ*, an infrared camera system was used for the first time. This allowed fish observation under the common low-light conditions in a commercial recirculation system for African catfish. During a 125-day trial, three groups of juvenile African catfish were fed a standard catfish diet containing 0.0%, 0.5%, and 2.0% 1g557. For ethological studies, video recordings (30 min per fish tank) were made at four times during the experiment. One day prior each video recording, welfare (skin lesions, plasma cortisol, blood glucose) and growth parameters were measured.

Using 1g557 as feed additive in a commercial recirculating system with African catfish (*Clarias gariepinus*, Burchell 1822) has shown that groups fed with 1g557 had significantly fewer bite wounds compared to an unsupplemented control group. This was supported by the video recordings which also indicated that treated fish showed less aggressive behaviour towards their conspecifics improving fish health and welfare.

References

Palm, H., Sörensen, H., & Knaus, U. (2015). Montmorillonite Clay Minerals With or Without Microalgae as a Feed Additive in Larval White Leg Shrimp (*Litopenaeus vannamei*). Annals of Aquaculture and Research, 2(1), 1008.

RELEVANCE OF SUSTAINABILITY DIMENSIONS AND ANIMAL WELFARE FOR CONSUMERS WHEN BUYING INNOVATIVE AQUACULTURE FISH PRODUCTS

A. Bermúdez*, L. López-Mas, L. Guerrero.

IRTA, Food Technology Program, Finca Camps i Armet, E-17121 Monells (Spain)
*E-mail: alejandra.bermudez@irta.cat

Introduction

A strategy of differentiation when launching new products is to incorporate added-value attributes that impact on consumers' choices. Previous research identified sustainability as a key factor in buying intention. Consumers are increasingly reflective in their food decisions and have broadened their understanding of sustainability (Mintel Food & Drink Industry Trend, 2020). Animal welfare has also been identified as a relevant driver of fish consumption in some circumstances. Therefore, it becomes necessary to undercover the concept of 'sustainability', but also animal welfare, into smaller parts (dimensions) and to investigate the role they play in innovative aquaculture fish products choice.

Self-reported measures, those given explicitly by consumers, are the most common way to grasp their opinion. Even so, in recent years has gained attention the advantages of combining explicit and implicit methods (i.e. physiological and emotional measures gathered by non-invasive sensors). This study, carried out within MedAID project (European Commission, Horizon 2020, No. 727315), aims to gain understanding on consumer's decision making process for new aquaculture fish products.

Material and methods

A combination of explicit and implicit methods was used during a choice experiment (i.e. ranking-based conjoint analysis) of a 'Meagre aquaculture fish burger with mushrooms', developed by AZTI (Spain). Explicit measures included a ranking task for six versions of the product with different factors and levels (Table1). Additionally, a questionnaire assessing willingness to try and buy the product, as well as product familiarity, healthiness, and convenience, the Single-item Food Choice Questionnaire (FCQ) (Onwezen et al., 2019), Spanish versions of Prosocialness Scale for Adults (Martínez-Pampliega et al., 2018) and New Environmental Paradigm Scale (Vozmediano et al., 2005) was used. Implicit methods consisted in Eye tracking (ET) to measure visual attention. Areas of interest (AOI) for all statements presented in the ranking task were assigned. Fixation count was used to identify relevant areas and duration of fixations to assess degree of involvement (Van Loo et al., 2015).

The study included 30 Spanish participants, divided in experimental and control group. Manipulation consisted in the viewing, previous to the ranking task, different versions of a video, differing in the information contained: general facts about aquaculture (control) and positive outcomes of aquaculture emphasising its sustainability (experimental).

Results

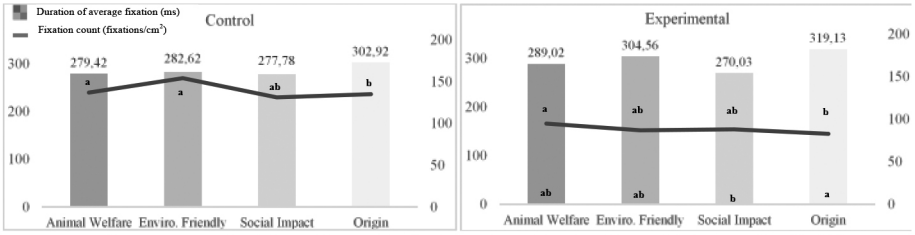
Low scores for familiarity confirmed the innovativeness of the 'Meagre fish burger with mushrooms'. Sensory, health, and natural were the most important attributes when choosing fish products, followed by items connected to sustainability as environmental issues, animal welfare, and social justice over price, convenience, familiarity, mood, or weight control. Participants revealed prominence of environmental and prosocial values. The results of the conjoint analysis showed that animal welfare was the most relevant factor, both in control and experimental conditions. In all cases, respondents preferred having information about positive outcomes on the different sustainability dimensions (environmental and social) and animal welfare. Besides, Spanish origin was chosen.

When comparing visual attention between factors (Figure 1), fixation count was higher for statements about environmental impact and animal welfare for the control group, and animal welfare for the experimental group. On the other hand, origin was the factor with longer fixation duration for both groups. In relation to the factor levels, social impact presented significant differences in attention between the level of information displayed, both in control and experimental conditions. Fixation count was higher when the statement referred had no information about social impact, whereas fixation duration was higher for the statement with information about positive outcomes. In addition, significant differences were found in the fixation count between groups for those statements that had no information about social impact or animal welfare, with lower counts for the informed condition

(Continued on next page)

Table 1. Factors and levels in the conjoint analysis design.

Factor	Levels
Environmental Impact	Fish farmed respecting the environment /No information
Animal Welfare	Fish farmed respecting the fish welfare /No information
Social Impact	Positive impact on local communities /No information
Origin	Spain / EU / Non-EU



Superscript a–b: different letters indicate statistically significant differences ($p < 0.05$).

Figure 1. Visual attention to factors control vs. experimental conditions.

Conclusions

For fish products perceived as innovative, animal welfare had greater importance in consumers' choices than origin, which traditionally has been one of the most important attributes for fish. When relevant information about positive outcomes of aquaculture (i.e., sustainability and animal welfare) was given, origin importance decreases. In line with respondents' choices, visual fixation to animal welfare statements was significantly higher than origin, highlighting its greater importance. On the other hand, higher fixation duration for origin indicated that participants noticed the attribute and they took longer time in the deliberation process. Considering visual attention between factor levels, social impact is perceived as a complex attribute. When relevant information about the positive outcomes was given, both fixation counts and fixation duration decreased, suggesting less complexity of the deliberation process.

Animal welfare and social impact concerns are gaining consumers' attention over environmental impact and origin. It is highly recommended to incorporate these added-value giving attributes when developing new aquaculture fish products. Communication that includes information about the outcomes of aquaculture and its sustainability could help consumers to select fish products aligned with their values and beliefs.

References

Martínez-Pampliega et al. (2018). Metric Goodness of the Adult Prosocialness Scale. Comparative Study of Italy and Spain. *Span. J. Psychol.*, *21*(e29), 1–10.

Onwezen et al. (2019). The development of a single-item Food Choice Questionnaire. *Food Qual. Prefer.*, *71*(August 2017), 34–45.

Van Loo et al. (2015). Sustainability labels on coffee: Consumer preferences, willingness-to-pay and visual attention to attributes. *Ecol. Econ.*, *118*, 215–225.

Vozmediano et al. (2005). Escala "Nuevo Paradigma Ecológico": propiedades psicométricas con una muestra española obtenida a través de Internet. *Medio Ambient. y Comport. Hum.*, *6*(1), 37–49.

HOST-MICROBIOTA INTERACTION IN THE MANILA CLAM (*Ruditapes philippinarum*) FARMED IN DIFFERENT SITES OF THE VENICE LAGOON

I. Bernardini*[1], A. Manuzzi[1], G. Dalla Rovere[1], M. Smits[1], J. Fabrello[2], L. Masiero[2], C. Breggion[2], A. Sambo[2], C. Bertolini[3], R. Pastres[3], C. Bettiol[3], E. Semenzin[3], C. Carrer[4], M. Varagnolo[5], Marco Ruffino[5], V. Matozzo[2], L. Bargelloni[1], T. Patarnello[1], M. Milan[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020, Legnaro (PD), Italy
[2] Department of Biology, University of Padova, Via Bassi 58/B, 35131, Padova, Italy
[3] Department of Environmental science, computer science and statistics, Ca' Foscari - University of Venice, 30173, Venezia, Italy
[4] Thetis Spa, Castello 2737/f, 30125 Venezia, Italy
[5] C.L.A.M. SOC.COOP., C.R.A.M.E. Chioggia, Società agricola Kappa S.s. Chioggia
E-mail contact: ilaria.bernardini.1@phd.unipd.it

Introduction

The Venice Lagoon represents a vulnerable ecosystem prone to significant variations of biotic and abiotic factors based on the spatiotemporal conditions as well as extensive anthropogenic interventions (Deheyn and Shaffer., 2007). These stressors can have consequences on the biology and ecology of sessile and highly sedentary organisms, such as bivalve mollusks, that cover, in turn, important ecological and economical roles. Shellfish, like the Manila clam (*Ruditapes philippinarum*), are extensively farmed in several areas of the Venice Lagoon, mostly due to their high tolerance and adaptability to di□erent conditions and to strong variations in environmental parameters (Boscolo Brusà et al., 2013). Recent studies have demonstrated that clam health depends also on the microbiota, or associated microbial community, which plays key roles in nutrient assimilation, homeostasis, immunological regulation (Aceves et al., 2018) and the protection against pathogens and environmental stressors (Milan et al., 2018). However, seasonal variations, adverse climatic conditions, chemical-physical features of different farming sites, and anthropogenic activities can influence the fitness of farmed clams as well as their associated microbial communities, with potential negative consequences on the clam farming aquaculture industry. In addition, the inauguration of the new Experimental Electromechanical Module (MoSE), system of flood barriers, designed to safeguard Venice and the lagoon from high tides, may lead to further modifications of the delicate Venice lagoon ecosystem. In this study, a long-term monitoring campaign was carried out on clams from four farming sites in the south of the Venice Lagoon characterized by different environmental conditions. The aim of this study was to investigate the correlations between seasonal trends of microbiota and gene expression profiles in the digestive gland and gills of clams, and the biometric characteristics, condition index, and site-specific environmental chemical-physical parameters. Overall, this study reports key information for predicting and identifying the potential impacts of the MoSE system on Manila clam farming activities.

Material and Methods

Twenty thousand individuals of Manila clam spat (*Ruditapes philippinarum*), supplied by the Satmar Company (France) in August 2018, were partitioned in 4 groups and placed at gradual distances from the Chioggia inlet, from the outmost to the innermost site. Clam sampling was performed at five intervals throughout a one-year period, representative of the seasonal variations (May 2019, July 2019, October 2019, February 2020 and May 2020). After each sampling, biometric parameters and condition index of clams were recorded. Digestive glands and gills were collected for transcriptomic analyses (RNA-sequencing) and microbiota characterization (16S rRNA Amplicon Sequencing). Raw data elaboration from molecular analyses is still ongoing. To link potential alterations in clam health with changes in environmental conditions, the sediments of each sampling area were collected to measure levels of dibenzo-p-dioxins/furan (PCDD/F), polychlorinated biphenylenes (PCB), hexachlorobenzene (HCB) and heavy metals at each sampling time. Moreover, two multiparametric probes located at different distances from the inlet continuously measured water temperature, turbidity, pH, chlorophyll-*a*, salinity, dissolved oxygen, and saturation throughout the year.

(Continued on next page)

Results

Results regarding biometric parameters showed no relevant differences between clams harvested in different sites at any single sampling time during the monitoring year. The highest growth rate was detected between May and July 2019, in all sites. The lowest condition index was recorded in October 2019 in all study areas, while the highest value was detected in the outermost site in May 2019 and February and May 2020. Detectable levels of the investigated pollutants were not different between investigated areas. Regarding environmental parameters, probes in both sites recorded a similar seasonal trend of each environmental parameter. Generally, the average temperatures, dissolved oxygen, and turbidity are always higher in the southern part (innermost probe), with the exception of the winter period in which a higher average temperature was observed near the inlet. Finally, the ongoing analyses on gene expression profiles and microbiota characterization performed in the two investigated tissues of clams will allow to evaluate seasonal alterations in clams farmed in different areas of the lagoon; their potential link to biometric analyses and environmental parameters will be also presented and discussed in this presentation.

Conclusion

By merging results obtained through the multidisciplinary approach applied in this study, important information regarding the influence of several environmental factors on the health status of farmed clams may be underlined. Furthermore, results of 16S rRNA sequencing and RNA-seq will make it possible to describe the host-microbiota interactions in the Venice lagoon farming areas, which may be associated with different environmental conditions. Overall, results obtained by this study will represent the "baseline" information which to evaluate the potential consequences of the MoSE system on Manila clam farming areas in the future from.

References

Aceves, A.K., Johnson, P., Bullard, S.A., Lafrentz, S., Arias, C.R., 2018. Description and characterization of the digestive gland microbiome in the freshwater mussel Villosa nebulosa (Bivalvia: Unionidae). J. Molluscan Stud. 84, 240–246. https://doi.org/10.1093/mollus/eyy014.

Boscolo Brusà R., Cacciatore F., Ponis E., Molin M., Delaney E.. 2013. Clam culture in the Venice lagoon: stock assessment of Manila clam (*Venerupis philippinarum*) populations at a nursery site and management proposals to increase clam farming sustainability. Aquat. Living Resour. 26. https://doi.org/10.1051/alr/2013042.

Deheyn D. D., Shaffer L. R., 2007. Saving Venice: Engineering and ecology in the Venice lagoon, Technology in Society, 29, https://doi.org/10.1016/j.techsoc.2007.01.014.

Milan M., Carraro L., Fariselli P., Martino M.E., Cavalieri D., Vitali F., Boffo L., Patarnello T., Bargelloni L., Cardazzo B.. 2018. Microbiota and environmental stress: how pollution affects microbial communities in Manila clams. Aquatic Toxicology, 194, https://doi.org/10.1016/j.aquatox.2017.11.019.

THE IMPROVEMENT OF SPERM CRYOPRESERVATION METHODS IN THE ENDEMIC TENCH (*Tinca tinca*) AND CRUCIAN CARP (*Carassius carassius*) FOR CONSERVATION PURPOSES

Gergely Bernáth[1], Levente Várkonyi[1], Balázs Csorbai[1], Levente Zete Láng[1], József Molnár[1], Tamás Bartucz[1], Borbála Nagy[1], István Lehoczky[2], Gergely Szabó[2], Béla Urbányi[1], Zoltán Bokor[1]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1., H-2100 Gödöllő/H-2484 Agárd, Hungary
[2]National Centre for Biodiversity and Gene Conservation Institute for Farm Animal Gene Conservation, Isaszegi u. 200., H-2100 Gödöllő
E-mail: Bernath.Gergely@uni-mate.hu

Introduction

Tench (*Tinca tinca*) and crucian carp (*Carassius carassius*) are endemic ecologically important fish species in Hungary. The angling interest and demand was increased for the two fish in the last decades. The size of their natural populations shows a drastic decreasing tendency in Hungary and in other European countries as well. Sperm cryopreservation is an efficient tool to support the reintroduction of the vulnerable natural populations using spermbanks (Horváth et al.2012; Martínez-Páramo et al. 2017).

Materials and methods

The aim of the study was to improve sperm cryopreservation methods in the two endemic species for conservational purposes. In our experiments, two different extenders (E1: 200 mM glucose, 40 mM KCl, 30 mM Tris, pH: 8.0±0.2; E2: 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM Na_2HPO_4 * $12H_2O$, 1 mM $MgCl^2$ * $6H_2O$, 1 mM $CaCl^2$ * $2H_2O$, 20 mM Tris, and 0.5% BSA, pH: 8.0 ± 0.2) and two different cryopreservation methods (Styrofoam box and controlled-rate freezer-CRF) were compared according to the experimental design. Sperm was frozen using the conventional 0.5 mL straw and 10 % methanol as cryoprotectant. Sperm motility (progressive motility) and other kinetic parameters (distance curved line, distance straight line, curvilinear velocity, straight line velocity, linearity) was investigated using a Computer-assisted Sperm Analysis (CASA) system.

Results

A significantly higher post-thaw progressive motility was recorded using E1 (34±4%) than in E2 (22±4%) in crucian carp. Contrary, no significant difference in progressive motility was observed between E1 (36±9%) and E2 (45±20%) in tench following thawing. However, motility reduced significantly in E1 in comparison with the fresh samples (Fig.1.). No significant difference was measured between the Styrofoam box (10±2%) and the controlled-rate freezer (6±1%) in thawed tench sperm. Furthermore, no significant difference was recorded in the other kinetic parameters between the different treated groups in tench and crucian carp as well.

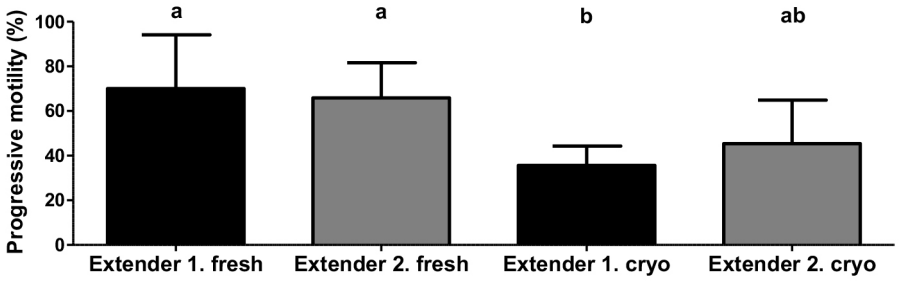


Fig.1. The progressive motility measured in tench sperm using two different extenders prior to cryopreservation and following thawing (*N*=6). The columns represent mean and SD. The different letters indicate significant difference between the columns at p>0.05.

(Continued on next page)

Discussion and conclusion

According to our results, different extenders can be efficient in the two cyprinid species. The reduced motility in tench sperm observed following thawing (cryopreserved in S. box and CRF) was caused probably by urine contamination (Król et al. 2018). Urine activated spermatozoa prior to the freezing process. Future improvement and standardization of the sampling method can prevent the mentioned undesirable phenomenon. The results can contribute to the establishment of future spermbanks in the case of the two endemic species.

Acknowledgements

The study was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12). The experiments were funded by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund. Our study was also supported by the project "The establishment and improvement of the gene bank strategies at the 21th century in different indigenous species, breeds and ecotypes of the Carpathian basin" VEKOP-2.3.2-16-2016-00012.

References

Król, J., Żarski, D., Bernáth, G., Palińska-Żarska, K., Krejszeff, S., Długoński, A., and Horváth, Á. (2018). Effect of urine contamination on semen quality variables in Eurasian perch *Perca fluviatilis* L. Anim Reprod Sci 197, 240–246.

Horváth, Á., Jesenšek, D., Csorbai, B., Bokor, Z., Raboczki, É., Kaczkó, D., Bernáth, G., Hoitsy, G., Urbányi, B., Bajec, S.S., et al. (2012). Application of sperm cryopreservation to hatchery practice and species conservation: A case of the Adriatic grayling (*Thymallus thymallus*). Aquaculture 358–359, 213–215.

Martínez-Páramo, S., Horváth, Á., Labbé, C., Zhang, T., Robles, V., Herráez, P., Suquet, M., Adams, S., Viveiros, A., Tiersch, T.R., et al. (2017). Cryobanking of aquatic species. Aquaculture 472, 156–177.

MODELLING THE *Sparicotyle chrysophrii* OUTBREAKS IN GILTHEAD SEABREAM (*Sparus aurata*) MEDITERRANEAN AQUACULTURE

M. Berrettini*[a], R. Barić[b], S. Čolak[b], M. Kolega[b], D. Mejdandžić[b], M.L. Fioravanti[c], A. Gustinelli[c], L. Parma[c] and C. Viroli[a]

[a]Department of Statistical Sciences, University of Bologna (Italy)
[b]Cromaris, Zadar (Croatia)
[c]Department of Veterinary Medical Sciences, University of Bologna (Italy)
E-mail: marco.berrettini2@unibo.it

Introduction

Sparicotyle chrysophrii represents one of the main parasitic threats for gilthead seabream (*Sparus Aurata*) cultures in the Mediterranean basin (see e.g. Alvarez-Pellitero, 2004), causing mortality and poor fish quality. A data set has been collected by the fish farm Cromaris, including ectoparasite counts and environmental hydrographic variables. In this study, a logistic regression model is fitted to identify factors that are connected to Sparicotyle outbreaks.

Data

The proposed analysis focuses on a single Cromaris farm, located in Bisage. In particular, seven cages have been monitored on eight examination days in the first year of life of the 2017 generation. For each of the resulting 56 observations, Sparicotyle presence has been checked on the left gill arches of 12 or 13 randomly drawn fishes. To understand the factors that may influence parasite presence, the dataset is integrated with some concomitant information about fishes (and the environment where they are bred) on the day of examination, such as actual water temperature (°C), average fish weight (g), actual fish density (kg/m^3) and average haematocrit value (%).

Model specification

Let Y_i be a random variable describing the observed number of Sparicotyle-infected fishes out of n_i (12 or 13) sampled from a single cage on a specific date. For each observed sub-sample $i = 1, ..., k = 56$, denote with $x_{i1}, ..., x_{iP}$ the values taken by P concomitant variables on the n_i sampled fishes (or the cage they are drawn from). Conditional on $x_i = (1, x_{i1}, ..., x_{iP})$, each Y_i is assumed to be independent and to follow a Binomial distribution with parameters n_i and π_i, where the latter represents the probability to observe the presence of *Sparicotyle chrysophrii* in one of the four left gill arches of a single fish. This probability is assumed to depend on the concomitant covariates x_i through a logistic function, as

$$\pi_i = \frac{\exp(\boldsymbol{x}_i\boldsymbol{\beta})}{1 + \exp(\boldsymbol{x}_i\boldsymbol{\beta})} \in [0,1], \qquad i = 1, \dots, n,$$

where $\boldsymbol{\beta} = (\beta_0, \beta_1, \dots, \beta_P)$ is a $(P+1)$-dimensional vector of unknown coefficients.

Results

All the covariates introduced in the Data Section have been included in the logistic regression model, together with the degree days (i.e. the sum of daily temperatures), which take into account the amount of time that passed between stocking fish on farm to the day of examination. Nevertheless, the latter has been removed from the model, as well as the average haematocrit value, during the variable selection phase, carried out via backward approach. The resulting model is adequate according to the X^2 goodness-of-fit test (p-value = 0.258).

	$\hat{\beta}$	exp ($\hat{\beta}$)	s. e. ($\hat{\beta}$)	p-value
intercept	-1.534	0.014	0.804	0.056
temperature (°C)	0.099	1.104	0.030	0.001
weight (g)	0.039	1.040	0.016	0.014
density (kg/m^3)	-1.053	0.349	0.447	0.018

Table 1: Maximum likelihood estimates of the regression coefficients $\widehat{\boldsymbol{\beta}}$ (numerically approximated via Fisher scoring algorithm), together with their exponential (for interpretation purpose), standard error and p-value of the corresponding Wald test.

(Continued on next page)

According to Table 1, the odds of a fish being sparicotyle-infected increase by about a 10.4% with each additional degree Celsius of temperature, and about a 4% with each additional gram of weight, keeping constant the values of the two remaining covariates. On the contrary, fish density seems to be negatively related with the parasite presence. However, it is worth noting that the latter coefficient estimate presents a sensibly higher standard error with respect to the two other variables.

Conclusions and further developments

The logistic regression model, fitted on the Cromaris data, highlights some statistically significant potential determinants of the *Sparicotyle chrysophrii* outbreaks in the gilthead seabream Mediterranean aquaculture. Nevertheless, the results presented are referred to a preliminary analysis which will be extended by integrating additional variables and observations. Moreover, some further work can be done to better fit the logistic regression model to the Cromaris data. For instance, random effects for each cage could be included in the analysis, as in the mixed paradigm (see e.g. McCulloch & Neuhaus, 2005). Furthermore, although a portion of the effect of time is already being caught by the variables considered (temperature and weight, in particular), the within-cage time dependence could be made explicit, e.g. by specifying an autocorrelation structure based on autoregressive-moving-average (ARMA) models (see e.g. Diggle et al., 2002).

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/).

References

Alvarez-Pellitero, P. (2004). Report about fish parasitic diseases. *Etudes et Recherches, Options Mediterranennes. CIHEAM/FAO, Zaragoza*, 103-130.

Diggle, P., Diggle, P. J., Heagerty, P., Liang, K. Y., Heagerty, P. J., & Zeger, S. (2002). *Analysis of longitudinal data*. Oxford University Press.

McCulloch, C. E., & Neuhaus, J. M. (2005). Generalized linear mixed models. *Encyclopedia of biostatistics*, *4*.

738
NON-DESTRUCTIVE METHOD FOR THE ASSESSMENT OF FISH QUALITY: A PROMISING TOOL FOR THE AIMS OF SUSTAINABLE AQUACULTURE PROJECT EIT FOOD "JUST ADD WATER"

Andrea Bertini*[1], Eleonora Iaccheri[2], Annachiara Berardinelli[1,3], Alessio Bonaldo[4], Francesco Parrino[3]

[1]Centre Agriculture Food Environment (C3A), University of Trento, San Michele All'Adige, Italy
[2]Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Cesena, Italy
[3]Department of Industrial Engineering, University of Trento, Trento, Italy
[4]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy
Email: andrea.bertini@unitn.it

Introduction
Mycotoxins and their effects have been examined in different animal species; however, more information about their impact on the fish immune response is still required. Fumonisins, produced by several Fusarium species, represent the most common mycotoxins in plant meals, which have been more frequently used in fish feed production in recent years. These toxins can cause major health problems in fish (Oliveira & Vasconcelos 2020), including both immunostimulation and immunosuppression (Riley et al. 1996; Pestka et al. 2004). Their effects need to be investigated more, especially in the main aquaculture species.

Materials and Methods
Physical and chemical changes occurring in the fish during storage were explored by using a non-destructive tool based on the dielectric property analysis. Specifically, dielectric constant (and loss factor () of trout eyes were acquired by using an open-ended coaxial probe (DAKS-3.5 probe, Speag). The probe was connected to a VNA (Vector Network Analyzer, Copper Mountains) and interfaced by USB with PC (DAK Software Installer 2.6.1.7). The calibration of the instrumental chain was conducted through the customized calibration kit (Speag DAK-3.5/1.2 Shorting Block, Metallic Strip Sets, and 0.6 l of Tissue Simulating Liquid). Acquisitions were carried out at 23 °C (± 1°C) on 7 fish in the GHz frequency range during 5 days of storage at 0°C (± 0.5°C).

Results
Non-specific stimulation showed a significant increase in proliferative activity after vaccination against Y. ruckeri in the fish from both fumonisin groups (vaccinated and non-vaccinated), indicating a pro-inflammatory immune reaction. Similar results were obtained in the non-vaccinated control. The vaccinated compared to the non-vaccinated fish from the control group showed significantly lower proliferation levels. With specific stimulation, significantly higher values were detected in the vaccinated fish from the fumonisin group compared to the vaccinated control.

Levels of specific antibodies were significantly increased in the vaccinated fumonisin group compared to the non-vaccinated fish at week 9. However, at week 10, the control fish showed similar results with even higher values. The enhanced immune reaction, which occurred very quickly in the vaccinated fish from the fumonisin group, may be indicative of pro-inflammatory changes (Ellis 1999).

Conclusion
Dielectric constant and loss factor of trout eyes appeared to describe the main changes occurring in the fish during storage. Further steps may include the validation of the techniques with other fish species and different rearing conditions. The setting up of a non-destructive and rapid tool will represent an important step in the project development.

(Continued on next page)

References

El Khaled, D., Castellano, N., Gázquez, J., Perea-Moreno, A.-J., Manzano-Agugliaro, F., 2016. Dielectric Spectroscopy in Biomaterials: Agrophysics. Materials 9, 310.

Franceschelli, L., Berardinelli, A., Dabbou, S., Ragni, L., Tartagni, M., 2021. Sensing Technology for Fish Freshness and Safety: A Review. Sensors 21, 1373.

Nelson, S., 2010. Fundamentals of Dielectric Properties Measurements and Agricultural Applications. J. Microw. Power Electromagn. Energy Publ. Int. Microw. Power Inst. 44, 98–113.

Renshaw, R.C., Dimitrakis, G.A., Robinson, J.P., Kingman, S.W., 2019. The relationship of dielectric response and water activity in food. J. Food Eng. 244, 80–90.

Sosa-Morales, M.E., Valerio-Junco, L., López-Malo, A., García, H.S., 2010. Dielectric properties of foods: Reported data in the 21st Century and their potential applications. LWT - Food Sci. Technol. 43, 1169–1179.

PHENOTYPIC AND GENOMIC CORRELATIONS BETWEEN FEED EFFICIENCY AND METABOLIC TRAITS IN EUROPEAN SEA BASS

M. Besson[a*], D.J. McKenzie[b], J. Nati[b], G. Salou[b], A. Vergnet[b], J. Brunier[c], A. Bajek[c], P. Haffray[a], M. Vandeputte[b,d] and F. Allal[b]

[a] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[b] MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34250 Palavas-les-Flots, France
[c] Ecloserie Marine de Graveline Ichtus, Route des Enrochements, 59820 Gravelines, France
[d] INRAE, GABI, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[*] Presenting author: mathieu.besson@inrae.fr

Introduction

Improvement of feed efficiency is an important factor in sustainability of the finfish aquaculture industry. Improving feed efficiency via selective breeding requires an ability to identify the most efficient individuals among selection candidates. A method recently developed for the European sea bass (*Dicentrarchus labrax*) is based on individual rearing of fish in aquariums under restricted feeding (Besson et al., 2019). In Besson et al., (2019), we showed that individual feed efficiency in the aquariums had a significant genetic basis (genomic $h^2 = 0.47$). There is a need, however, for greater understanding of physiological mechanisms underlying individual variation in feed efficiency in fishesdespite its potential. This paper reviews past work to improve FE in fish using selective breeding and assess future directions. Direct selection on FE traits requires methods to measure individual feed consumption and estimate FE efficiently and accurately. This is particularly difficult to do in fish because of the environment in which they live. Many of the published studies on FE were found to be inaccurate because of methodological problems. The relatively low heritability estimates of FE traits in fish published to date are probably partly as a result of inaccurate measurements of feed intake. Improving ways to measure the individual feed intake with high accuracy will be critical to the successful application of genetics to improving FE. Indirect selection criteria that could be used to improve FE (including growth after starvation/refeeding, body composition, neuropeptides or hormone levels. It is generally assumed that growth and, more specifically, lean growth are major contributing factors to individual feed efficiency (Knap and Kause, 2018). However, growth and body composition are only expected to explain 60-87 % of individual variation in feed efficiency in fishes; the remaining variation may be caused by individual basal metabolic rate, immune response functions, physical activity, and responses to social factorsinflammatory challenges or lactation have been studied. After nine generations of selection, the divergent selection for RFI led to highly significant ($P<0.001$. Our aim was therefore to explore the phenotypic and genomic correlations between individual feed efficiency and metabolic traits in European sea bass.

Material and Methods

First, 458 sea bass were phenotyped in aquariums to estimate their feed efficiency following the procedure of Besson et al., (2019). Briefly, the fish stayed 3 periods of 2 weeks in 10L individual aquariums. Fish were individually fed a restricted daily ration based on their body weight and corresponding to 50% of the optimal ration given by the feed manufacturer (Le Gouessant – NEO MARIN). Because feed was restricted, individual feed efficiency was estimated as the residual of the final body weight on initial body weight at the start of the experiment (rFBW).

Second, 934 sea bass (336 previously phenotyped in aquariums and 598 of their sibs) were placed, in consecutive batches, in 32 individual respirometry chambers. Measurements of oxygen uptake (MO_2) were made by intermittent stopped-flow respirometry as described in McKenzie et al. (2014). The procedure was based on 15-minutes cycles comprising 8 minutes stopped-flow and 7 minutes flushing with aerated water. Water oxygen levels in the chambers were measured every ten seconds. During stopped-flow, oxygen uptake by the fish was calculated as the slope of the decrease in oxygen quantity over the 8 minutes (MO_2 in mg O2 h^{-1}). Measurements were done for 36h following 12h of acclimation allowing us to potentially estimate 144 MO_2 values per fish. With all the values of MO_2 obtained, we estimated the standard metabolic rate (SMR in mg O_2 h^{-1}) of the fish by averaging the lowest 10% of the MO_2 measurements. After the 48h, fish were weighed to correct the SMR for the effect of metabolic body weight ($BW^{0.8}$) and generate residual SMR (rSMR).

All fish were genotyped on the 57k SNP array DlabChip (Griot et al., 2021). Genomic links between fish enabled the estimation of genetic parameters of rFBW and SMR and genetic correlations between those traits using AIREMLF90 software.

(Continued on next page)

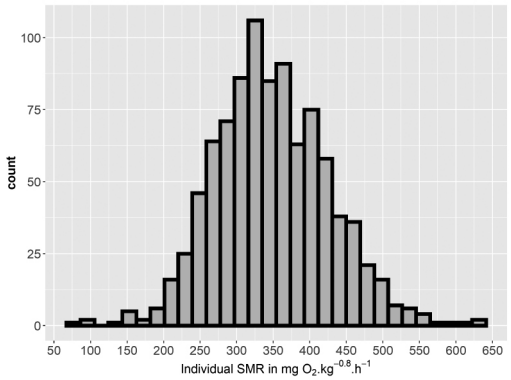


Figure 1. Histogram of SMR for the 934 sea bass phenotyped in the metabolic chambers.

Table 1. Genomic heritability (on the diagonal) of residual body weight gain in aquarium (rFBW) and residual standard metabolic rate (rSMR). Above the diagonal is the genomic correlation between the two traits.

	rFBW	rSMR
rFBW	0.23 (0.08)	-0.77 (0.41)
SMR		0.10 (0.04)

Results

We found large phenotypic variation in SMR (Figure 1). The mean SMR was 354 mg O2.kg-0.8.h-1 with a CV of 27%. Additionally, despite being weak, the phenotypic correlation between rFBW and rSMR was significant (r = -0.15, $F_{1,335}$ = 7.45, p-value = 0.0066). This correlation suggests that the most efficient individuals were also those with low standard metabolic rate, as expected.

Both rFBW and rSMR were heritable (Table 1) and the genomic correlation between them was strong and negative (-0.77 ± 0.41). This result supports the phenotypic correlation found between the two traits and indicates that SMR plays a role in the genetic basis of feed efficiency in sea bass.

Discussion

This study confirms that the individual efficiency under restricted feeding in aquariums is heritable. Furthermore, to our knowledge, this is the first study to phenotype several hundreds of fish for their individual oxygen consumption, allowing reliable estimation of genomic parameters for rSMR. We found that rSMR had a genetic basis and that individual feed efficiency of sea bass was partly explained by their metabolic rate. This finding is valuable for sustainable fish farming, in understanding how the physiology of fishes may relate to their robustness and efficiency.

Acknowledgements

SELFIE – Selection for Feed Efficiency project (n° P FEA 4700 18FA 100 0021) was funded by the French Government and the European Union (EMFF, European Maritime and Fisheries Fund) at the "Appels à projets Innovants" managed by the France Agrimer Office.

References

Besson, M., F. Allal, B. Chatain, A. Vergnet, F. Clota, and M. Vandeputte. 2019. Combining individual phenotypes of feed intake with genomic data to improve feed efficiency in sea bass. Front. Genet. 10:219. doi:10.3389/fgene.2019.00219.

Griot, R., F. Allal, F. Phocas, S. Brard-Fudulea, R. Morvezen, A. Bestin, P. Haffray, Y. François, T. Morin, C. Poncet, A. Vergnet, S. Cariou, J. Brunier, J.S. Bruant, B. Peyrou, P.A. Gagnaire, M. Vandeputte. 2021. Genome-wide association studies for resistance to viral nervous necrosis in three populations of European sea bass (*Dicentrarchus labrax*) using a novel 57k SNP array DlabChip. Aquaculture 530, 735930

Knap, P. W., and A. Kause. 2018. Phenotyping for genetic improvement of feed efficiency in fish: Lessons from pig breeding. Front. Genet. 9:184. doi:10.3389/fgene.2018.00184.

McKenzie, D. J., A. Vergnet, B. Chatain, M. Vandeputte, E. Desmarais, J. F. Steffensen, and B. Guinand. 2014. Physiological mechanisms underlying individual variation in tolerance of food deprivation in juvenile European sea bass, Dicentrarchus labrax. J. Exp. Biol. 217:3283–3292. doi:10.1242/jeb.101857.

GENETIC CORRELATIONS BETWEEN PROCESSING TRAITS AND INDIVIDUAL FEED EFFICIENCY IN A GILTHEAD SEA BREAM (*Sparus aurata*) COMMERCIAL LINE

A. Bestin*[a], M. Besson[a], A. Vergnet[d], F. Allal[d], F. Clota[c,d], S. Cariou[b], J.-S. Bruant[b], P. Haffray[a], M. Vandeputte[c,d]

[a] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[b] FMDS (Ferme Marine du Soleil), 17840 La Brée-les-Bains, France
[c] INRAE, GABI, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[d] MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34192 Palavas-les-Flots, France
E-mail: anastasia.bestin@inrae.fr

Introduction

In fish farming, costs of feed range from 30 to 70% of the total production cost (Doupé and Lymbery, 2004; Kolstad et al., 2004). The gilthead sea bream (*Sparus aurata*) is the most important species of Mediterranean aquaculture, with 228,000 tons produced in 2018 (FAO, 2020). Feed efficiency is a key driver of profitability in this species, and its improvement would contribute to reduce environmental impacts of farming. Selective breeding could be used to improve this trait, provided individual phenotypes of feed efficiency are available. An experimental setting was implemented for this purpose at Ifremer (Palavas-les-Flots, France) and tested in a sea bream population of the FMDS line as described in Besson et al. (2019) for sea bass. We estimated then the heritability of individual feed efficiency, production traits and their genetic correlations.

Material and Methods

The cohort was derived from the FMDS breeding program in September 2018, using 61 sires and 28 dams mated in a partial full factorial design. The fertilized spawn of each dam was incubated separately. Larvae were transferred and reared in a single tank to minimize environmental variance. About 800 fish (~2.7g, at random) were individually tagged at 91 days post hatching (dph) and sent to Ifremer to undergo the individual feed efficiency trial, starting at 161 dph. About 1530 of their sibs were tagged and sent in a sea-cage for growing from 128 to 432 dph. All progeny and their parents were tissue-sampled for DNA parentage assignment (cage-reared fish) or for genotyping with the DlabChip 57K SNP chip for fish tested for individual feed efficiency.

From the feed efficiency trial we obtained the individual residual body weight gain (rBWG) as a proxy of individual feed efficiency according to this formula:

$$\mathrm{rBWG} = \mathrm{BWG} - (\beta_0 + \beta_1 \times \mathrm{MBW} + \beta_2 \times \mathrm{IFI})$$

MBW is the initial metabolic body weight of the fish ($BW^{0.8}$). β_0 is the regression intercept, β_1 is the partial regression coefficient of an animal's BWG on its metabolic weight and β_2 is the partial regression coefficient of an animal's BWG on IFI, its cumulated feed intake during the trial period.

From the slaughtering after sea-cage rearing we obtained Harvest Body Weight (HBW), Fat content (Distell® FatMeter, Scotland), gutted carcass weight and headless and gutted carcass weights, which were regressed on HBW to obtain phenotypic and genetic parameters for residual headless and gutted carcass weight (rHC_W) and residual carcass weight (rC_W) as surrogates for the yield of those body parts relative to HBW.

Heritabilities and genetic correlations for all traits were computed based on multivariate linear mixed animal models fitted by restricted maximum likelihood in AIREMLF90 using a single-step approach, using genomic information for fish with individual feed efficiency phenotypes and pedigree information for cage-reared fish (Misztal et al., 2002).

Results and Discussion

As shown in Table 1, individual feed efficiency is a heritable trait in sea bream (0.23 ± 0.09). None of the processing traits measured at slaughtering in the sea cage was significantly genetically correlated with feed efficiency. Nonetheless, there might be a tendency where leaner fish have a higher feed efficiency (genetic correlations of -0.20 with Fat and +0.55 with rHC_W). Efficient fish may also be the fastest growers (genetic correlation of +0.22 between harvest body weight and feed efficiency).

(Continued on next page)

Table 1: Heritabilites and genomic correlations of processing traits on cage-reared sibs with individual residual body weight gain. Heritabilites are on the diagonal and genomic correlations are above.

rBWG	HBW	Fat	rC_W	rHC_W
0.23 (0.09)	0.22 (0.56)	-0.20 (0.35)	0.24 (0.30)	0.55 (0.44)
	0.23 (0.08)	0.59 (0.24)	0.68 (0.18)	0.98 (0.12)
		0.25 (0.08)	0.02 (0.25)	0.16 (0.48)
			0.40 (0.09)	0.79 (0.31)
				0.16 (0.07)

Conclusion

Individual feed efficiency measured in a commercial line of sea bream is a heritable trait, which can thus be improved by selective breeding. This first result in sea bream supports the study by Besson et al. (2019) on sea bass in which individual feed efficiency traits measured in aquaria were also heritable. This trait is positively genetically correlated with growth and processing traits, which would enable selection on feed efficiency without impairing other production traits of interest.

Acknowledgement

The data presented here were obtained in the PerformFISH project which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 727610. This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein.

References

Besson, M., Aubin, J., Komen, H., Poelman, M., Quillet, E., Vandeputte, M., Van Arendonk, J.A.M., De Boer, I.J.M., 2016. Environmental impacts of genetic improvement of growth rate and feed conversion ratio in fish farming under rearing density and nitrogen output limitations. J. Clean. Prod. 116, 100–109. https://doi.org/10.1016/j.jclepro.2015.12.084

Besson, M., Allal, F., Chatain, B., Vergnet, A., Clota, F., Vandeputte, M., 2019. Combining Individual Phenotypes of Feed Intake With Genomic Data to Improve Feed Efficiency in Sea Bass. Front. Genet. 10, 1–14. https://doi.org/10.3389/fgene.2019.00219

Doupé, R.G., Lymbery, A.J., 2004. Indicators of genetic variation for feed conversion efficiency in black bream. Aquac. Res. 35, 1305–1309.

Kolstad, K., Grisdale-Helland, B., Gjerde, B., 2004. Family differences in feed efficiency in Atlantic salmon (Salmo salar). Aquaculture 241, 169–177.

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D.H., 2002. BLUPF90 and related programs (BGF90), in: Proceedings of the 7th WCGALP. Montpellier, pp. 28–071.

DIETARY METHYLMERCURY AND SELENIUM INTERACTIONS IN RAINBOW TROUT JUVENILES (*Oncorhynchus mykiss*)

M. Bidon[1]*, C. Marchan Moreno[2], K. El Hanafi[2], S. Queipo Abad[2], J. Roy[1], D. Amouroux[2], M. Bueno[2], Z. Pedrero-Zayas[2], S. Fontagné-Dicharry[1]

[1]INRAE, Univ. Pau & Pays Adour, E2S UPPA, NUMEA, 64310 Saint-Pée-sur-Nivelle, France
[2]Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les matériaux, Pau, France
E-mail: marius.bidon@inrae.fr

Introduction
Mercury (Hg), especially the organic, methylated form (MeHg), is a toxic heavy metal that can accumulate in large amounts in fish through time in various organs, like liver and brain. Brain, with neurons, is the principal target of MeHg toxicity (Ni et al. 2012). MeHg induces its toxicity through induction of oxidative stress and inflammation (Morcillo et al. 2017). Selenium (Se), an essential micronutrient mainly involved in antioxidant protection, has been shown to reduce MeHg toxicity. However, Se and MeHg interactions are far to be totally understood. As relatively high levels of these two elements can be found in marine ingredients, a better understanding of their interactions is important to improve aquafeed formulations.

Material and Methods
Six plant protein-rich diets were formulated to contain two different MeHg levels (0 and 2.5 mg Hg/kg in HgC and HgH diets) combined with 3 different Se levels and forms (0.3 mg Se/kg in SeC diets and 2.6 mg Se/kg supplied either as sodium selenite in SeI diets or as L-selenomethionine from Orffa in SeO diets). Each diet was distributed to 3 replicate tanks of 50 fish (initial mean body weight: 26±1 g) to apparent satiation over a 3-week feeding trial at a water temperature of 17.5°C. Fish growth performance (n=3 tanks/diet) was analysed as well as total Hg and Se contents (n=3 individuals/diet) and relative gene expression levels (RT-PCR, n=9 individuals/diet) in liver and brain.

Results
Reduced growth performance of fish fed MeHg was recorded only within the non-Se supplemented SeC-group (Table 1). Feeding MeHg significantly increased total Hg concentrations in both liver and brain but to a significant smaller extent in liver when fish were supplemented with Se. Along with the hepatic increase of total Hg, dietary MeHg supplementation decreased total Se content in liver. Dietary inorganic Se supplementation increased total Se concentrations in liver to a larger extent than dietary organic Se supplementation but inorganic Se intake did not increase significantly Se content in brain contrary to organic Se intake.

In liver, MeHg intake increased transcript levels of enzymes involved in cellular stress response and antioxidant defences such as GSTπ and MsrB3 (Table 1). Both dietary organic and inorganic Se supplementation increased hepatic transcript levels of the selenoprotein GPX1a and also SelPa1 but only in HgC groups. In brain, dietary MeHg supplementation decreased transcript levels of RBFOX3, a neural marker, and up-regulated expression of IL-1β, a pro-inflammatory cytokine.

Discussion and conclusion
The growth inhibition by MeHg after only 21 days of feeding could be due to the prooxidant and proinflammatory properties highlighted by gene expression analysis in brain and liver. Fighting against MeHg-induced oxidative stress and inflammation consumes energy that is thus less available for growth (Morcillo et al. 2017). The mitigation by dietary Se supplementation that promoted antioxidant genes lends further support to this hypothesis.

Indeed, at the transcript level we found that MeHg induced inflammation in brain and antioxidant defences in liver to fight against oxidative stress. The antioxidant support by dietary Se supplementation in plant-based diets at the transcript level, in agreement with previous studies (Fontagné-Dicharry et al., 2020), could provide better protection against xenobiotics like MeHg in rainbow trout juveniles, even if the controlled genes by dietary MeHg and Se are not systematically similar and deserved further investigation for a better understanding.

(Continued on next page)

Table 1. Effects of dietary MeHg and Se supplementation and their interaction on growth performance, total Hg, total Se and gene expression (normalised with luciferase and EF1α to the control group HgC-SeC) in liver and brain of rainbow trout juveniles fed for 3 weeks using two-way ANOVA. GSTπ, glutathione S-transferase; MsrB3, methionine sulfoxide reductase B3; GPX1a, glutathione peroxidase 1a; SelPa1, selenoprotein Pa1; IL-1β, interleukin 1β; RBOX3, RNA binding fox-1 homolog 3.

	Dietary MeHg group		Dietary Se group			Interaction
	HgC	HgH	SeC	SeI	SeO	
Final mean body weight (g)	42.7	41.9	41.7	42.6	42.6	**0.025**
Liver total Hg content (µg/g)	0.3^{b}	5.9^{a}	4.1^{y}	2.5^{z}	2.6^{z}	**0.008**
Liver total Se content (µg/g)	18.8^{a}	16.0^{b}	2.6^{z}	33.4^{x}	16.1^{y}	**0.002**
Brain total Hg content (µg/g)	0.4^{b}	3.6^{a}	1.9	1.8	2.2	0.624
Brain total Se content (µg/g)	1.2	1.1	0.8^{y}	1.0^{y}	1.7^{z}	0.920
Liver GSTπ transcript level	1.4^{b}	2.1^{a}	1.4	2.1	1.8	0.858
Liver MsrB3 transcript level	1.9^{b}	2.4^{a}	1.8	2.3	2.3	**0.040**
Liver GPX1a transcript level	1.3	1.3	1.1^{y}	1.4^{z}	1.4^{z}	0.230
Liver SelPa1 transcript level	1.5	1.6	1.5	1.6	1.7	**0.002**
Brain IL-1β transcript level	1.6^{b}	2.5^{a}	1.7	2.2	2.3	0.190
Brain RBFOX3 transcript level	1.1^{a}	1.0^{b}	1.0	1.1	1.0	0.740

The present results provide evidence of interactions between dietary MeHg and Se on tissue Hg and Se concentrations, especially in liver. The difference of Hg and Se storage between the two tissues suggest post-absorptive interacting effects between Hg and Se maybe at the depuration level due to their strong affinity for each other as suggested by Amlund et al. (2015) in zebrafish but do not exclude interactive effects at the digestive or absorption level that deserve further investigation. As muscle, the edible part, has been shown to readily accumulate MeHg (Berntssen et al. 2004), this tissue should be included in the study to ascertain this finding with different dietary Se levels and forms.

Our data showed that the dietary level of 2 mg Hg/kg diet supplied as organic mercury induced adverse effects as early as 21 days of feeding, especially in Se-deficient diets, in agreement with the threshold levels of dietary MeHg set as 5 mg Hg/kg by Berntssen et al. (2004) for salmonid diets. In brain, our RBFOX3 results confirm the high sensitivity of neurons to MeHg toxicity (Ni et al. 2014). Longer exposure, for example up to 6 months, should be assessed in rainbow trout juveniles for a better understanding on the adverse effects of MeHg and the interacting effects with dietary Se.

Acknowledgements

This project was supported by the French National Research Agency (ANR-18-CE34-0004), the Region Nouvelle-Aquitaine (BENESEL) and I-site E2S UPPA (contract 2020-32) and has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska- Curie grant agreement No. 101007962.

References

Amlund, H., Lundebye, A.-K., Boyle, D., Ellingsen, S., 2015. Dietary selenomethionine influences the accumulation and depuration of dietary methylmercury in zebrafish (*Danio rerio*). Aquat. Toxicol. 158: 211-217.

Berntssen, M.H.G., Hylland, K., Julshamn, K., Lundebye, A.-K., Waagbø, R., 2004. Maximum limits of organic and inorganic mercury in fish feed. Aquac. Nutr. 10: 83-97.

Fontagné-Dicharry, S., Véron, V., Larroquet, L., Godin, S., Wischhusen, P., Aguirre, P., Terrier, F., Richard, N., Bueno, M., Bouyssière, B., Antony Jesu Prabhu, P., Tacon, P., Kaushik, S.J., 2020. Effect of selenium sources in plant-based diets on antioxidant status and oxidative stress-related parameters in rainbow trout juveniles under chronic stress exposure. Aquaculture 529: 735684.

Morcillo, P., Esteban, M.A., Cuesta, A., 2017. Mercury and its toxic effects on fish. AIMS Environ. Sci. 4: 386-402.

Ni, M., Li, X., Rocha, J.B.T., Farina, M., Aschner, M., 2012. Glia and methylmercury neurotoxicity. J. Toxicol. Environ. Health A 75: 1091-1101.

USING OSMOTIC PUMPS TO INDUCE SEXUAL MATURATION IN MALES AND FEMALES OF EUROPEAN EEL (*Anguilla anguilla)*

M. Blanes-García*, P. García Salinas, M. Morini, L. Pérez, J.F. Asturiano, V. Gallego

Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n. Edificio 7G 46022, Valencia, Spain
marblaga@posgrado.upv.es

Introduction

The European eel (*Anguilla anguilla*) is a high commercial valued species for aquaculture. Nowadays, the control of the reproduction and the ability to produce eels seems to be the unique sustainable solution to reduce the pressure on eel natural populations (Kagawa et al., 2009; 2013). Traditionally, the European eel has been induced to sexual maturation by hormonal injections (Asturiano, 2020), but the weekly injections require repetitive handling, which causes stress to the fish. In this sense, the study of new methods must be a powerful tool to optimize the sexual maturation protocols in this species. The goals of this study were *i)* to validate osmotic pumps as a system to induce the sexual maturation in both sexes and *ii*) to evaluate the production and quality of gametes both from males and females.

Material and methods

Eels were moved to our facilities in the Universitat Politècnica de València, Spain. Regarding males, the eels were divided in Control, OP-100 and OP-200 groups (n=10 per group). The control group received weekly injections of hormonal treatment (hCG_{rec}, 1.5 IU/g fish) for 15 weeks. The induction of sexual maturation of osmotic pumps (OP) groups was carried out by implanted hCG_{rec}-loaded osmotic pumps (ALZET 1004 and ALZET 2006, respectively). Sperm samples were collected weekly by stripping and several sperm parameters were assessed using software CASA. Biometric parameters such as fin color and gonadosomatic index were evaluated

Concerning females, the eels were divided in Control (n=11) and OP-2ML4 group (n=10). The control group received weekly injections carp pituitary extract (CPE; 20 mg/kg fish) for 15-20 weeks. The induction of sexual maturation of OP-2ML4 was carried out by CPE-loaded osmotic pump ALZET 2ML4. The females were weekly monitoring and samples of oocytes were collected using a cannula. The ripe females were carried out to spawning using DHP injections. The eggs were collected by stripping and some fertilization trials were done. Biometric parameters such as gonadosomatic index were also evaluated.

Results and discussion

Concerning males, outcomes from study showed that the implantation of osmotic pumps ALZET 1004 and ALZET 2006 were able to induce the sexual maturation and gamete production in European eel males (Fig. 1A). In that sense, OP groups reached gonadosomatic index values of 2.23 ± 0.81 (OP-100) and 3.06 ± 1.43 (OP-200), also showing a fin colour darker throughout the maturation treatment.

Regarding gamete production, while the Control group generated 90% of spermiating males from 8th week, and sperm samples from this group showed high quality (>70% of motility) during the peak of the spermiation period (12-15th week), the Osmotic Pumps groups (OP-100 and OP-200) did not show enough values of volume (Fig. 1B), density (Fig. 1C), and motility (Fig. 1D).

Concerning females, the use of osmotic pumps also became as an effective method to induce the sexual maturation. The 80% of females of OP-2ML4 group reached the sexual maturation and the 50% showed an unusual early maturation between 4th and 10th week (Fig. 2). Several females were successfully induced to spawning, reaching embryos stage.

Therefore, this study showed that the use of osmotic pumps was able to induce sexual maturation, spermiation and ovulation on European eel. In the case of males, results showed that osmotic-controlled release delivery system have not still enough potential to produce acceptable values of sperm quality parameters for aquaculture purposes. However, in European eel females the osmotic pumps were able to generate spawning females so this method could become and viable alternative for eel hatchery procedures.

(Continued on next page)

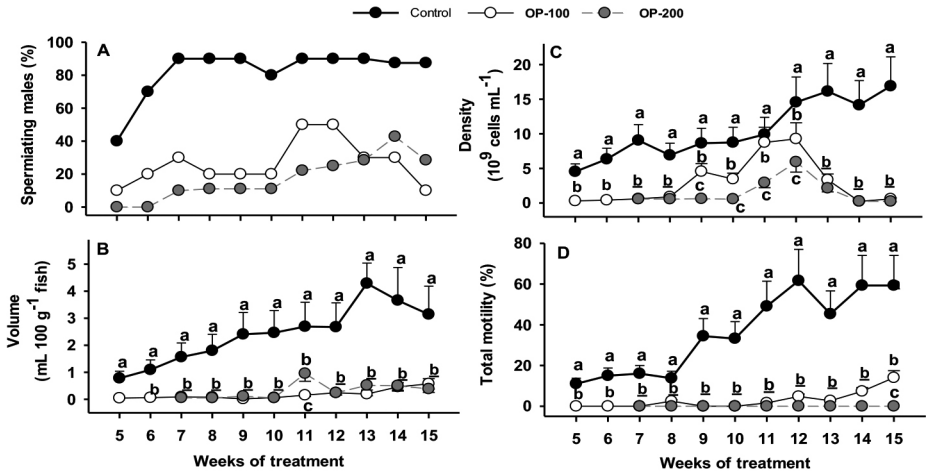


Figure 1. Evolution of sperm production parameters throughout the hormonal treatments (Control, OP-100 and OP-200): A) Spermiating males; B) Sperm volume; C) Sperm density; and D) Percentage of motile cells. Different letters mean significant differences.

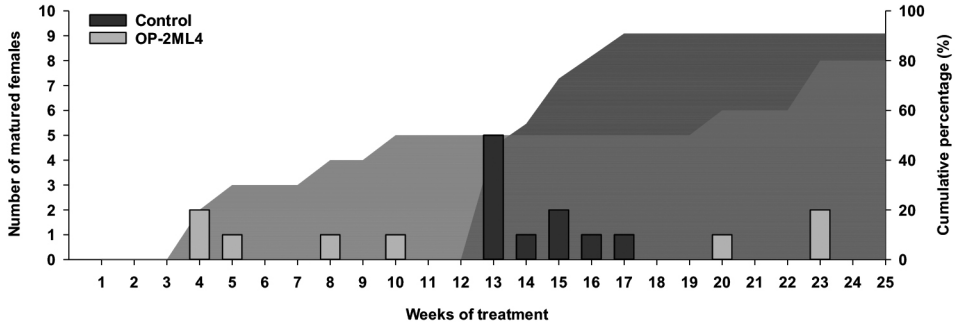


Figure 2. Bars show the number of mature females in each week of treatment (Control vs OP-2ML4), and shaded area shows the accumulative percentage of mature females.

Bibliography

Asturiano, J.F., 2020. Improvements on the Reproductive Control of the European Eel, in: Yoshida, M., Asturiano, J. F. (Eds.), Reproduction in Aquatic Animals: From Basic Biology to Aquaculture Technology. pp. 293–320.

Kagawa, *et al*. 2013. Using osmotic pumps to deliver hormones to induce sexual maturation of female Japanese eels, *Anguilla japonica*. Aquaculture 388–391, 30–34.

Kagawa *et al*. 2009. Effects of continuous administration of hCG, salmon pituitary extract, and gonadotropin-releasing hormone using osmotic pumps on induction of sexual maturation in male Japanese eel, *Anguilla japonica*. Aquaculture 296, 117–122.

FIRST SPERM QUALITY ASSESSMENT AND CRYOPRESERVATION OF TWO ENDANGERED SPECIES: IBERIAN TOOTHCARP (*Aphanius iberus*) AND VALENCIA TOOTHCARP (*Valencia hispanica*)

M. Blanes-García[a*], P. Risueño[b], L. Pérez[a], J.F. Asturiano[a], V. Gallego[a]

[a]Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n. Edificio 7G 46022, Valencia, Spain
vicgalal@upvnet.upv.es
[b]Centro de Conservación de Especies Dulceacuícolas de la Comunitat Valenciana (CCEDCV). Plaza 04, El Palmar, Valencia, Spain.

Introduction

The sensitive state of conservation of several endemic fish species such as Iberian toothcarp (*A.iberus*) and Valencia toothcarp (*V. hispanica*) has led local government to consider the implementation of conservation measures to preserve their populations (GVA, 2015). In fact, they are included in the IUCN Red List of Threatened Species, classified as "Endangered" (*A. iberus*) and "Critically endangered" (*V. hispanica*).

The *in-situ* measurements carried out during the last few decades have been successfully supplemented with the *ex-situ* conservation action (captive breeding programs and periodic repopulations) carried out at the Center for the Conservation of Freshwater Species of the Valencian Community (CCEDCV, El Palmar, Valencia). However, the limited knowledge about the reproductive biology of these species makes necessary to investigate different aspects of their reproductive cycle, to improve their population management. Besides, to complement the conservation tasks, one strategy currently applied in fish management is the creation of genetic resources banks (GRBs) by cryobiology techniques (Martínez-Páramo et al., 2017). In this sense, the main objectives of this work were *i*) to improve the knowledge on the breeding biology of both species, and *ii*) to develop protocols for the conservation of gametes for the future management and conservation.

Material and methods

Temporal series of samplings were carried out in different places in the Comunitat. Valenciana (Spain) for catching the fish. Sperm samples were collected by stripping. Sperm motion (motility and velocity) and sperm morphometric parameters were assessed by using CASA and ASMA software, respectively (see Gallego & Asturiano; 2018). A protocol based on PBS medium and 10% (v/v) of methanol as cryoprotectant was applied for cryobanking the sperm of these threatened species, where high quality sperm samples (>60% motility) were used at the beginning of the trial.

Results and discussion

Sperm kinetic patterns were similar in both species, which showed high motility values until 30 s post-activation, reaching maximum motility values of 71.2 and 74.8% (*A. iberus,* Fig. 1A; *V. hispanica*, Fig. 1C). Both species displayed high curvilinear velocity values at the beginning of the activation process, reaching the highest values of 169.5 and 198.3 μm/s (*A. iberus,* Fig. 1B; *V. hispanica*, Fig. 1D). Spermatozoa from both species showed similar features to other external fertilizers belonging to Cyprinodontiformes, with spherical heads, uniflagellated and without acrosomes (Table 1).

Cryopreserved samples showed lower motility than fresh samples but reaching acceptable percentages of motile cells after thawing of around 20 and 25% (*A. iberus* and *V. hispanica*, respectively). Several authors have reported in other fish species with similar technical limitations (hard management, tiny sperm volume, *etc*), similar motility results in cryopreservation trials (Diogo et al., 2018; Fernandes et al., 2019).

This study improves our knowledge on the reproductive biology of *A. iberus* and *V. hispanica* by reporting sperm motion parameters and spermatozoa morphometric features. In addition, this study is the first of its kind to achieve gamete cryopreservation of these two endemic and endangered fish species. These are all new tools which can be used to complement the management and conservation programs that are being developed.

(Continued on next page)

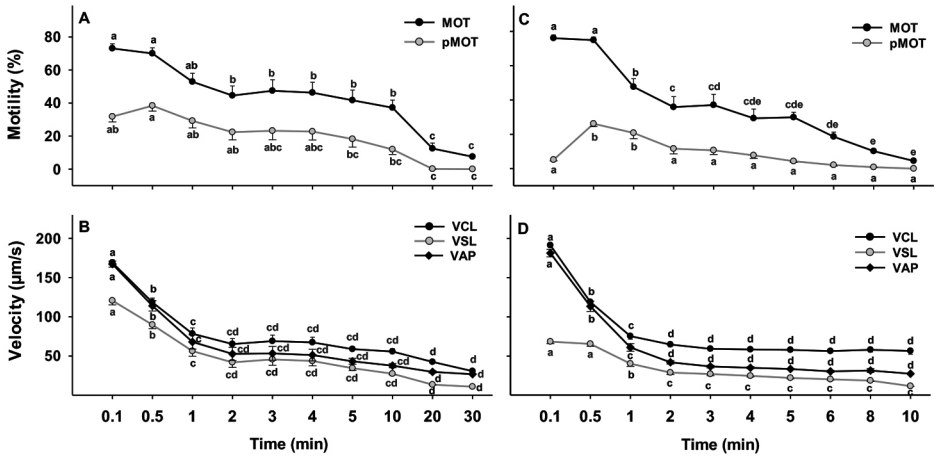


Figure 1. Sperm kinetic parameters in *A. iberus* (A and B; n=7) and *V. hispanica* (C and D; n=13) at different post-activation times. Data are expressed as the mean ± SEM. Different letters mean significant differences over time (p-value ≤ 0.05).

Table 1. Morphometric parameters of sperm head of *A. iberus* (n=9) and *V. hispanica* (n=8) measured with ASMA software. Data are expressed as the mean ± SEM. Different letters mean significant differences between species (p-value ≤ 0.05).

	A. iberus	***V. hispanica***
Area (µm^2)	4.84 ± 0.06	4.81 ± 0.04
Perimeter (µm)	8.04 ± 0.05	8.02 ± 0.04
Length (µm)	2.55 ± 0.01	2.55 ± 0.01
Width (µm)	2.35 ± 0.01	2.32 ± 0.01
Ellipticity	1.09 ± 0.01 ^{b}	1.10 ± 0.004 ^{a}
Elongation	0.04 ± 0.001 ^{b}	0.05 ± 0.002 ^{a}
Rugosity	0.95 ± 0.001 ^{a}	0.94 ± 0.001 ^{b}
Regularity	0.97 ± 0.003	0.97 ± 0.004

Bibliography

Diogo, P., et al. 2018. Electric ultrafreezer (-150ºC) as an alternative for zebrafish cryopreservation and storage. Fish Physiol. Biochem. 44, 1443-1455.

Fernandes M.D.O., et al. 2019. Cryopreservation of sperm in annual fish *Austrolebias minuano* (Cyprinodontiformes; Rivulidae). Aquaculture Research, 1-8.

Gallego, V., & Asturiano, J. F. (2018). Sperm motility in fish: technical applications and perspectives through CASA-Mot systems. *Reproduction, Fertility and Development*, *30*(6), 820-832.

GVA, 2015. Technical report. Conservación de peces marismeños en la Comunitat Valenciana. Balance de 25 años de trabajo. Valencia, Spain: Direcció General de Medi Natural i d'Avaluació Ambiental.

Martínez-Páramo, S., et al. 2017. Cryobanking of aquatic species. Aquaculture 472, 156–177.

APPLICATION OF MARINE BASED COMPOUNDS IN CHEESE PRODUCTION: A REVIEW

Marijana Blažić[1]*, Dora Herljević[1], Sandra Zavadlav[1], Valentina Belavić[1], Ana-Marija Cikoš[2], Jurislav Babić[2], Karmen Matković Melki[3]

[1]Karlovac University of Applied Sciences, Trg J.J. Strossmayera 9, 47000 Karlovac, Croatia
[2]Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek, Croatia
[3]University College Aspira, Mike Tripala 6, 21000 Split, Croatia
E-mail: marijana.blazic@vuka.hr

Introduction

Marine organisms are subjected to various extreme environmental conditions (temperature, salinity, light, pH, nutrients availability) which affect their chemical composition and lead to the production of diverse metabolites that can be used as ingredients in industrial food production (Kim, 2015). It is important to find the appropriate extraction method of marine metabolites to preserve their biological potency as they give a nutritional value and health benefits when applied in the food products. Many marine-based food ingredients belong to the category of nutraceuticals, which are defined as bioactive substances that have physiological benefits or can provide protection against chronic diseases (Rasmussen and Morrissey, 2007).

Encapsulation

Rapid development of technology and increased consumer awareness of the close relationship between diet and health has forced food researchers to develop and create new products with functional properties (Waterhouse and Sun-Waterhouse, 2018). Among many ways of incorporating the beneficial compounds into the food, encapsulation is one of the most emerging. It is defined as a physicochemical or mechanical process of entrapping active agents within a carrier material (Kostov et al. 2016). This protective barrier is "temporarily isolating" the substances from the surrounding environment (e.g. light, temperature, moisture or oxygen) and enables its controlled release at a target site (Oliver and Augustin, 2009). Carrier materials, known as encapsulants, can be made of various carbohydrates, proteins and lipids.

Application in cheese

Encapsulation process finds its application in cheese production where extracted compounds from marine organisms can enhance functional properties of cheese. Various types of fish, microalgae, sponges, marine microorganisms, like bacteria and fungi, are capable of synthesizing polyphenols, polysaccharides, pigments, vitamins, minerals and other compounds that can be entraped and secured in microcapsules and then added in cheese during its production (Parte et al., 2017). For example, derived marine enzymes are extremely resistant to temperature and pH oscillations. Hence, they can provide numerous advantages over traditional enzymes used in cheese production. Proteinase can be used in protein coagulation of various cheese types or they can help removing the milk oxidized flavour (Rossano et al., 2011). Furthermore, marine polysaccharides, can improve cheese quality acting as stabilizers (Rasmussen and Morrissey, 2007), enhancing its texture, creaminess and controlling syneresis. Also, sulfated polysaccharides and polyphenol extracts exhibit good antioxidant and antimicrobial activities (Fleita et al., 2015) that contribute to the longer shelf-life of cheese. Various bioactives have antifungal properties against *Aspergillus* spp. that can cause spoilage and contamination of cheese by producing mycotoxins, such as aflatoxins that are known for their potential to cause liver damage and acute poisoning (Bhosale et al., 2000). Compounds isolated from the algae are known for their health benefits, for instance vitamins A and B, fatty acids and pigments are known for their body weight, blood pressure and blood sugar regulations (Krishna Koyande et al., 2019). The addition of calcium-rich algae in cheese can help in absorption of that element and prevent hypocalcemia (Ścieszka and Klewicka, 2018).

Conclusion

Incorporation of marine-based bioactive compounds into cheese would greatly contribute, not only to the technological and sensory properties of cheese, but also to the improvement of the consumers health, thus making it a possible new trend in cheese production.

(Continued on next page)

Acknowledgement
This work has been supported by the Croatian Government and the European Union through the European Structural and Investment Funds - the Competitiveness and Cohesion Operational Programme (KK.01.1.1.04.) under the project "Modification of cheese ripening process and development of whey-based products—SIRENA".

Literature:

Bhosale, S.H., Jagtap, T.G. & Naik, C.G. (2000). *Antifungal activity of some marine organisms from India, against food spoilage Aspergillus strains*. National Institute of Oceanography Dona Paula, Goa-403004, India. *Mycopathologia,147(3),133-0138*

Fleita, D., El-Sayed, M., & Rifaat, D. (2015). *Evaluation of the antioxidant activity of enzymatically-hydrolyzed sulfated polysaccharides extracted from red algae; Pterocladia capillacea. LWT - Food Science and Technology, 63(2), 1236–1244*.

Kim, S.-K. (2015). *Springer handbook of marine biotechnology. Springer-Verlag Berlin Heidelberg: Berlin. ISBN 978-3-642-53970-1. XLVI, 1512*

Kostov, G., Shopska, V., Denkova, R., Ivanova, M., Balabanova, T., & Vlaseva, R. (2016). *Encapsulation of plant and animal oils used in dairy industry: A review. Acta Universitatis Cibiniensis. Series E: Food Technology, 20(1), 21–40*.

Krishna Koyande, A., Chew, K. W., Rambabu, K., Tao, Y., Chu, D.-T., & Show, P.-L. (2019). *Microalgae: A potential alternative to health supplementation for humans. Food Science and Human Wellness*.

Oliver, C. M., & Augustin, M. A. (2009). *Using dairy ingredients for encapsulation. Dairy-Derived Ingredients,565-588*.

Parte, S., Sirisha, V. L., & D'Souza, J. S. (2017). *Biotechnological Applications of Marine Enzymes From Algae, Bacteria, Fungi, and Sponges*. Advances in Food and Nutrition Research, 75–106.

Rasmussen, R. S., & Morrissey, M. T. (2007). *Marine Biotechnology for Production of Food Ingredients. Advances in Food and Nutrition Research, 237–292*.

Rossano R, Larocca M, Riccio P. (2011). Digestive enzymes of the crustaceans Munida and their application in cheese manufacturing: a review. *Mar Drugs*; 9(7):1220-1231.

Ścieszka, S., & Klewicka, E. (2018). *Algae in food- a general review. Critical Reviews in Food Science and Nutrition, 1–23*.

Waterhouse, G. I. N., & Sun-Waterhouse, D. (2018). *Encapsulation Systems Containing Multi-Nutrients/Bioactives: From Molecular Scale to Industrial Scale. Reference Module in Food Science*.

JELLYFISH BASED NOVEL FOOD PRODUCTS IN EUROPE. A STORY IS STARTING

Ramires FA[1], De Domenico S[1,2], Gallo A[1], De Rinaldis G[1], Migoni D[2], Albano C[1], Fanizzi FP[2], Slizyte R[4], Angel D[3], Klun K[5], Javidpour J[6], Piraino S[2,7], Leone A[1,7], Bleve G[1]

[1]National Research Council, Institute of Science of Food Production (CNR-ISPA, Lecce), Via Prov.le Lecce – Monteroni, 73100 – Lecce, Italy
[2]Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Campus Ecotekne, S.P. 6, Lecce-Monteroni, 73100 - Lecce Italy
[3]Department of Maritime Civilizations, The Charney School for Marine Science, University of Haifa, Haifa, Israel
[4]SINTEF Ocean, 7465 Trondheim, Norway
[5]National Institute of Biology, Marine Biology Station, Piran, Slovenia
[6]University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark
[7]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), P.le Flaminio, 9, 00196 Rome, Italy

Introduction:
In Europe, as well as in many other western countries, jellyfish (JF) are not a traditional food, they are not usually consumed and the JF market is probably limited to Asiatic communities. The use and marketing of JF in Europe is still hindered by the regulation on novel food (Commission Regulation EU 2283/2015) and mainly by the absence of standard methods for the treatment and processing of the raw material according to the EU safety standards. JF is considered a traditional food in Asian countries, such as China, Japan, Korea, Thailand which host the majority of consumers and producers. Although JF did not make its way to European consumers, some JF species originating from the EU were exported to East Asia for consumption.

Traditionally JF are soaked in a mixture of NaCl and aluminum salts. This procedure reduces the water content and changes the JF gelatinous tissue into the consistency expected for the final edible product. This product is generally characterized by a crispy and firm texture highly appreciated by the Eastern market. However, aluminium, that is so extensively used in this procedure, is supposed to cause memory impairment and cognitive dysfunctions, which would lead to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Then, a reduction of dietary intake of aluminium is highly recommended. In Europe a Tolerable Weekly Intake (TWI) was established of 1 mg aluminium /kg body weight/week; however, the limits could even be more restrictive for overexposed populations.

Then, new processes have to be designed to obtain final products observing safety and quality standards, maintaining nutritional traits, having sensory properties suitable for Western consumers. This is particularly true for raw material that has no history as food in western Countries and specifically in the European area (Torri et al., 2019). In within the GoJelly H2020 project, a new process and jellyfish-based products were developed.

Material & Methods:
An accurate search of possible already existing methods for JF processing/treatment for food uses was performed. Then, the laws in force in terms of food safety and process hygiene criteria were considered to establish the most important quality and safety parameters, in compliance with European Union laws, to be applied to JF-based foods.

Rhizostoma pulmo was used as a model of edible JF for the setting-up of the processing procedure. Among all already available food additives and information on their possible use in EU, U.S.A., Australia and New Zealand, some of them were chosen for treating JF tissue, in order to reduce the development of enzymatic activities and undesired microorganisms and to improve the texture and nutraceutical traits of the semi-finished product.

Safety assays (microbiological, biogenic amines), physico-chemical (texture, pH, salinity), nutritional (fatty acids, protein content, antioxidant activity, elemental content, aminoacids, etc.) analyses were performed on JF treated samples. Accredited standard parameters established by the law in force regarding food safety and process hygiene criteria were also applied.

A completely new jellyfish processing chain for the design of human foods in a "western style" was developed at CNR-ISPA, Italy, on Mediterranean species, and treated JF were used to design and preparation of different new jellyfish-based food prototypes. They were tested for safety, quality, sensory traits, as well as for their shelf-life. The procedure was also tested on JF species sent to Italy from the other GoJelly partners.

(Continued on next page)

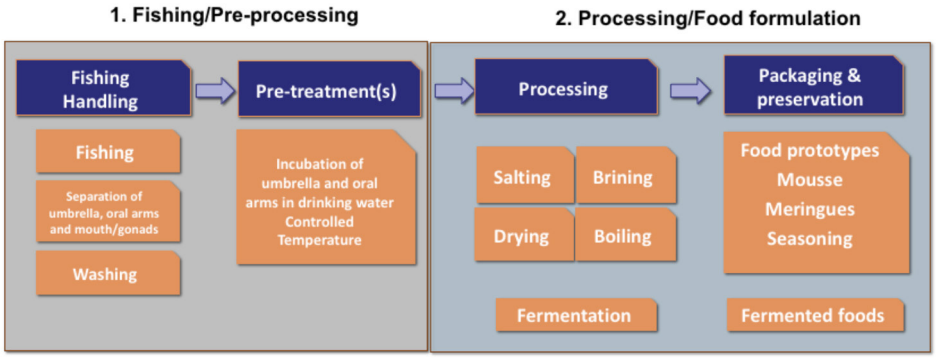


Figure 1. Flow chart of the new food chain for JF treatment for food uses developed at CNR-ISPA.

***Results**:*
In the absence of specific indications, a set of market safety standards in force in Europe for food products comparable to JF, together with diverse essential hygienic criteria valid for fish and fish-derived foods in some European Member States, were considered and successfully applied to the barrel JF, *Rhizostoma pulmo*, from the Ionian Sea Italian coasts, that was assessed as a model for human food.

A protocol for the stabilization and processing of *R. pulmo* JF into a semi-finished food product without using alum was developed at CNR-ISPA, Italy. Safety and quality parameters, together with a series of technological and nutritional traits were used to monitor and characterize the JF obtained products (Bleve et al., 2019). Some food thickening/stabilizing agents allowed in the EU were able to control possible microbial pathogens and spoilage species to increase the texture and nutritional traits.

Different new food prototypes obtained by treated JF were studied at laboratory scale: a) drying; b) cooking under vacuum; c) formulation of new JF-based foods. Laboratory-scale prepared food prototypes were tested for main quality and sensory traits and preliminary data on the final product shelf-life were produced. Finally in collaboration with professional chefs JF-based recipes were proposed.

Discussion & Conclusion:
A new flow chain for JF preparations, from the starting material to semi-finished food products, was developed (Figure 1).

The obtained products are very different from the traditional Asiatic ones. Still the possible production of JF as human food by replacing alum with salts allowed by the EU regulations as stabilizing and thickening agents, was demonstrated. This new procedure can offer new food chain opportunities based on JF and JF-derived foods to be considered novel food acceptable in the EU. A European Patent describing the developed procedure was deposited by CNR-ISPA, Italy, on July 2020.

The proposed method can also be applied for the stabilization/treatment of other abundant and putatively edible JF species in Mediterranean Basin and in other Seas. New JF based food prototypes following a "Western Style" have been designed.

Literature
Bleve, G., Ramires, F.A., Gallo, G. & Leone, A. 2019. Identification of Safety and Quality Parameters for Preparation of Jellyfish Based Novel Food Products. Foods 2019, 8(7), 263; https://doi.org/10.3390/foods8070263
Leone, A. 2020. European Jellyfish CookBook. @CnrEdizioni,2020, https://doi.org/10.48257/BLE-001
Torri, L., Tuccillo, F., Bonelli, S., Piraino, S. & Leone, A. 2019. The attitudes of Italian consumers towards jellyfish as novel food. Food Quality and Preference, 79 (2019) 103782, https://doi.org/10.1016/j.foodqual.2019.103782.

NEW GRADING DEVICE WITH NOVEL STRUCTURES INCREASES SELF- GRADING SUCCESS OF WHITELEG SHRIMPS *L. vannamei* IN AQUACULTURE CULTIVATION SYSTEMS

Mirko Bögner*, Amirhossein Karamyar, Christian W.G. Detsch, Matthew J. Slater

Alfred Wegener Institute Helmholtz Center for Polar- and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

*Corresponding author: E-Mail: Mirko.Boegner@awi.de

Introduction

Uneven growth of aquacultured shrimp leads to an increase in cannibalism, untargeted feeding and uneven product size. Size grading can prevent these effects. However, current sorting methods are highly invasive and cause high stress levels and flight responses. The animals can injure themselves and may even jump out of the tank.

In this study, a size grading device was developed that allows the animals to sort themselves voluntarily according to size in the cultivation tank. For this purpose, innovative structures were developed that are specifically adapted to the body shape and behaviour of the animals. These structures are conceptualised to either encourage or discourage passing of the grading device. Results demonstrate strong grading effects (75 %) on the distribution of shrimps between different tank compartments and the ability to separate shrimps of different size voluntarily (up to 85%). Small animals can be separated from the cohort without stress or any personnel effort and a uniform size distribution of the shrimps can be guaranteed.

This voluntary grading system is expected to increase animal welfare and growth performance in shrimp farms while it reduces labour.

Material and Methods

In the first experiments sorting elements (through which shrimp could pass) with different structures (tubes, wedges, bars, V- shaped) were installed in the middle of the experimental tanks (2.5 x 0.7 x 0.7 m) (N=3). The control group had a perforated sorting element plate as a subdivision (N=3). Twenty-five shrimp (18.4 g ± 2.9) were placed on each side of the tank and shrimp were of a size theoretically capable of passing the structures and perforated plates in both directions. The structures facilitate or make it harder to pass the device in one direction and thus divide the tank into a “sorting” and “non sorting” area. For each structure the distribution of the shrimps was documented hourly for 36 h. The most effective structure was used in subsequent experiment. In this experiment, twenty-five small (2.6 g ± 0.6) and twenty-five large shrimp (14.6 g ± 2.9) were placed in the “unsorted” area of the tank. The structures were narrowed so that only the small animals could cross it into the “sorting” area. Shrimps in both tank areas were counted hourly for 72 h.

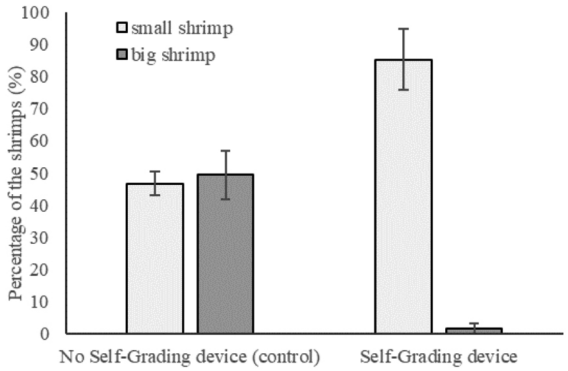


Fig.1: Proportion of large and small shrimp in the “sorting” area after 72 h using grading structure and without.

(Continued on next page)

Results and Conclusion

In the first experiments, tubular structures resulted in 73% of animals moved into the sorting area. The use of wedges (59%) and bars (57%) had little supporting effect. The combination of wedges and tubes caused 75% movement to the sorting area as did the V-shaped structure. In the second experiment with mixed size groups, the V-shaped structure caused about 85% of the small individuals to migrate into the sorting area (Fig. 1).

Tested devices with novel structures are effective and achieve high levels of self grading in shrimp.

UP-SCALLING THE APPLICATION OF HYDROGEN PEROXIDE AS DESINFECTION METHOD IN A COMMERCIAL RAS REARING ATLANTIC SALMON (*Salmo salar*): A CASE STUDY

Desislava Bögner*[1], Gregor Jähne[1], Kyra M. Böckmann[1], Matthew J. Slater[1]

[1] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
E-mail: desislava.boegner@awi.de

Introduction

Disinfection is a very important part of recirculation aquaculture systems (RAS). RAS allows for controllable environments in which main variables relevant to animal welfare and a successful production can be manipulated as required to improve efficiency and profitability. Common disinfection methods include chemical disinfectants, antibiotics, biocides, UV radiation and ozone. They can be used to treat disease outbreaks, or to reduce the bacterial load of the system which otherwise could lead to the overgrowth of potential pathogens or opportunistic bacterial groups competing with biofilter bacteria for space and resources. Ozone is the most used disinfection method requiring expensive technology and trained stuff. Hydrogen peroxide (H_2O_2) has been on focus as a "green" alternative. High H_2O_2 doses are associated with acute toxicity symptoms for some species. Low doses are harmless, offer additional system oxygenation and contribute to water quality improvement. After testing the use of low doses in a small research RAS, the present study aims to describe the first case study up-scaling a continuous hydrogen peroxide application to commercial fish production in RAS with focus on the determination of required concentrations, application monitoring and variations on microbiome composition.

Material and Methods

The present study was performed at RAS facilities of Danish Salmon A/S in Hirtshals, Denmark, one of the European pioneers in rearing salmon in land based aquaculture facilities and producing about 1.200 metric tons/year of Atlantic salmon (*Salmo salar*). Two identical Grow-out RAS with own water treatment elements and eight pre-grow tanks were used as treatment and control systems to compare the effects of continuous H_2O_2 application in combination with ozone to common operational practice. Oxygen Cones and additional aeration stones ensured the basic Oxygen supply in the tanks. Defined H_2O_2 quantities were applied with a dosing lance connected to a peristaltic pump and an International Bulk Container with PERSYNT® 50, (EVONIK Industries) into the distribution pipe feeding a collection tank from where the water was evenly distributed to all tanks. Based on previous experiments, a final dosing of about 20 l/h was projected. Water samples were collected at the start (REF) and after slowly enhancing the dosage over time (24h and 30d), for the determination of the total microbial count (certified chromogenic Compact Dry TC plates from R-Biopharm), microbial viability (BacLight Viability Kit) and bacterial community composition (FISH). Water parameters (Ammonia, Nitrite, Nitrate, Phosphate, COD, Turbidity and H_2O_2 concentration) and production related information (feeding rate, fish biomass, oxygen consumption, ozone production) were also regularly evaluated.

Results and Discussion

H_2O_2 application started on August 06, 2020 with 1 l/h (0.51mg/L) and was increased over time up to 14 l/h (7.09 mg/L) (Fig1). On September 30, 2020 the application was stopped due to detected changes on feeding behavior of the fish as well as incorrect redox measurements in the treatment system which could probably be attributed to accumulation of oxidative species not having enough organic material to react. The test and control systems had similar biomass during the experimental period (test: 34.9-52.5 tones and control 36.4-51.4 tones) and the feed intake was accordingly adjusted (mean feed intake test: 496 kg/day; control 450 kg/day). The oxygen demand registered on the treated system (82 l/min - 90 l/min) was lower than the control (104 l/min - 115 l/min). In general, there was a reduction of turbidity and decreased nitrogen species and phosphate in the treated system. System maintenance (biofilter cleaning and backwashing) might had influenced the COD and total microbial counts measurements. Total microbial counts reflected a steady increase in the number of CFU/ml in both systems (Fig.2) and evidenced microbial accommodation. There was an increased rate of microbial mortality according to the viability results with higher values in the treated system reflecting the longer exposure of this system to oxidative stress (Fig 3). The community composition varied according to the treatment (Fig.4) and changes in the abundance of the different bacterial groups analyzed could be attributed to variable vulnerability of members of these groups to the disinfection potential of H_2O_2 or its combination with ozone.

(Continued on next page)

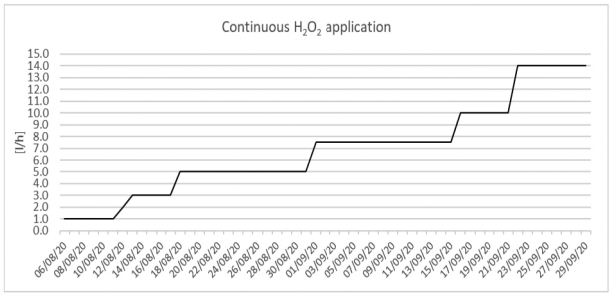


Fig.1 Hydrogen peroxide application in the initial phase of the study.

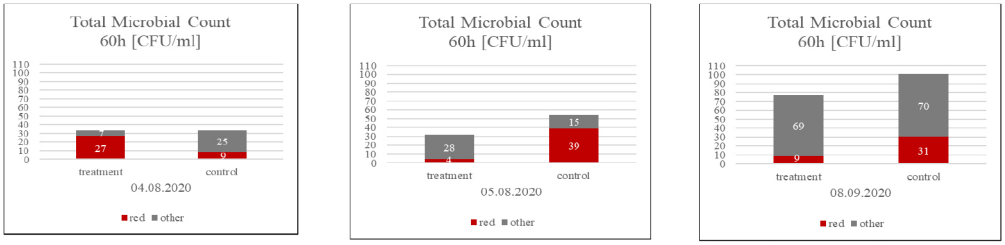


Fig.2 Total microbial counts (CFU/ml). a) reference samples before starting; b) sampling after 24 h by 1l/h application; c) sampling after 30 days application by 7.5l/h

Fig.3 BacLight Viability results. A) Reference samples obtained before starting application from both systems while operating with ozone. B) Sampling after 24 h application by1l/h together with ozone disinfection in the test

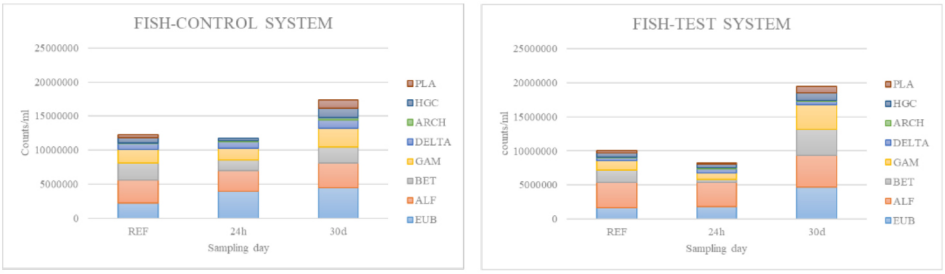


Fig.4 Fluorescence in situ hybridisation analysis of water samples from control and test system before starting application (REF), and after 24 h application by 1l/h and 30d application by 7.5 l/h combined with ozone.

This study was part of the industrial project “Follow up test of hydrogen peroxide as disinfection method in aquaculture facilities rearing Atlantic Salmon” in cooperation with Danish Salmon and EVONIK.

ULTRASOUND DESINFECTION IN FRESHWATER AND MARINE RECIRCULATING SYSTEMS

Desislava Bögner*[1], Mirko Bögner[1], Kyra M. Böckmann[1], Kira Szidat[1] Anna Lenfers[1], Yara Zimmer[1], Christopher Behrendt[1], Marcus Salzwedel[1], Kerstin Klemm[1], Matthew J. Slater1, Joachim Henjes[1]

[1] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
E-mail: desislava.boegner@awi.de

Introduction

Further development of recirculating aquaculture systems (RAS) towards zero-exchange depends mostly on the improvement of water treatment technologies. Ozone and UV radiation are leading technologies requiring high energy demand and educated staff able to manage the hurdles of their application. Moreover, there are some constrains for systems with poor mechanical filtration or where accumulation of particles higher than 50 μm significantly reduce the penetration potential of UV application. An alternative method commonly used in wastewater treatment to eliminate particulate aggregates is sonication. This method is based on cavitation effects which contribute to disrupt bacterial bioflocs and to break microbial cell walls leading to reduced viability. The present study aims to evaluate the disinfection capacity of a prototype created to treat process water in a RAS rearing aquaculture relevant freshwater and saltwater species with three sonication frequencies. The potential impact on the microbiome of the system in different compartments beside the reactor as well as bacterial viability was evaluated.

Material and Methods

An ultrasound prototype composed of 12 inducers connected to control devices was created in the frame of this project and adapted to a 5 m^3 research RAS composed of three rearing tanks, a drum filter, 2 biofiltration units (nitrification-denitrification), a sump and a protein skimmer with ozone disinfection. For the experiments the system was initially prepared for rearing European Seabass (*Dicentrarchus labrax*) and in a second experiment for rearing tilapia (*Oreochromis niloticus*). Process water coming from the sump was conducted into the prototype at a flow rate of 10 l/min and treated with 575 kHz, 862 kHz and 1142 kHz without further disinfection. For the saltwater experiments we tested 50% and 75% frequencies amplitude while only 75% amplitude was used for freshwater experiments. Each frequency was applied for 96 h and daily sampling was conducted to determine variations on microbial viability (BacLight Viability Kit) and bacterial community composition (FISH) with respect to reference samples collected before treatment. For FISH analysis (Fig. 1) generic FAM labelled DNA probes for Eubacteria (EUB) and Archaea (ARCH) as well as more specific probes for α-, β-, γ-, δ-Proteobacteria (ALF, BET, GAM, DELTA) and Actinobacteria (HGC) were included. When available, also non-labelled competitor DNA probes in equimolar concentration as the respective labelled probe were used. All samples were counterstained with DAPI.

Results and Discussion

Marine RAS:
The sterilizing effect was impacted by the amplitude used. Frequencies 575 kHz and 1142 kHz showed higher disinfection potential by 75% amplitude than 50%. The proportion of dead cells increased with the frequency. At 1142 kHz, a decrease in the total number of most of the selected bacterial groups was detected (Fig. 2) while the total numbers of bacteria at the end of application did not significantly change when using 575 kHz and 860 kHz. Sonication with all tested frequencies lead to changes in the bacterial community. Especially at 1142 kHz, a strong decrease in ALF, BET, GAM and ARC and an increase in DEL was observed (Table 1). This suggests a selective effect of US treatment on microbial community.

Freshwater RAS:
The sonication treatment of the system while rearing freshwater species did not show a defined impact with respect to changes in bacterial composition over time. At a frequency of 860 kHz, there was an increase in the number of counted bacteria over time (Fig. 3) while a slightly drop was observed by 575 and 1142 kHz. No marked changes in the composition of the bacterial community were detected for the latter frequencies. For all frequencies tested there was no conspicuous change in the percentage of dead cells.

(Continued on next page)

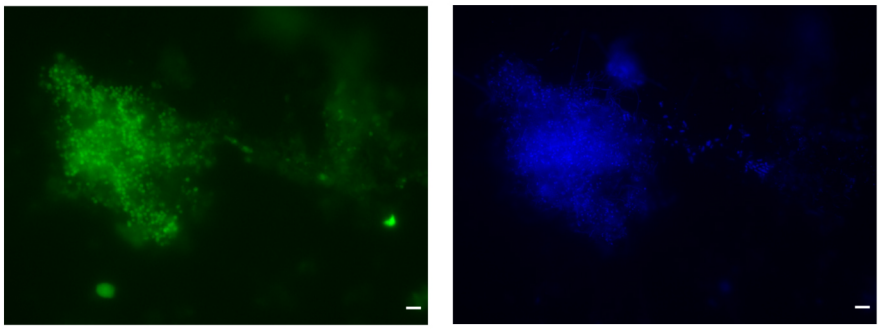


Fig. 1 FISH assessment: Samples hybridized with DNA probes labelled with FAM fluorophore (in green) and counterstained with DAPI (in blue). Scale bar = 5µm.

Table 1. Temporal change in abundance of selected bacterial groups during US treatment.

	Marine RAS				
Frequencies	ALF	BET	GAM	DELTA	ARCH
575 kHz	↓	↑	↓	↓	↓
862 kHz	↑	↓	↑	↓	↑
1142 kHz	↓	↓	↓	↑	↓

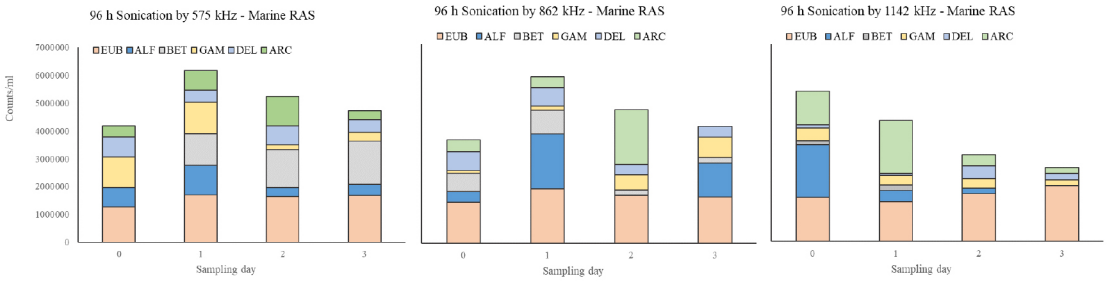


Fig. 2 Bacterial community composition on saltwater samples exposed to three frequencies for 96 h

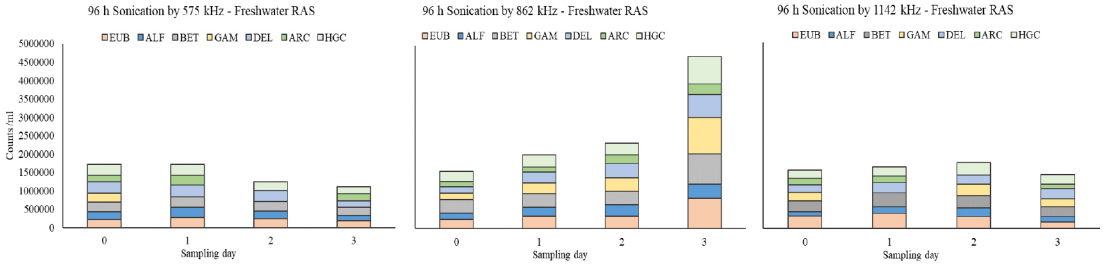


Fig. 3 Bacterial community composition on freshwater samples exposed to three frequencies for 96 h

This study is part of the BMBF financed project „Ultraschall-Behandlung von Prozesswasser in Aquakultur- sowie Abwasser-Anlagen“ (UvPAA), grant agreement number 02WQ1432B

VIRAL INFECTION DRIVES THE REGULATION OF FEEDING BEHAVIOUR RELATED GENES IN *Salmo salar*

David Muñoz, Andrea Aguilar and Senastian Boltaña*

Departamento de Oceanografía, Centro de Biotecnología, Universidad de Concepción, 4030000 Concepción, Chile
*sboltana@udec.cl

The feeding behaviour in fish is a complex activity that relies on the ability of the brain to integrate multiple signals to produce appropriate responses in terms of food intake, energy expenditure, and metabolic activity. Upon cues stresses, like infection by virus or activating mediators such as proinflammatory cytokines, Prostaglandins and Cortisol, both POMPC and NPY/AgRP neurons from the hypothalamus are stimulated, thus triggering a response that controls both energy storage and expenditure. However, how appetite modulators or neuro-immune cues link pathogenesis and energy homeostasis in fishes remains poorly understood. Here, we provide the first evidence of a molecular linkage between inflammation and food intake in *Salmon salar*. We show that in vivo viral challenge with IPNV impacts food consumption by activating anorexic genes as *mc4r, crf* and *pomcb-1*, and 5-HT in the brain of *Salmon salar*. At the molecular level, viral infection induces an overall reduction of lipid content in the liver, favouring the production of AA and EPA associated with the increment of *elovl2* gene. In addition, infection upregulates leptin signalling and inhibits insulin signalling. These changes are accompanied by a robust inflammatory response represented by the increment of IL-1β, IL-6, TNFα, and PGE2; also, an increased cortisol levels *in vivo*. Thus, we propose a model in which hypothalamic neurons respond to inflammatory cytokines and stress-related molecules and interact with appetite induction/inhibition. These findings provide evidence of crosstalk between pathogenesis-driven inflammation and

NEW INSIGHTS INTO CLOCK GENES AND THE CIRCADIAN SYSTEM IN SALMONIDS

C.M. Bolton [1]*, M. Bekaert [1], M. Eilertsen [2], R. Karlsen [2], D. Dolan [2], J. Taylor [1], J.V. Helvik [2], and H. Migaud [1].

1. Institute of Aquaculture, University of Stirling, Scotland, UK.
2. Department of Biological Sciences, University of Bergen, Norway.
Email: c.m.bolton@stir.ac.uk

Introduction

Circadian rhythms or 'clocks' are the visual expression of endogenous oscillatory expression of genes and proteins lasting approximately 24-hours, which synchronises biochemical, physiological, and behavioural responses enabling organisms to respond to diel environmental changes (Cox and Takahashi, 2019). They can be directly linked to altered metabolic, physiological and behavioural traits and influence most biological processes (Wulund and Reddy, 2015). The circadian mechanism is highly conserved across organisms however, our understanding of core clock mechanisms and circadian control of fish physiology remains limited (Frøland Steindal and Whitmore, 2019). There is an unusually large complement of clock genes in salmonids as a direct result of the two rounds of whole genome duplication (WGD) events (Ts3R, teleost specific and SSr4, salmonid specific) resulting in an abundance of circadian related genes (Lien *et al.*, 2016). As such deciphering the circadian clock mechanism in salmonids is complex and the functional divergence remains largely unknown (West *et al.*, 2020).

The aims of this study were to describe the effects of the salmonid specific WGD on clock gene diversity in Atlantic salmon. Through phylogenetic analysis, core clock gene ohnologs were classified and renamed. This was confirmed by transcriptomic analyses during early freshwater development of salmon reared under controlled lighting regimes including photoperiodic manipulations and narrow bandwidth lights. New findings provide a tool for further circadian clock research in salmon.

Materials and Methods

The genomes of five species of salmonids (*Salmo salar, Salvelinus alpinus, Oncorhynchus mykiss, Oncorhynchus kisutch, and Oncorhynchus tshawytscha*) were interrogated using *Danio rerio* clock genes [*clock, arntl, period, cryptochrome, nr1d, ror and csnk1e/d*] and *Esox lucius* [the ancestor of all salmonids – a pre-SSr4 duplication outgroup] to further explore the effect of the salmonid specific WGD SSr4 on the clock genes in salmonids. The transcriptomic isoforms with the highest percentage identity to the reference were selected and aligned. Maximum Likelihood (ML) trees were inferred to identify the relationship between identified clock genes and those of the latest common ancestors. Identified putative clock genes were re-classified based on the zebrafish nomenclature and were renamed from their predicted names after zebrafish orthologues.

Two separate cohorts of *S. salar* at different life stages [early development (UiB) and pre-smolt juveniles (UoS)] were kept under controlled lighting regimes and sampled over 24-hours at 4 hrs intervals. A range of lighting conditions were tested during egg incubation including photoperiod (14L:10D-LD, continuous darkness DD, and continuous light-LL), intensity (0.01 W/m^2, 0.1 W/m^2 and 1.0 W/m^2), and spectra (blue, green, red, and white) and at the juvenile/parr stage (12:12 LD vs. LL). RNA was extracted from dissected embryos or tissues (brain and liver) and sent away for RNA sequencing. RNA sequences were aligned to the published genome and gene expression patterns were statistically analysed.

Results

Most clock genes identified in salmonids are duplicated as a direct result of the salmonid specific WGD event SSr4. There are individual gene families in which some members display reciprocal gene loss and gene retention from the latest common ancestors (*E. lucius* and *D. rerio*). RNA sequencing identified that all *S. salar* putative core clock genes identified and classified *in silico* were expressed at both life stages sampled. Expression varied throughout early development from eye pigmentation through to first feeding (255dd – 690dd). Despite noise caused by individual variance two clock genes displayed significantly rhythmic expression patterns over a 24-hour sampling period pre-first feeding (690dd). Exposure to DD appeared to significantly down regulate some clock genes, whereas exposure to LL appeared to significantly up regulate clock gene expression of several clock genes when compared to those on the LD treatment. Spectral composition appeared to significantly influence the regulation of several clock genes. With red and green light treatments appearing to significantly down regulate a few of the identified genes when compared to white light during the midpoint of the light and dark periods. In late fresh water developmental stages (UoS) 262 genes were significantly rhythmically expressed in *S. salar* parr amongst which 13 of the putative core clock genes were identified.

Discussion/ Conclusion

Clock genes are expressed from early development in *S. salar*. Whilst there appears to be variance between individuals relating to the time at which clock genes peak and trough, it appears that as the salmon develop there is an increase in the number of putative core clock genes which are significantly rhythmically expressed. This is indicating maturation of the 'biological clock' or circadian mechanism in *S. salar* throughout freshwater developmental stages. There was a greater influence of the LL photoperiod on the significant regulation of clock genes than DD when compared to the LD group, indicating that the presence of light signals has a greater influence over clock gene regulation than the absence of lighting cues. Spectral composition to a lesser extent than photoperiod also influences the regulation of clock gene expression. However, the effect of intensity at early developmental stages was negligible. The role of feeding from first feeding in entraining the circadian system can be hypothesised. Additional work is required to further elucidate the complexity the circadian mechanism in salmonids and how the complement of clock genes identified function as components of this mechanism.

References

Cox, K. H. and Takahashi, J. S. (2019) 'Circadian clock genes and the transcriptional architecture of the clock mechanism', *Journal of Molecular Endocrinology*, 63(4), pp. R93–R102. doi: 10.1530/JME-19-0153.

Frøland Steindal, I. and Whitmore, D. (2019) 'Circadian Clocks in Fish—What Have We Learned so far?', *Biology*. MDPI AG, 8(1), p. 17. doi: 10.3390/biology8010017.

Lien, S. *et al*. (2016) 'The Atlantic salmon genome provides insights into rediploidization', *Nature*. Nature Publishing Group, 533, pp. 200–205. doi: 10.1038/nature17164.

West, A. C. *et al*. (2020) 'Diversified regulation of circadian clock gene expression following whole genome duplication', *PLoS Genetics*, 16(10), p. e1009097. doi: 10.1371/journal.pgen.1009097.

Wulund, L. and Reddy, A. B. (2015) 'A brief history of circadian time: The emergence of redox oscillations as a novel component of biological rhythms', *Perspectives in Science*. Elsevier BV, 6, pp. 27–37. doi: 10.1016/j.pisc.2015.08.002.

WATER QUALITY PARAMETERS IN A PARTIALLY RECIRCULATED, LAND-BASED, COMMERCIAL ABALONE/*ULVA* IMTA SYSTEM, AND EFFECTS OF INCREASED RECIRCULATION RATES

Joseph A de Prisco[1], Mark D Cyrus[1,2], Brett M Macey[1,2], John J Bolton[1]*

[1]Department of Biological Sciences, University of Cape Town, 7701, South Africa
[2]Department of Forestry, Fisheries and the Environment, Cape Town, 8001 South Africa.
Email: john.bolton@uct.ac.za

Introduction

Several land-based abalone (*Haliotis midae*) farms in South Africa have practiced commercial integrated multitrophic aquaculture (IMTA) for many years, using the green seaweed *Ulva rigida* to enable partial water recirculation of bioremediated effluent and to re-use waste nitrogen as feed (Bolton et al. 2009, 2016; Nobre et al. 2010). Buffeljags Abalone Farm (Viking Aquaculture) (34.7550° S, 19.6154° E) produce over 500t of *Ulva* yr^{-1} in abalone effluent in raceway ponds, principally to remove ammonia enabling 50% water recirculation, thus saving ca. 40% of pumping costs. The aim of this study was to measure water parameters to assess the functioning of the system, and to conduct a preliminary trial to assess the effects of increasing the recirculation rate. The latter was carried out on a particularly warm day in summer and included 100% recirculation, which was tested in case of the need for the farm to be isolated from the inbound water stream in the occurrence of a Harmful Algal Bloom (HAB).

Methods

Experiments were conducted on fully commercial abalone systems containing ca. 10 000kg of abalone per module. Two experimental data collections were carried out. Environmental parameters were measured where water enters and leaves the abalone tanks, and where it exits the *Ulva* paddle raceway, in three replicate commercial modules running at the customary 50% recirculation rate, over the course of 24 hours. In addition, over a 3-day period in the summer, single commercial modules were adjusted to compare the 50% recirculation rate with 75% and 100% recirculation, with parameters measured at the sump which directly supplies the abalone tanks. Parameters presented are temperature, pH, Total Ammonia Nitrogen (TAN) and Free Ammonia Nitrogen (FAN), as well as the % removal of TAN and FAN in the *Ulva* paddle raceways at 50% recirculation.

Results and Discussion

Over a 24-hour period at 50% recirculation, temperature varied minimally between sampling points during the experiment, with slight daytime increases throughout. The pH was higher in abalone inbound and *Ulva* effluent water compared to the abalone effluent water. Total ammonia nitrogen percentage removal across the seaweed biofilters ranged from 65%-85% (mean 73%). Free ammonia nitrogen removal across the seaweed biofilters ranged from 41%-80% (mean 63%). A regression analysis demonstrates a strong positive linear relationship between TAN removal and TAN load to the seaweed biofilter (r^2= 0.90). Principal component analysis revealed a strong negative correlation between FAN removal and pH, as pH increased across the seaweed biofilters the proportion of FAN removal decreased.

There were no statistically significant differences (Mood's Median Test, $p>0.05$) between the 50% and 75% recirculation cluster for temperature, pH, TAN or FAN. Statistically significant differences occurred between 100% and 50% recirculation, 100% and 75% recirculation and 100% recirculation and ambient conditions for pH, TAN and FAN, whilst no statistically significant differences occurred for temperature. At 100% recirculation, TAN and FAN increased rapidly, though the commensurate rapid and considerable decrease in pH meant the FAN increase was not as high as it would be at a normal seawater pH of ca. 8.2. Abalone suffered no mortalities at 100% recirculation for three days and later reports from the farmers suggested no effects on growth rate in the months following the experiment. TAN levels breached WWF guideline maximum effluent concentrations for abalone aquaculture (0.6mg/L = 35.23 μM/L) only in the 100% recirculation cluster, and only then during three of the thirteen sampling runs. The TAN concentrations in 50% and 75% recirculation treatments were far below the WWF guideline maximum effluent concentration with maximum concentrations of 7.15 µM/L in 50% and 13.46 μM/L at 75%.

Some increase, up to 75%, in recirculation rate seems feasible for maintenance of normal farm functioning, and these results suggest that short-term 100% recirculation to reduce or prevent HAB impact can be a practical solution.

(Continued on next page)

50% recirculation (24 hours)

(MIN.) MEAN (MAX)

TEMPERATURE	ABALONE IN	(14.1) **14.9** (16.1)
	ABALONE OUT	(13.9) **14.7** (16.2)
	ULVA OUT	(13.6) **14.6** (16.4)
pH	ABALONE IN	(7.71) **7.84** (8.03)
	ABALONE OUT	(7.57) **7.64** (7.79)
	ULVA OUT	(7.52) **7.73** (8.15)

Ammonia % removal (*Ulva* paddle raceway)

TAN (% removal)	(64.55) **73.40** (84.84)
FAN (% removal)	(40.84) **62.96** (80.29)

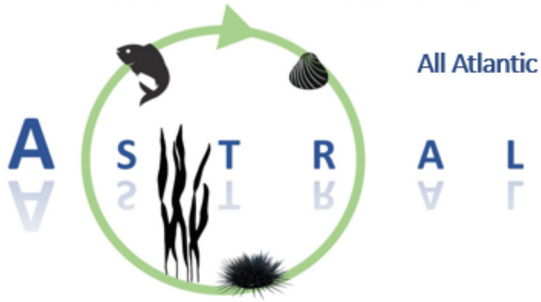
Increased recirculation (72 hours)

(MIN.) MEAN (MAX)

TEMPERATURE	50%	(18.3) **19.2** (21.0)
	75%	(18.2) **19.1** (20.5)
	100%	(18.2) **19.5** (22.4)
pH	50%	(7.51) **8.11** (8.49)
	75%	(7.50) **8.09** (8.41)
	100%	(7.02) **7.64** (8.14)

Ammonia concentrations

	TAN (µM/L)	FAN (µM/L)
50%	(0.15) **1.50** (7.15)	(0.04) **0.85** (4.03)
75%	(0.15) **2.96** (13.46)	(0.06) **1.32** (5.22)
100%	(19.08) **33.75** (59.85)	(3.38) **7.17** (19.72)



All Atlantic Ocean Sustainable Profitable and Resilient Aquaculture

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 863034.

References

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

Bolton JJ, Robertson-Andersson DV, Shuuluka D & Kandjengo L (2009). Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. J. Appl. Phycol. 21: 575-583.

Nobre AM, Robertson-Andersson D, Neori A & Sankar K (2010). Ecological–economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture 306: 116–126.

PARTIAL REPLACEMENT OF DIETARY PLANT-BASED INGREDIENTS WITH *Nannochloropsis gaditana* FROM BIOREFINERY IMPROVES GROWTH PERFORMANCE IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

T. Bongiorno*[1], L. Proietti[1], L. Foglio[1], F. Castillo-Cascino[1], A. Di Biase[2], A. Morillas[3], F.G. Acién Fernández[3], A.J. Vizcaíno[4], A. Galafat[4], F.J. Alarcón[4], K. Parati[1]

[1]Istituto Sperimentale Italiano L. Spallanzani - Loc. La Quercia, 26027 Rivolta d'Adda, Italy
[2]AIA-Agricola Italiana Alimentare, Spa, Brand Veronesi Mangimi, 37036 San Martino Buon Aalbergo, Italy
[3]Department of Chemical Engineering, University of Almería, 04120 Almería, Spain
[4]Department of Biology and Geology, Ceimar-University of Almería, 04120 Almería, Spain
E-mail: t.bongiorno@tstechnologies.it

Introduction

Plant-based protein (mainly soybean meal) is one the major protein source for fish feed, however since EU has a 70-80% protein deficit, it massively imports soybean from third countries. On the other hand, in order to produce enough plant-based proteins to satisfy the needs of the whole animal and aquaculture sector, about 20-30% of EU arable land should be dedicated to this purpose (Martin, 2014). Furthermore, soy and other plant-based ingredients, naturally contains anti-nutritional factors (ANFs) such as trypsin inhibitors and oligosaccharides that can hinder digestibility. Therefore, alternatives to plant-based protein sources are needed. The introduction of low-cost microalgae meal from biorefinery would improve sustainability of the entire aquaculture chain by: i) promoting the recovery and reuse of resources, ii) providing an alternative to conventional fish and soy protein and lipid sources and iii) maintaining a high nutritional quality. Previously results about inclusion in aquafeed of microalgae from biorefinery, as partial substitution of fishmeal, suggest that this biomass is a valuable nutrient source able to ensure an adequate growth performance, especially when the microalgae biomass was previously subjected to the hydrolysis treatment (Bongiorno *et al*., 2020). In fact, the release of low molecular weight bioactive peptides and free amino acids induces positive effects on the health *status* of GUT of fish (Galafat *et al*., 2020). No data are yet available regarding the potential replacement of plant-based ingredients with microalgae deriving from biorefineries. This study aims to evaluate the effects of partial replacement of dietary plant-based nutrient sources with different microalgae meals based on *N. gaditana* on growth performance of gilthead seabream (*S. aurata*) juveniles. The replacement included crude and hydrolyzed microalgae biomasses, coming from both biorefinery and conventional cultivation system.

Materials and methods

N. gaditana microalgae, grown on conventional Synthetic Medium (SM) and diluted Pig Manure (PM), were included in aquafeeds as hydrolyzed (Galafat *et al*., 2020) or crude biomasses. In particular, five complete experimental diets were formulated to be grossly iso-proteic and iso-lipidic. Control diet (C) was prepared using a blend of conventional animal and vegetal protein sources while experimental diets were prepared replacing the 5% of plant-based protein and lipid by *N. gaditana* grown on Synthetic Medium (crude (SM-C) or hydrolyzed (SM-H)) and grown on Pig Manure (crude (PM-C) or hydrolyzed (PM-H)). Each diet was randomly assigned to tanks and tested in triplicate according to a mono-factorial design. Microalgae dried biomass and diets were analyzed microbiologically and verified for nutritional quality. To carried out the feeding trials at pilot-scale, 900 *S. aurata* juveniles (average 36.1±0.02g each) were randomly allocated among 15 square-shaped 500-L-fiberglass tanks, (60 fish/tank) in a RAS under controlled rearing conditions (temperature, 23.1°C, dissolved oxygen 8.3 mg/L, artificial day-length, 12h, 300 lux). Diets were offered in two daily meals with a feed ratio between 2-3% body weight, over 12 weeks and each group were weighted every month under moderate anesthesia. At the end of the trial, Survival Rate (SR), Final Body Weight (FBW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Feed Intake (FI) were evaluated, and fifteen fish per group were subjected to individual biometry and dissected to determine biometric, somatic indices and slaughter yield. All the data were evaluated for normality distribution and differences among treatments were analyzed by one-way analysis of variance (ANOVA) and, if adequate, means were compared using the Duncan's test, set for $P < 0.05$, using SPSS-PC release 17.0 (SPSS Inc., Chicago, IL, USA).

(Continued on next page)

Table 1 Growth performance, nutrient utilization, somatic indices and yields of *S. aurata* juveniles fed the test diets over 12 weeks.

	C	C-SM	C-PM	H-SM	H-PM
Growth parameters (n=3 tanks)					
IBW[1] (g)	36.14±0.02	36.16±0.01	36.14±0.05	36.13±0.01	36.15±0.03
FBW[2] (g)	150.94±1.69[a]	154.98±0.68[b]	155.23±1.35[b]	153.98±1.37[ab]	156.45±2.84[b]
SGR[3]	1.66±0.01[a]	1.69±0.00[b]	1.69±0.01[b]	1.69±0.01[ab]	1.70±0.02[b]
FI[4] (g)	9737.16±75.14	9819.02±45.54	9861.43±78.57	9810.66±63.24	9844.41±103.10
FCR[5]	1.42±0.03[b]	1.39±0.01[ab]	1.38±0.01[a]	1.39±0.01[ab]	1.36±0.03[a]
SR[6] (%)	98.89±0.96	98.89±0.96	99.44±0.96	99.44±0.96	100.00±0.00
Biometric, somatic indices and yields (n=15 fish)					
VSI[7] (%)	4.82±0.75	5.04±0.98	5.17±0.79	4.90±0.55	5.24±0.57
HSI[8] (%)	1.26±0.25	1.22±0.37	1.35±0.26	1.34±0.33	1.45±0.28
MFI[9] (%)	0.83±0.32[a]	1.16±0.42[b]	1.07±0.50[ab]	0.81±0.31[a]	1.07±0.33[b]
CY[10] (%)	94.16±0.76	93.93±0.91	93.90±0.98	93.75±0.77	93.67±0.76
FY[11] (%)	53.75±2.26	55.71±2.31	53.24±3.70	55.42±3.33	54.36±2.26
K[12]	1.81±0.14	1.79±0.17	1.78±0.09	1.75±0.11	1.77±0.09

Data are reported as mean ± SD. [1]IBW: Initial biomass (g)/number of fish in the tank; [2]FBW (g): Final biomass (g)/number of fish in the tank; [3]SGR: [(ln final body weight - ln initial body weight) / days × 100; [4]FI (g): [pre-weighed feed amount- undistributed feed weight – (n. of uneaten feed pellets x average weight of individual feed pellet)]; [5]FCR: feed intake /weight gain; [6]SR: (final number of live fish/initial number of live fish) × 100; [7]VSI, viscerosomatic index = viscera weight (g)/body weight (g) × 100; [8]HSI, hepatosomatic index = liver weight (g)/body weight (g) × 100]; [9]MFI, mesenteric fat index = [100 × (total mesenteric fat weight (g)/body weight (g))]; [10]CY, carcass yield = (gutted body weight/body weight) × 100; [11]FY, fillet yield = fillet with skin weight/body weight) × 100.; [12]K, condition factor = body weight/standard length^3 × 100.

Results and discussion

All the experimental diets resulted similar for their proximate and fatty acid composition as well as for their microbiological quality (data not reported). Growth performance, nutrient utilization, biometric and somatic indices of *S. aurata* juveniles fed the test diets over 12 weeks are shown in Table 1. No significative differences were observed in IBW, FI, SR and biometric and somatic indices ($P>0.05$) except MFI ($P<0.05$). Dietary treatments significantly affected growth parameters, FBW SGR, FCR ($P<0.05$). To date, no data are available on the potential inclusion of microalgae obtained from a biorefinery as plant-based ingredients substitution in aquafeeds. The results obtained in this study suggest that a dietary inclusion of 5% of *N. gaditana,* as partial replacement of some vegetable origin ingredients (mainly soy protein concentrate), improves the growth performance in seabream juveniles after 90 days of feeding, independently of the biomass treatment and the growth medium used. Further studies are underway to verify both the quality of the fillets and the health *status* of the GUT in those seabream specimens.

Acknowledgments

This work has received funding from the European Union HORIZON 2020 Research and Innovation Program under the Grant Agreement No. 727874 (project SABANA).

References

Bongiorno T., Foglio L., Proietti L. *et al*. Microalgae from biorefinery as potential protein source for Siberian sturgeon (*A. baerii*) aquafeed. Sustainability 12(21): 8779 (2020). https://doi.org/10.3390/su12218779

EVALUATION OF *Nannochloropsis gaditana* BIOMASS CULTIVATED IN AGRO-INDUSTRIAL WASTEWATER AS POTENTIAL INGREDIENT FOR FEEDING GILTHEAD SEABREAM (*Sparus aurata*). EFFECT ON DIGESTIVE ENZYMES

A.J. Vizcaíno[1]; A. Galafat[1]; M.I. Sáez[1]; T.F. Martínez[1]; T. Bongiorno*[2], L. Proietti[2], L. Foglio[2], F. Castillo Cascino[2], A. Di Biase[3], A. Morillas[4], F.G. Acién Fernández[4], K. Parati[2], F.J. Alarcón[1]

[1]Department of Biology and Geology, Ceimar-University of Almería, 04120 Almería, Spain
[2]Istituto Sperimentale Italiano L. Spallanzani - Loc. La Quercia, 26027 Rivolta d'Adda (CR), Italy
[3]AIA-Agricola Italiana Alimentare, Spa, Brand Veronesi Mangimi, 37036 San Martino Buon Aalbergo (VR), Italy
[4]Department of Chemical Engineering, University of Almería, 04120 Almería, Spain
E-mail: t.bongiorno@tstechnologies.it

Introduction

Conventional land-based crops like grains, pulses and their derivatives are commonly used as feasible alternative for partial replacement of fishmeal and fish oil in aquafeed production. However, these ingredients usually have imbalanced nutrient profiles as well as anti-nutritive factors that may negatively affect nutrient digestibility and bioavailability, and hinder their use in aquafeeds, especially at high inclusion level. In this regard, microalgae can be considered as a potential alternative to plant-based protein sources. Microalgae has been successfully evaluated as dietary ingredients in different fish species, although their high production costs and the existence of a cellulosic-rich cell wall, that could limit the bioavailability of nutrients, are two of the main bottlenecks for their generalized use in commercial feed formulation. Because of this, growing microalgae in agro-industrial waste could be of interest in the context of a circular economy, not only for recycling valuable nutrients but also for reducing the microalgae production cost. In addition, the use of enzymatic hydrolysis treatment of microalgal biomass before inclusion to aquafeeds could be a valuable tool for improving its performance as dietary ingredient. Given these considerations, this study aims to assess the effect of the dietary inclusion of crude and hydrolyzed *Nannochloropsis gaditana* biomasses produced in a sustainable bio-refinery on the activity of digestive enzymes in gilthead seabream (*Sparus aurata*) juveniles.

Materials and methods

Nannochloropsis gaditana biomasses produced on synthetic medium (SM) and on 1:10 (v/v) diluted pig manure (PM) were obtained from the SABANA facilities (Universidad de Almería, Spain). Enzymatic hydrolysis of microalgae biomasses was carried out using the procedure described by Galafat *et al*. (2020). A pilot scale feeding trial was carried out at the Istituto Sperimentale Italiano L. Spallanzani (Italy). After the acclimatation period, 900 gilthead seabream (*S. aurata*) juveniles (36.1 g) were randomly distributed in fifteen 500-L fiberglass tanks (60 fish/tank) and fed (2-3% fish biomass) with five experimental diets; i) a microalgae-free diet (Control, CT), and ii) four diets replacing 5% of plant-based protein and lipid ingredients by *N. gaditana* grown on synthetic medium [crude (SM-C) or hydrolyzed (SM-H)] and grown on pig manure [crude (PM-C) or hydrolyzed (PM-H)]. All the diets were tested in triplicate over 12 weeks. At the end of the feeding trial, fish were euthanized and sacrificed by spine severing and the whole digestive system were obtained from four specimens per tank. The proximal and distal intestinal segments were separated for analysis. Tissue samples were randomly pooled to obtain twelve different enzyme extracts per dietary treatment, and total alkaline protease, trypsin, chymotrypsin, leucine aminopeptidase and alkaline phosphatase activities were measured according to Vizcaíno *et al*. (2014). In addition, the effect of dietary treatments was evaluated considering three different factors: i) use of microalgae (with or without dietary inclusion), ii) type of fertilizer used for producing microalgae biomasses (SM vs PM), and iii) enzymatic hydrolysis of microalgae biomasses (crude biomasses vs hydrolyzed biomasses).

Results and discussion

The results of the analysis of digestive enzyme activities are summarized in Table 1. Overall, the use of microalgae biomass did not negatively affect none of the digestive enzymes studied. All the enzymes, except for alkaline phosphatase, showed significantly higher activity levels in the distal region of the intestine compared to the proximal region ($p<0.05$). Trypsin, chymotrypsin, and total alkaline protease activity levels were higher in fish fed on *Nannochloropsis*-supplemented diets ($p<0.05$), which might increase the availability of small peptides favouring the digestion process. Leucine aminopeptidase and alkaline phosphatase remained unaffected. A significant effect of the type of fertilizer used for growing microalgae was observed. Chymotrypsin and total alkaline protease activities were significantly higher in fish fed on diets supplemented with microalgae grown on SM in both proximal and distal intestinal regions ($p<0.05$). Similarly, feeding fish on diets

(Continued on next page)

Table 1. Digestive enzyme activities (U/g tissue) measured in the intestinal extracts obtained from the proximal and distal intestinal regions of juvenile gilthead seabream at the end of the feeding trial.

	LEU	AKP	TRY	CHY	TAP
Proximal intestine					
CT	0.27 ± 0.08	4.75 ± 0.67 ab	0.30 ± 0.07	4.95 ± 0.092 a	843.57 ± 121.77 a
C-SM	0.30 ± 0.07	4.89 ± 0.57 a	0.32 ± 0.09	6.87 ± 0.094 b	1013.21 ± 120.61 b
C-PM	0.25 ±0.06	4.10 ± 0.46 ab	0.26 ± 0.05	8.35 ± 1.08 c	952.27 ± 137.92 ab
H-SM	0.24 ± 0.07	4.40 ± 0.82 ab	0.24 ± 0.07	6.08 ± .93 ab	879.51 ± 52.12 a
H-PM	0.31 ± 0.09	5.02 ± 0.76 b	0.36 ± 0.10	6.70 ± 0.96 b	1180.29 ± 186.15 c
p value	*0.066*	*0.025*	*0.082*	*<0.001*	*<0.001*
Factor					
microalgae	*0.978*	*0.511*	*0.813*	*<0.001*	*0.005*
fertilizer	*0.970*	*0.397*	*0.663*	*0.002*	*0.421*
treatment	*0.616*	*0.730*	*0.329*	*0.012*	*0.013*
Distal intestine					
CT	0.41 ± 0.90	4.50 ± 0.52	0.82 ± 0.18 a	19.45 ± 2.02 a	2228.59 ± 265.61 a
C-SM	0.41 ± 0.05	4.36 ± 0.68	1.06 ± 0.21 ab	22.33 ± 1.67 ab	2731.42 ± 268.69 c
C-PM	0.40 ± 0.09	4.42 ± 0.83	0.84 ± 0.22 a	23.36 ± 3.03 b	2506.60 ± 187.77 b
H-SM	0.42 ± 0.09	3.96 ± 0.56	1.12 ± 0.20 b	20.06 ± 1.88 a	2425.56 ± 188.20 b
H-PM	0.38 ± 0.10	4.23 ± 0.45	0.88 ± 0.20 a	22.04 ± 2.51 ab	2519.46 ± 157.68 b
p value	*0.859*	*0.236*	*0.001*	*0.015*	*0.007*
Factor					
microalgae	*0.738*	*0.263*	*0.048*	*0.007*	*0.004*
fertilizer	*0.760*	*0.130*	*0.446*	*0.029*	*0.032*
treatment	*0.393*	*0.417*	*0.004*	*0.067*	*0.354*

Data are mean ± SD (n=12). Codes: LEU (leucine aminopeptidase); AKP (alkaline phosphatase); TRY (trypsin); CHY (chymotrypsin); TAP (total alkaline protease). Different small letters in the same row denote significant differences among dietary treatments (ANOVA, $p<0.05$). Factors are: i) Microalgae (with or without microalgae inclusion), ii) Fertilizer (synthetic medium vs diluted pig manure), and iii) Treatment (crude biomass vs hydrolyzed biomass). For the different factors, significant differences were stablished by a comparison of means (Student's t-test).

supplemented with microalgae hydrolysate induced an increase in the activity level of these last enzymes, but only in the proximal intestine. As a conclusion, the positive effect observed in this study confirmed the usefulness of *N. gaditana* produced in a sustainable bio-refinery for feeding gilthead seabream juveniles. Additional research is needed to evaluate the effect on different aspects of the physiological status of fish as well as to determine whether these biomasses meet the nutrient standard required to be used as dietary ingredients for feeding this fish species.

Acknowledgments

This work has received funding from the European Union HORIZON 2020 Research and Innovation Program under the Grant Agreement No. 727874 (project SABANA).

References

Galafat A., Vizcaíno A.J., Sáez M.I. *et al*. Evaluation of *Arthrospira sp*. enzyme hydrolysate as dietary additive in gilthead seabream *(Sparus aurata)* juveniles. *J Appl Phycol* 32: 3089-3100 (2020).

Vizcaíno A.J., López G., Sáez M.I. *et al*. Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. *Aquaculture*, *431*: 34–43v (2014).

GROWTH, SEAWATER ADAPTATION AND STRESS RESPONSE OF DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar*) FED TWO DIFFERENT DIETS DURING SMOLTIFICATION

M. Bortoletti[1*], L. Maccatrozzo[1], S. Peruzzi[2], M. Vascellari[3], E. Melchiotti[3], G. Radaelli[1] and D. Bertotto[1]

[1] Department of Comparative Biomedicine and Food Science, University of Padova, Italy
[2] Department of Arctic and Marine Biology, The Arctic University of Norway, Norway
[3] Department of Histopathology, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
E-mail: martina.bortoletti@phd.unipd.it

Introduction

Recently, the use of artificially induced triploid fish has received great attention in the Atlantic salmon (*Salmo salar*) farming industry. Triploidy is generally induced in fish to produce sterile individuals for reproductive control and genetic containment. Moreover, triploidy induction represents one of the easiest and suitable methods to avoid adverse effects of pre-harvest sexual maturation, such as reduced growth and flesh quality deterioration (Piferrer et al., 2009). Nevertheless, triploidy may display some critical issues related to smoltification, a delicate process of physiological changes controlled by the GH/IGF axis that enables young salmonids transition from freshwater to seawater. Moreover, it may lead to growth impairments. In fact, previous studies have reported a higher incidence of vertebral deformities in triploid than diploid salmons due to the different dietary requirements for phosphorus and amino acids compared with their diploid counterparts (Smedley et al., 2016). Hydrolyzed fish proteins have high concentrations of free amino acids that may be absorbed more efficiently, enhancing nutrient utilization and fish growth (Olsen and Toppe, 2017; Peruzzi et al., 2018). On this basis, the inclusion of these two elements in aquafeeds for triploid fish may guarantee an adequate growth performance and improved fish health. Hence, the present study evaluated growth, seawater adaptation and stress status in diploid and triploid salmon fed high-protein phosphorus-rich fishmeal-based diets to better understand if the physiological changes associated with triploidy and a different feeding regime may influence these aspects during smoltification.

Materials and methods

A total of 60 fish (30 fish per ploidy) were allocated to twelve 200L circular indoor tanks. Triplicate tanks per ploidy were fed either a standard fish meal (STD) diet or a modified diet in which 45% of the fish meal fraction was replaced with hydrolyzed fish proteins (HFM) (15 fish per diet) (Skretting AS, Stavanger, Norway). Phosphorus inclusion in STD and HFM diets (MasterLab, Boxmeer, Netherlands) was equal to 19 g kg^{-1} and 18 g kg^{-1}, respectively, which is considerably higher than the reported requirement of Atlantic salmon (8 g phosphorus kg^{-1} diet). Fish were reared under 12h of light (12L:12D) and at low temperature (10.0±0.5°C) to simulate winter conditions and to induce parr-smolt transformation. Samplings took place every month from October to December and fish muscle and liver were collected. Radioimmunoassay (RIA) and Real Time PCR were used, respectively, to evaluate muscle cortisol levels and to assess growth hormone (GH) and its receptor (GHrec), insulin-like growth factor I (IGF-I), Myostatin (MSTN) and heat shock protein (HSP70) muscle gene expression. An in-situ hybridization was performed on liver's sections using a novel and highly sensitive multiplex nucleic acid technology called RNAscope®, which allows to detect and localize at the cellular level IGF-I mRNA in formalin-fixed paraffin-embedded organs. Data of gene expression and IGF-I mRNA were expressed as mean ± standard error. Prior to statistical analysis, all the data were evaluated for normality distribution. Differences between treatments were analysed using a mixed model and, if adequate, means were compared using the Satterthwaite's method, set for $p < 0.05$, using R software.

Results and discussion

Cortisol levels showed significant differences according to both ploidy and sampling time, with higher levels in triploids and in November, suggesting this month as crucial for salmon smoltification. No significant changes were found in GH, GHrec and IGF-I expression according to diet or ploidy, whereas variations were found according to sampling time, where gene expression increased from October to November. The in-situ hybridization of liver sections mirrored this trend, as the parenchyma of the November samples was evidently more positive to IGF-I probe than that of the October ones. Indeed, during salmon smoltification, GH by mean of IGF-I along with cortisol can improve salinity tolerance, as they are key molecules involved in fish osmoregulation and saltwater adaptation, and the observed increase in their levels underlines once again how they act in synergy. As for MSTN expression, it did not show significant changes, indicating that myogenesis was not affected by any of the three factors studied. The same result was obtained for HSP70, given the optimal conditions and low temperatures in which the animals were reared.

(Continued on next page)

Conclusions

The obtained results confirmed that smoltification period was successfully induced, given the significant changes in cortisol levels and in GH, GHrec and IGF-I expression over time. Given that ploidy and diet seemed to have little or no effect on growth, seawater adaptation and stress response, we can conclude that no significant physiological changes associated with triploidy and feeding regime have negatively impacted these aspects. Besides, the use of artificially induced triploid salmons is a proper solution to contain the drawbacks resulting from farmed escapes and pre-harvest sexual maturation cases, which are two main concerns for the Atlantic salmon farming industry.

Acknowledgment

This study was supported by the Norwegian Research Council, Regional Research Fund - RFF-NORD (Grant no. 248028) to the Arctic University of Norway and by the University of Padua (2018 - prot. BIRD184589) to G. Radaelli.

References

Olsen, R.L., Toppe, J., 2017. Fish silage hydrolysates: Not only a feed nutrient, but also a useful feed additive. *Trends Food Sci Tech* 66, 93:97.

Peruzzi, S., Puvanendran, V., Riesen, G., Seim, R.R., Hagen, Ø., Martínez-Llorens, S., Falk-Petersen, I.B., Fernandes, J.M.O., Jobling, M. 2018. Growth and development of skeletal anomalies in diploid and triploid Atlantic salmon (*Salmo salar*) fed phosphorus-rich diets with fish meal and hydrolyzed fish protein. *PLoS One*. 13, e0194340.

Piferrer, F., Beaumont, A., Falguière, J.C., Flajs Ãhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293, 125:156.

Smedley, M.A., Clokie, B.G.J., Migaud, H., Campbell, P., Walton, J., Hunter, D., et al., 2016. Dietary phosphorous and protein supplementation enhances seawater growth and reduces severity of vertebral malformation in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* 451, 357:368.

BIOFLOC-BASED PHENOTYPE SWITCHING IN *Vibrio parahaemolyticus* PROTECTS AGAINST AHPND IN SHRIMP

P. Bossier, V. Kumar, M. Wille, T. Margarida Lourenço

Lab of Aquaculture & Artemia Reference Center, Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
Peter.Bossier@ugent.be

This study aimed to describe the underlying mechanism behind the AHPND protective effect of a biofloc system in *Litopenaeus vannamei*. First, the results confirmed that a biofloc system maintained at a C/N ratio of 15, improves the water quality and contributes to the nutrition of cultured animals as bioflocs might serve as an additional protein source. Secondly, the study demonstrated that the biofloc system enhances the survival of *L. vannamei* upon challenge with a *V. parahaemolyticus* AHPND strain. Remarkably, the results highlight that in the biofloc system, AHPND-causing *V. parahaemolyticus* possibly switch from virulent planktonic phenotype, producing AHPND toxins, to a non-virulent biofilm phenotype (not producing APHND toxins), as demonstrated by a decreased transcription of flagella-related motility genes (*flaA*, *CheR*, and *fliS*), Pir toxin ($PirB^{VP}$), and AHPND plasmid genes (*ORF14*). In contrast an increased expression of the phenotype switching marker *AlkPhoX* gene was observed in both *in vitro* (in the biofloc) and *in vivo* (in the stomach of biofloc-based shrimp) conditions. Taken together, results suggest that bioflocs steer phenotype switching, contributing to the decreased virulence of *V. parahaemolyticus* AHPND strain towards shrimp postlarvae. This information opens the possibility to combat AHPND not only by trying to eliminate the AHPND-causing *V. parahaemolyticus* from the system but rather to steer the system allowing for a phenotypic switch of *V. parahaemolyticus*.

CELLULAR AND MOLECULAR EFFECTS OF POLYMETHYLMETHACRYLATE NANOPLASTICS IN *Sparus aurata*

I. Brandts[a,b*], C. Barría[c], L. Franco-Martínez[d], A. Barreto[e], M.A. Martins[f], A. Tvarijonaviciute[d], L. Tort[a], M. Oliveira[e] and M. Teles[a,b]

aDepartment of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
bInstitute of Biotechnology and Biomedicine, Universitat Aut`onoma de Barcelona, 08193 Barcelona, Spain
cCentro de Investigación y Gestión de Recursos Naturales (CIGREN), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
[d]Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, Regional Campus of International Excellence Mare Nostrum, University of Murcia, Espinardo, Murcia
30100, Spain
[e]Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[f]Department of Physics & CICECO, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: irene.brandts@uab.cat

Introduction

Nanoplastics, nanometric-sized plastic particles formed from the breakdown of larger plastic objects (<1,000 nm, NPs) (Hartmann et al., 2019), are considered emerging pollutants of global importance, as they have the potential to enter the environment and cause adverse ecological effects (Geissen et al., 2015). Nevertheless, the scientific community's understanding of their effects on biota and ecological impact is still limited. The gilthead seabream (*Sparus aurata*) is a top predator and one of the most relevant fish species for human consumption in the Mediterranean region, with a joint aquaculture and capture fisheries production of over 195 thousand tons in 2016. These circumstances, taken together with the fact that more than 8 million tons of plastic end up in the ocean each year (Jambeck et al., 2015), highlight the need to investigate the effect that NPs may have on commercially cultured fish such as gilthead seabream. In the present study, we aimed to investigate the effects that a short-term exposure (24 and 96 h) to polymethylmethacrylate (PMMA) NPs might have on the gilthead seabream, using biomarkers of different levels: gene expression endpoints in liver, biochemical markers in liver and plasma and erythrocyte nuclear anomalies (ENAs) in blood.

Material and methods

S. aurata juveniles weighing (8.7 ± 0.4 g) were obtained from an aquaculture facility in Spain and acclimatized in 1,000 L aquaria with aerated ASW (salinity: 30) at 19 ± 1 ◦C and natural photoperiod (14 h light: 10 h darkness) for 2 months. Fish were then randomly distributed in six different experimental groups: 0, 0.001, 0.01, 0.1, 1 and 10 mg L^{-1} of PMMA-NPs. Each experimental condition consisted of replicate 3 tanks with 15 L of experimental media each and 6 fish in the tank. The animals were exposed to NPs generally following OECD Guideline 203 regarding acute toxicity tests (OECD, 1992) and sampled at two different time points, 24 and 96 h. For each time-point, 3 animals per replicate tank were sampled (n = 9) and blood and liver samples were collected, for the determination of cellular, biochemical, and molecular markers.

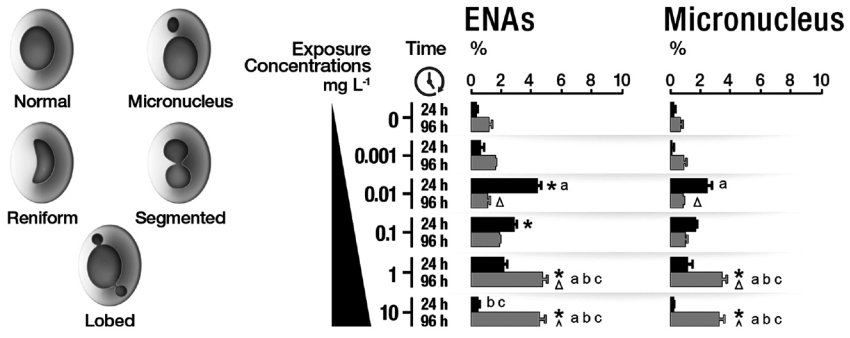


Fig. 1. Erythrocytic nuclear anomalies (ENAs) and micronuclei in the blood of gilthead seabream after 24 and 96 h exposure to polymethylmethacrylate nanoplastics (PMMA-NPs). Significant differences are marked as follows: Asterisk (*) vs. control, a versus 0.001 mg L^{-1} PMMA-NPs, b versus 0.01 mg L^{-1} PMMA-NPs and c versus 0.1 mg L^{-1} PMMA-NPs, within the same exposure time. Significant differences between sampling time points are marked at 96 h with a triangle (Δ) versus 24 h, $p < 0.05$.

(Continued on next page)

Results and discussion

In the present study, we sought to assess response of two hepatic key functions, lipid metabolism and antioxidant response. All the tested PMMA-NPs' concentrations induced a significant overexpression of hepatic *ppar**β**, rxr* and *apoa1* levels at 24 h, whereas *ppar**α*** and *pparγ* levels remained unaltered at this time point. After 96 h of exposure, expression levels of *ppar**α*** (0.001, 1 and 10 mg L-1), *pparγ* (10 mg L-1) and *rxr* were up-regulated, while *ppar**β*** and *apoa1* returned to control levels. Additionally, increased cholesterol levels were found in liver and plasma at 96 of exposure to PMMA-NPs, while triglycerides were also altered in plasma. This changes in values of both lipid compounds, together with the alterations in the expression of genes responsible for regulating lipid metabolism (*ppar**α**, pparγ, rxr, apoa1*) could suggest that PMMA-NPs are somehow preventing fish from properly using their fat stores and that fish could be exploiting alternative pathways in lipid metabolism. Regarding the genes encoding antioxidant enzymes evaluated in the liver of the gilthead seabream, we observed an overall increase in gpx1, sod2 and gr expression levels at an early time-point, which returned to control levels at 96 h. Moreover, we observed an increase of total antioxidant capacity (TAC) levels at both 24 and 96 h, and an increase in total oxidative status (TOS) at 96 h. Oxidative stress can cause damage to biological molecules such as DNA, therefore potentially inducing the formation of ENAs. An increase in the total number of ENAs at both exposure times was found, with micronuclei being the most frequently recorded ENAs. The observed increase in ENAs found in our study could derive from the direct interaction of NPs with the nuclei of erythrocytes or from increased oxidative stress after exposure to PMMA-NPs. Comparing results obtained at the different sampling times, it is possible that at 24 h the observed ENAs come from the action of NPs on cells, while at 96 h the effects of oxidative species might be added.

Conclusions

Increased transcriptional levels of key genes related with lipid metabolism and changes in plasmatic levels of cholesterol and triglycerides suggest an alteration of lipid pathways after a short exposure to PMMA-NPs. An early up-regulation of genes related with the antioxidant status indicates that fish were able to activate antioxidant defences, however increased TOS levels at 96 h suggests that antioxidant defences might not be able to completely counteract the exposure's effects. The increased frequency of ENAs suggests that PMMA-NPs can induce clastogenic/aneugenic damage in fish erythrocytes. Taken as a whole, the results of this study show that the gilthead seabream is affected by the exposure to PMMA-NPs in the short-term, as reflected by the alteration of parameters at different levels of biological organization.

References

Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., van der Ploeg, M., van de Zee, S. E.A.T.M., Ritsema, C.J., 2015. Emerging pollutants in the environment: a challenge for water resource management. Int. Soil Water Conserv. Res. 3 (1), 57–65.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassell¨ov, M., Verschoor, A., Daugaard, A. E., Rist, A., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayian, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347 (6223), 768–771.

MICROBIOME INCEPTION: AN INTESTINAL CESTODE CAUSES A HIERARCHICAL LANDSCAPE OF DISTINCT MICROBIAL COMMUNITIES NESTED WITHIN FARMED ATLANTIC SALMON (*Salmo salar*)

Jaelle C. Brealey*

Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
Email: jaelle.brealey@ntnu.no

Introduction
Cestodes represent a major health concern for both human and animal populations. The cestode *Eubothrium*, which parasitises Atlantic salmon (*Salmo salar*), impacts salmon health and is of significant economic burden to the salmon aquaculture industry. As intestinal parasites, cestodes share the space with the host gut microbiome. Studies suggest that parasitism perturbs the gut microbiome, potentially disrupting the important role that the microbiome plays in maintaining host health. Cestodes may also carry their own internal microbiome, despite lacking a digestive tract, and the extent to which this endomicrobiome resembles the surrounding host gut environment is currently unclear.

Methods
In one of the first such studies performed in a cestode, we investigated the microbiome of *Eubothrium*. We sampled the host gut mucosa, the surface of the cestode (the tegument) and the cestode endomicrobiome in 30 sea-farmed, harvest-aged Atlantic salmon and profiled the microbiota with 16S amplicon sequencing (Figure 1). We also used shotgun metagenomics sequencing of eight cestode endomicrobiome samples to further characterise putative functional differences among strains detected by the 16S data.

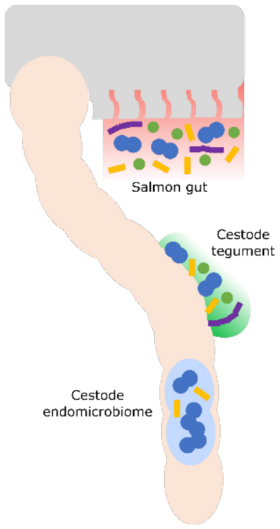


Figure 1. Schematic of the three sampling sites (salmon gut mucosa, cestode tegument surface and cestode endomicrobiome) profiled with 16S amplicon sequencing.

Results
We determined that cestode presence altered the salmon gut microbial community, with an increase in pathobionts and a decrease in the dominating commensal *Mycoplasma* phylotypes. We also established that despite lacking a gut, the cestode carried a distinct endomicrobiome, while the tegument harboured an intermediate microbial community including bacteria from both the cestode and the salmon microbiomes. Shotgun metagenomics revealed distinct *Mycoplasma* phylotypes in the cestode endomicrobiome with functional potential that differed from the *Mycoplasma* phylotypes abundant in the salmon gut.

Conclusions
Our results indicate that cestode infection is associated with gut dysbiosis in the salmon host by simultaneously serving as a potential source of novel bacterial species as well as a selective force benefiting putative pathogens. Our results highlight the importance of taking a hologenomic approach to understanding parasite infections, where the parasite and its associated microorganisms are considered as a holobiont with combined effects on the host microbiome and overall host health.

AQUACULTURE AND MARITIME SPATIAL PLANNING: LONGLINE FARMS AS ELEMENTS OF DESIGN FOR THE COASTAL SEASCAPE

D. Brigolin[1], E.M.D. Porporato[2], R. Pastres[3]

[1]Dipartimento di Culture del Progetto, Università IUAV di Venezia, 30135 Venezia, Italy
[2]IMC—International Marine Centre, Loc. Sa Mardini, 09170 Oristano, Italy
[3]Dipartimento di Scienze Ambientali, Informatica e Statistica, Università Ca' Foscari di Venezia 30172 Venezia, Italy
* dbrigolin@iuav.it [Tel. +39 041 2571318; e-mail: dbrigolin@iuav.it]

Introduction
In EU, the Maritime Spatial Planning Directive (Directive 2014/89/EU) provides the basis for an integrated management approach of maritime activities, and most of the states are currently finalizing their national plans of the sea. A sound site selection and zoning of aquaculture, carried out in the framework of MSP implementation, represent a priority for a sustainable expansion of the sector (Sanchez-Jerez et al., 2016).

Here we present an attempt to consider shellfish farm as an element for designing the costal seascape, by exploring the potential of different layouts of areas allocated to shellfish aquaculture. Our analysis was carried out in two contiguous regions of the Northern Adriatic Sea, and considered both the suitability for aquaculture expansion in the area, and the interplay between shellfish aquaculture and other coastal uses, namely: nature conservation, maritime traffic, and trawling fishery.

Methods
In the first step of the analysis, the work presented by Brigolin et al. (2017), based on a spatial multi-criteria optimization technique was expanded, in order to cover the coastal area of Veneto and Emilia Romagna regions, representing one of the most productive spots along the Norther Adriatic coast. Within this region, Intermediate Level Criteria (ILC) (Radiarta et al., 2011) were considered according to: i) optimal growth conditions; ii) environmental interactions and iii) socio economic evaluation. Based on the suitability maps obtained, we identified potential areas of interest for aquaculture expansion. We took into consideration the uncertainty in the weights assigned to different criteria, by identifying high-suitability stable areas, i.e. areas in which the different weights assigned a-priori in the analysis produced similar results.

As a second step, we explored different solutions for areas of shellfish aquaculture expansion, by considering the following factors:

- Creating continuity between existing conservation areas, in which trawling activities are prevented;
- Imposing a physical constraint to limit traffic outside navigation corridors in the area.

The second step of the analysis was carried out by using a goal function to compare different solutions and considering existing data on sea traffic intensity derived by Emodnet, the locations of existing Natura 2000 sites, and the maps of sea currents obtained from CMEMS Copernicus Marine Service data portal.

Results and discussion
Based on the output of growth and deposition models, and on the multi-criteria analysis, it was possible to map areas characterized by high (0.75-0.90) and very high >0.90 suitability, with a spatial resolution of 1 km (Fig.1). Existing mussel cultivation areas, and perimeters of the Natura 2000 sites present in the area were also mapped, and superimposed to the layer representing traffic intensity in this maritime region. Two scenarios of increase of the surface covered by mussel leases were considered, namely +50% and doubling the surface within the region, and allocation distributed between three maritime areas selected within the portion of sea characterized by high and very high suitability, and marked with A1, A2 and A3 in Figure 1. Results of this exercise highlighted that the optimal allocation of the increase in mussel farms among A1, A2 and A3, and different shapes of the farms within these areas, can drive to different results when considering aquaculture suitability alone, and considering the effects produced by the farms on conservation and maritime traffic. Results are discussed with respect to the influence of weighting factors on the outcomes of the analysis and the perspectives for including additional regulation services, such as eutrophication control and carbon sequestration in the analysis.

(Continued on next page)

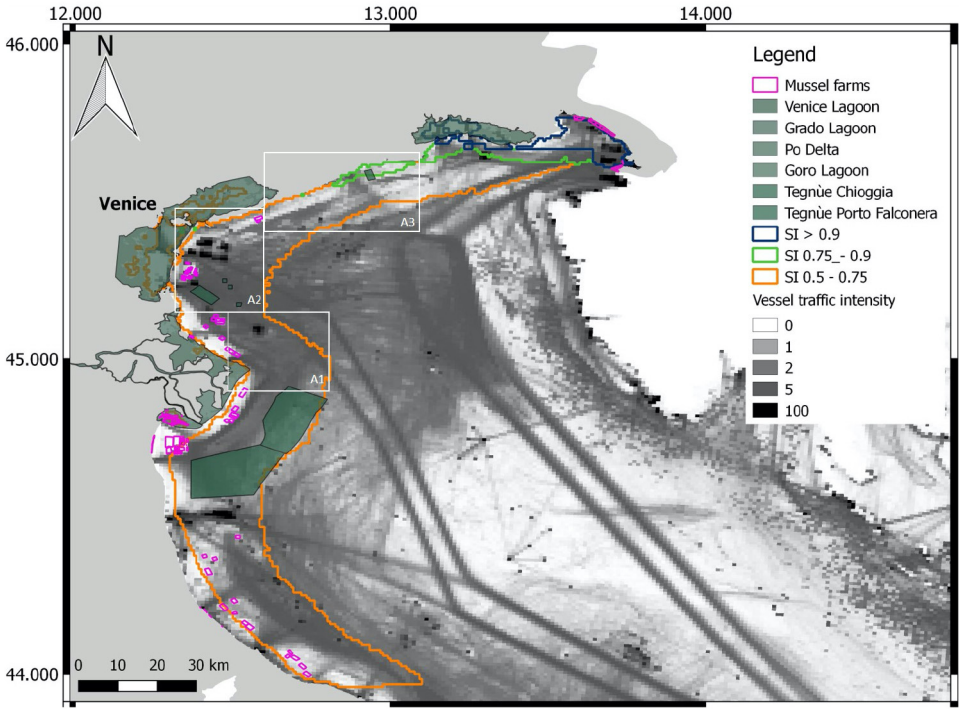


Figure 1. Mussel farming suitability map, existing longline farms, Natura 2000 sites, and traffic intensity in the Northern Adriatic Sea. A1, A2 and A3 mark the three zones investigated in the second step of the analysis.

Acknowledgements

DB carried out this research in the framework of the MSP-MED project "Towards the operational implementation of MSP in our common Mediterranean Sea", EMFF Work Programme 2018 EASME tender EASME/EMFF/2018/1.2.1.5.

References

Brigolin, D., Porporato, E.M.D., Prioli, G., Pastres, R., 2017. Making space for shellfish farming along the Adriatic coast. ICES-Journal of marine science 74(6), 1540-1551.

European Community, 2014. Directive 2014/89/EU of the European parliament and of the council of 23 July 2014 establishing a Making space for shellfish farming along the Adriatic coast 1549

framework for maritime spatial planning. Off. J. Eur. Union L257 (Bruxelles).

Radiarta, I. N., Saitoh, S.-I., and Yasui, H. 2011. Aquaculture site selection for Japanese kelp (*Laminaria japonica*) in southern Hokkaido, Japan, using satellite remote sensing and GIS-based models. ICES – Journal of Marine Science, 68: 773–780.

Sanchez-Jerez, P., Karakassis, I., Massa, F., Fezzardi, D., Aguilar-Manjarrez, J., Soto, D., Chapela, R., Avila, P., Macias, J. C., Tomassetti, P., et al. 2016. Aquaculture's struggle for space: the

need for coastal spatial planning and the potential benefits of Allocated Zones for Aquaculture (AZAs) to avoid conflict and promote sustainability. Aquaculture Environment Interactions, 8: 41–54.

TOWARDS SUSTAINABLE ADRIATIC MARICULTURE USING NEW FEED FORMULATIONS: THE ADRIAQUANET PROJECT

Daniele Brigolin[1,2*], Tanja Segvic-Bubic[3] Roberto Pastres [1], Edouard Royer[1], Fabio Mina[4], Emilio Tibaldi[4]

(1) Bluefarm Ltd, Venezia, Italy
(2) Università IUAV di Venezia, 30123 Venezia, Italy
(3) IZOR, Split, Croatia
(4) DI4A, Università di Udine, Udine, Italy

Email: dbrigolin@iuav.it

Introduction

The INTERREG project AdriAquaNet (https://www.italy-croatia.eu/web/adriaquanet), coordinated by Udine University, aims at enhancing the sustainable development of Adriatic mariculture and fostering cooperation between Italian and Croatian operators. In order to achieve these goals, the project is developing innovations concerning the whole seabass and seabream supply chain. In particular, the project focuses on: 1) farm management, fish nutrition and waste disposal; 2) fish health and welfare; 3) new added value products. The project also tests a comprehensive monitoring system, which integrates the real time monitoring of relevant environmental parameters, i.e. water temperature and dissolved oxygen, footage captured with high resolution video camera, which allows monitoring fish behaviour and simulation models. In this paper, preliminary results of fish growth performance using a novel feed formulation including insects and poultry PAP at lab scale and in at operational level, are also presented.

Methods

Feeding innovations have been tested on the basis of a comprehensive approach assessing both the zootechnical performance and local environmental impact. The systematic approach included: 1) designing novel aquafeed low in fish meal, including insect and poultry by product meals; 2) comparing new feeds to standard formulations at a laboratory scale (TRL 4-5) under controlled environmental conditions, in order to determine Key Performance Indicators, e.g. Specific Growth Rate, FCR; 3) validation of lab results in a trial at an actual farm (TRL 8) in order to estimate the above KPIs at an operational level; 4) assessment of the potential local environmental impact due to faeces and uneaten feed pellets using FiCIM, Fish Cage Integrated model, (Brigolin et al. 2014). This dynamic model, available for demonstration at https://www.bluefarmenvironment.com/, includes four modules. The "individual" module allows fish growth estimation, oxygen demands and ammonia excretion rate in relation to feed composition and water temperature. This module can also be used as a stand-alone model for quickly simulation of site-specific growth trajectories, based on water temperature climatology. The user-friendly interface was developed and presented to AdriAquaNet end-users as part of the project training activities. The second module, "population" upscales the output of the individual model at a population level, in order to simulate the change of the total biomass in a fish cage, as well as the total oxygen consumption, total ammonia and organic particulate (uneaten feed + faeces) emissions. Time-dependent emissions are taken as input by the "dispersion" module, which allows one to estimate the potential effects on the water column and the deposition of organic carbon particle on surface sediment underneath a fish cage. The fourth module cumulates daily flows of organic carbon and convert them into an increase in the concentration of organic carbon above the background level in surface sediment, considering the dynamics of early diagenesis processes. As a result, FiCIM can provide maps of organic carbon enrichment in a form on which potential damage to the benthic community can be assessed. FiCIM as the main inputs requires feed composition and time series of feed quantities provided to the fish, water temperature, and current. The two latter parameters were collected by deploying a buoy equipped with dissolved oxygen, water temperature sensors and a current meter. Data, collected every 15 minutes, were sent to a cloud and available in real time.

Results & Discussion

The AAN approach was applied to a new feed formulation for gilthead seabream. The new feed was tested on 100g sea bream over a 25 weeks long trial at laboratory scale: the results were very encouraging, showing improved FCR (1.37 vs. 1.50, $p<0.05$) when compared to conventional FM or Veg-protein rich feeds. Based on these findings, a 7-month trial started in cooperation with the project partner – Friskina at the inshore fish farm located in the middle Adriatic area (Hr), with seabream kept in cages and comparing the test feed with a commercial one in duplicate. The test feed resulted in improved weight (447 vs. 417g, $p<0.08$), SGR (0.57 vs. 0.53% d^{-1}) and FCR (1.7 vs. 1.9). Measured fish weights in cages (n=50) throughout the trial are compared with the output of FiCIM individual module (Fig. 1). As presented, the model correctly predicts the final weight, as well as the stagnation of the weight in cage 2, towards the end of the trial when fish was not fed.

(Continued on next page)

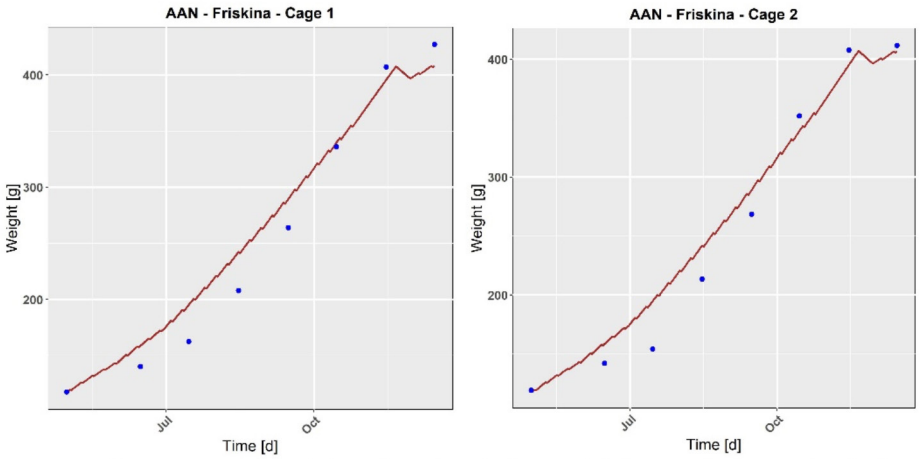


Figure 1 Comparison of model predicted and observed fish growth during the feeding trial at Friskina farm.

Concluding remark

The results presented here indicates that novel feed formulations with limited reliance on fish meal and conventional feed proteins are effective and sustainable under operational conditions. In addition, the FiCIM model can effectively simulate the growth of seabream in relation to the environmental forcings and feed features. As a next step, FiCIM will be applied for mapping the organic carbon enrichment of surface sediment at both AdriAquaNet pilot sites, located at Friskina and Orada farms, in order to complete the integrated assessment of the novel feeding protocol.

Acknowledgements

The research leading to these results has received funding from the project AdriAquaNet - Improving innovation and sustainability in Adriatic aquaculture, is supported, to the extent of 85% of the total funding, by the European Regional Development Fund (ERDF) through the Interreg V-A Italy-Croatia 2014-2020 Program, Blue innovation.

References

Brigolin, D., Meccia, V.L., Venier, C., Tomassetti, P., Porrello, S., Pastres, R., 2014. Modelling biogeochemical fluxes across a Mediterranean fish cage farm. Aquaculture Environment Interactions. Vol. 5 (2014): 71-88.

DIETARY EFFECTS ON THE REPRODUCTIVE PERFORMANCE OF THE SEA URCHIN *Tripneustes gratilla*

Marissa Brink-Hull*[12], Mark D. Cyrus[23], Brett M. Macey[23], Clint Rhode[1], Kelvin L. Hull[1], Rouvay Roodt-Wilding[1]

[1] Stellenbosch University, Stellenbosch, 7600, South Africa
[2] University of Cape Town, Rondebosch, Cape Town, 7700, South Africa
[3] Department of Forestry, Fisheries and the Environment, Cape Town, 8001, South Africa

Email: marissa.brink-hull@uct.ac.za

Introduction

Tripneustes gratilla has been identified as a commercially viable species for aquaculture production in South Africa, with the potential for inclusion in integrated multi-trophic aquaculture (IMTA) systems. However, these broadcast spawning animals often display differential parental contributions within aquaculture environments. This reproductive strategy can reduce genetic variation through a bottleneck effect, subsequently resulting in a poor response to artificial selection and poor production output in cultured populations (Grant *et al*., 2017). Additionally, feed-type and quality, as well as the feeding regimes used for broodstock conditioning, will impact feed intake, digestion and eventual nutrient availability, affecting gametogenesis and reproductive performance of these animals (Azad *et al*., 2011). This study assessed various biological and genetic factors for *T. gratilla*, as well as different feeding regimes that could affect reproductive competition, larval growth and juvenile performance after implementing a factorial breeding design.

Materials and Methods

First-generation (F1) broodstock animals (n = 32) were conditioned on 4 diets [formulated feed (20% *Ulva* inclusion (Cyrus *et al*., 2014)), kelp (*Ecklonia maxima)*, green seaweed (*Ulva rigida*) and mixture of the three diets] for approximately four months. Spawning was induced by injecting 0.5M KCl. Sperm or eggs were collected for quantification and subsequent morphological assessments, as well as for the quantification of egg lipids, proteins, carbohydrates and fatty acid profile assessments using gas chromatography flame ionisation detection (GC-FID). Remaining gametes were used in a factorial breeding design, where a standardised number of sperm and eggs of each individual were combined with that of other individuals fed the same broodstock conditioning diet. After spawning, broodstock animals were dissected, gonad weight and colour assessed and gonads processed for routine paraffin histology to evaluate gonad maturity.

Larvae were reared in 130L conical tanks. Larval morphology and survival were assessed for 20 days. Larvae from the kelp and mixed diet fed broodstock survived for the full duration of larval rearing and were transferred to 87L settlement containers, pre-coated with *Ulvella lens*. Approximately 3 months post-settlement, DNA was extracted and 10 species-specific microsatellite markers (Carlton and Lippé 2007; Wainwright *et al*., 2012) were amplified across 16 F1 broodstock (8 fed kelp and 8 fed mixed diet) and a total of 364 second-generation (F2) offspring. Genetic diversity of F1 broodstock relative to the F2 offspring, parental contribution through parentage assignment and heritability (h^2) estimates for growth were evaluated.

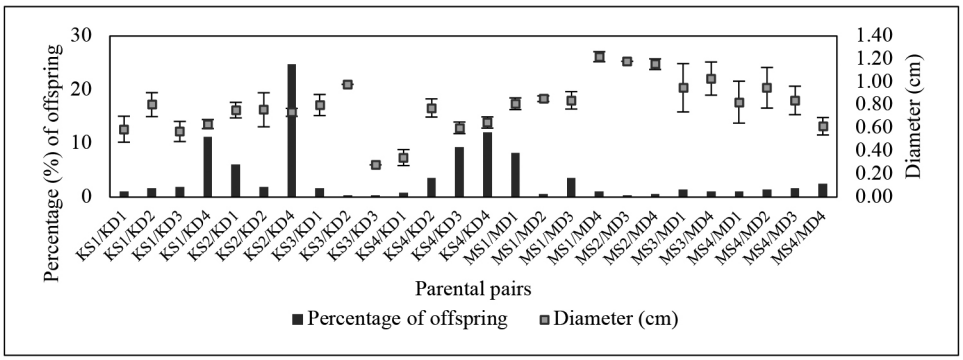


Figure 1 Percentage of *Tripneustes gratilla* offspring assigned to parental pairs in F2 cultured cohort, where 26 full-sib families were identified and mean family body diameters (cm) are indicated (K: Kelp, M: Mixed, S: Sire, D: Dam).

(Continued on next page)

Results and Discussion

Across broodstock, significant differences ($P < 0.05$; ANOVA) in gonad and egg colour suggest a formulated feed (20U) should not be fed in isolation for reproductive purposes. Dietary carotenoids (red, orange and yellow pigments) incorporated in sea urchin gonads and eggs could have downstream effects on urchin health and reproduction; via pro-vitamin A, photoprotective, and immunity-related roles, resulting in improved hatching and larval survival. Excess linoleic acid (C18:2n6) was observed in samples from animals fed a formulated feed. Although these fatty acids may elicit inflammatory response in urchins, there are potential benefits to including a formulated feed in conjunction with natural feeds in a mixed feeding regime. The egg fatty acid profile associated with the mixed diet clustered separately (principal component analysis) from the singular feeds, possibly as a result of the interactions between the combination of compounds obtained from the individual diets.

Larvae from broodstock fed kelp and the mixed diet displayed similar growth rates, with larvae from broodstock fed a mixed diet displaying a greater extent of phenotypic plasticity. Genetic diversity analyses showed no statistically significant ($P > 0.05$) differences between F1 broodstock animals and their F2 offspring, with an average allelic richness (A_R) of 5.31 ± 0.52 and 5.18 ± 0.42, respectively. A total of 26 out of 32 possible parent pairs contributed to the F2 generation (Fig. 1) and juveniles assigning to broodstock fed a mixed diet were significantly ($P < 0.05$) larger (average offspring size of 0.94 ± 0.10 cm) than broodstock fed kelp (average offspring size of 0.66 ± 0.07 cm). However, a larger number of offspring (79.65%) were assigned to kelp fed broodstock (Fig. 1). An assessment of offspring phenotypic performance showed low heritability estimates ($h^2 = 0.050 \pm 0.058$) for body diameter, suggesting that additive genetic effects play limited roles in this trait.

Conclusion

Aquaculture establishments could take advantage of the maternal provisioning strategy of sea urchins to benefit future commercial production. Animals fed the mixed feeding regime outperformed the other feeds across various measurements, with relatively equal parental contributions to offspring. The varied nutrient content of the included feeds, carotenoid content, diverse array of essential amino acids and fatty acids supplied by the respective singular feeds, as well as improved digestibility through the enzymatic activity of the bacterial communities of the natural feeds, could have contributed to the reproductive success of the mixed diet fed broodstock. This study also showed that the implementation of a factorial breeding design maximises genetic diversity in subsequent generations by negating unequal parental contributions to some extent.

References

Azad, A.K., Pearce, C.M., McKinley, R.S., 2011. Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). *Aquaculture* 317: 187–196.

Carlton, D.B., Lippé, C., 2007. Eleven new microsatellite markers for the tropical sea urchin *Tripneustes gratilla* and cross-amplification in *Tripneustes ventricosa*. *Molecular Ecology Resources* 7: 1002–1004.

Cyrus, M.D., Bolton, J.J., de Wet, L., Macey, B.M., 2014. The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla*. *Aquaculture Research* 45: 159–176.

Grant, W.S., Jasper, J., Bekkevold, D., Adkison, M., 2017. Responsible genetic approach to stock restoration, sea ranching and stock enhancement of marine fishes and invertebrates. *Reviews in Fish Biology and Fisheries* 27: 615–649.

Wainwright, B.J., Arlyza, I.S., Karl, S.A., 2012. Isolation and characterization of nineteen microsatellite loci for the collector sea urchin, *Tripneustes gratilla*. *Conservation Genetic Resources* 4: 963–965.

THE SIGNIFICANCE OF INTRODUCING A DESIGN CODE TO THE AQUACULTURE INDUSTRY IN NORWAY AND THE EFFECT ON FISH ESCAPE

Joachim Buarø

STIM Knowledge Services, Kjøpmannsgata 37, N-7011 Trondheim (Norway)

Introduction

From the initiation in the 70s to the end of the 90s there was an accelerating development in the growth of the aquaculture industry in Norway. It was observed that the increase in activity led to an increased number of incidents of structural failures leading to fish escape. This led to the development of regulations to make the aquaculture systems more reliable.

Regulations and design code

In 2003 the first revision of the NYTEK-regulations and the relating standard NS9415 was ready to be implemented. Further with the introduction of the aquaculture act in 2005 the authorities got tools that helped improve the robustness of the aquaculture plants and decrease the risk for fish escape. The NYTEK-regulation and NS9415 has been through revisions and is currently being revised.

Effects on fish escape

The regulations design code has proven to be effective as the number of fish escapes have plummeted in a period when the production volume has nearly tripled. The number of escapes due to system collapses are close to none and the few fish escape that happen is related to operations and human error.

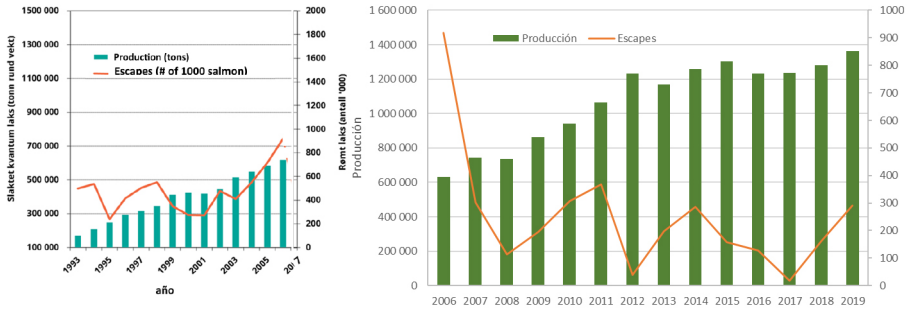


Figure 1: Historical relation between production and fish escape

GENOME EDITING TO PRODUCE MONOSEX AND STERILE FISH FOR AQUACULTURE

John Buchanan, Spencer Herbert, Takeshi Umazume, and Xavier Lauth

Center for Aquaculture Technologies, 8395 Camino Santa Fe Suite E, San Diego, California, USA
jbuchanan@aquatechcenter.com

Introduction

The ability to produce sterile progeny from broodstock for aquaculture has significant benefits to culture productivity and environmental sustainability. We describe the development of strategies to generate, breed and mass-produce infertile fish. Our solutions rely on precise genetic modifications to create broodstock lines that can be incorporated into breeding programs. These approaches were validated in tilapia but are transferrable to multiple species of fish. We expect that adoption of these technologies will result in broad economic and environmental benefits for aquaculture.

Methods and Results

One strategy for mass producing sterile fish is designed to produce monosex, sterile populations in culture. In addition to the benefit of sterile fish, this allows the benefit of sexually dimorphic performance traits in culture. We first investigated gene mutations in two evolutionarily conserved pathways, one governing sex differentiation and the other sex competency. We created edits in genes necessary for spermiogenesis and steroid hormone synthesis causing male sterility and masculinization, respectively. Double gene edit combinations for these genes produced all-male sterile populations. Likewise, we created variants in genes whose inactivation caused females to develop atrophic ovaries arrested at a previtellogenic stage or string-like ovaries lacking oocytes. We further disrupted genes causing genetic males to sex reverse into females. Double gene edit combinations for these genes produced all-female, sterile populations.

Propagation of the double KO broodstock lines was achieved via germ cell transplantation from a juvenile mutant donor into a germ cell free wild-type recipient embryo. In the resulting recipients, the induced edits had no effect as the genes targeted are not expressed in germ cells. With this approach, we generated fertile broodstock that successfully mas- produced sterile, monosex populations.

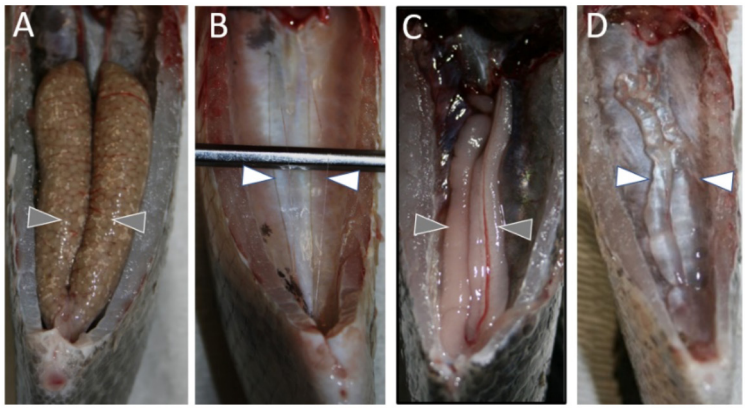


Fig 1 A-D. Dissected gonads of fertile and sterile Nile tilapia. Female (B) and male (D) with genome edited changes show string-like ovaries and translucid testes devoid of oocytes and spermatozoa, respectively. Age matched control female (A) and male (C) display mature gonads. Gray arrow heads point to gonads from fertile fish and white arrow heads point to the gonad from sterile fish.

EVALUATION OF STARFISH (*Asterias rubens*) AS A RAW MATERIAL FOR THE MANUFACTURE OF AQUACULTURE DIETS

B. Budiño[1*], M. Turull[2], A. Nebot[3], B. Fandiño[1], M. Rambla-Alegre[4], P. Savarino[5], N. Mallo[1], S. Díez[2], S. Cabaleiro[1]

[1] Centro Tecnológico del Cluster de la Acuicultura. Punta Couso S-N, Ribeira 15965, Spain.
[2] Instituto de Diagnóstico Ambiental y Estudios del Agua - CSIC. Carrer de Jordi Girona 18-26, Barcelona 08034, Spain.
[3] Aiguanatura dels Ports. Alfara de Carles S-N, Tarragona 43528, Spain.
[4] IRTA. Ctra. Poble Nou, km. 5.5, Sant Carles de la Ràpita, Tarragona 43540, Spain.
[5] Organic Synthesis and Mass Spectrometry Laboratory, University of Mons—UMONS, 23 Place du Parc, 7000 Mons, Belgium.

budino@cetga.org

Introduction

At present, and for over a decade, the superpopulation of starfish has been wreaking damages on the bivalve populations along the Galician coast, reducing productivity and forcing the fishermen's associations to carry out costly cleaning work for their removal. The valorization of this marine resource, currently treated as a non-usable waste, would open a new business opportunity for the shellfish sector, helping its revitalization and job creation, as well as contributing to the implementation of sustainable aquaculture. The objective of this work is to analyze the viability of using starfish as raw material for the production of organic feed for aquaculture, with the purpose of reducing the dependence on fishmeal, opening new opportunities in the fish nutrition market.

Material and Methods

Starfish sampling: Starfish (*A. rubens*), obtained from 3 predator cleanings on the west coast of Galicia (NW Spain), were sacrificed by thermal shock and frozen at -20ºC.

Starfish microbiological and biochemical analysis: The presence of *E.coli* and *Salmonella spp*. was determined by qPCR with specific primers. Metal determination was performed by total digestion with hydrogen peroxide and nitric acid in microwave and subsequent analysis with ICP-MS for trace metals and with ICP-OES for major metals. Quantification of saponins was performed by UPLC-MS on the samples previously extracted with acetonitrile. Lipophilic toxins were determined by HPLC-MS/MS, amnesic toxins were quantified by HPLC-UV and paralytic toxins were analyzed by UPLC-FLV.

Manufacture of starfish meal and experimental diets: Two diets, control diet (CD) and experimental diet (ED), were formulated and manufactured (Acuinuga, Spain), with the only difference that ED substituted 15% of fish meal for the obtained starfish meal.

Determination of the fatty acid profile and proximal composition: Samples were lyophilized (Virtis; 24 h, 25 mtorr, -50 ºC) and. fatty acid methyl esters obtained (Bligh&Dyer,1959). The fatty acid profile was determined using a gas chromatograph with flame ionization detector (Agilent 8860 GC), with a DB-FastFAME column (Agilent, 30 m length, 0.25 mm internal diameter, 0.25 µm phase thickVirtness), using FAMEs standard of 10 mg ml^{-1}. The percentages of crude protein (volumetry), ash, fiber, fat and humidity (gravimetry), and total sugars (Luff-Schoorl method) were determined in the elaborated starfish meal and diets.

Feeding trials: The trial with rainbow trout (*Oncorhynchus mykiss*) was conducted at the facilities of Aiguanatura dels Ports, and the trials with turbot (*Psetta maxima*) and sea bream (*Sparus aurata*) at the facilities of CETGA. The diets (CD and ED) were fed to groups of 75 trouts in 2000L tanks, and to groups of 50 turbots and sea breams in 400L tanks, supplied with circulating filtered sea water or spring water, with dissolved oxygen >8 mg l^{-1}, temperature ranging 15-20ºC and natural photoperiod. Fish were fed at a feed rate of 1.5% weight, and weighed every 30 days. Results were compared by ANOVA and Fisher LSD test (BioStat, AnalystSoft).

(Continued on next page)

Results and Discussion

According to our results, major metals in starfish were Ca (86,6-203,58 g Kg^{-1}) and Na (17,77-27,63 g Kg^{-1}), and none of the legislated trace metals (As, Cd, Hg and Pb) were above the maximum admissible values. Paralytic, amnesic or lipophilic toxins above the permitted limits for animal feed (Regulation (EC) No. 853/2004) were not detected.

The most common saponins in the analyzed *A. rubens* were the ruberosides A-F.The highest ones were ruberoside A and ruberoside F, with no biological activity reported for them (Singh & Ort, 2020). Forbeside A, which possesses anti-inflammatory and antiviral activity (Findlay *et al.,* 1987), was detected in 2 of the 3 samples.

Starfish meal shows a lower content of protein, fat and fiber, and a higher ash content than fish meals. Regarding metal content, Ca predominates in starfish meal (8-20%), with Na and Mg values slightly higher than those of fish meal, and slightly lower values of P, K, Fe and Mn. In addition, the presence of Cr, As, Cu and Pb stands out, although the maximum limits allowed by legislation for raw materials are not reached.

The two diets tested (CD and ED) show a protein content of 41.2-42.7%, a fat content ranging 11-13%, with an ash content exceeding any of the commercial fish feeds (12-17% *vs.* 8-12). The inclusion of 15% starfish meal in the CD results in a small decrease in protein content (from 41.2 to 42.7%), an increase in fat content (from 11 to 13%) and a significant increase in ash content (from 11.7 to 16.9%), also lowering the fiber content (from 9.5 to 5.7%). Fatty acid profile of the CD is not significantly altered by replacing 15% fish meal with starfish meal.

The diet efficiency was assessed in rainbow trout (103 day trial) and in turbot and sea bream (30 day trial, still in progress). In trout and turbot trials, weight gain was significantly higher in the group fed the CD compared to the group fed ED ($p<0.001$ in both cases). In the sea bream trial, however, no significant differences were observed in the weight gain of both groups ($p=0.732$). Specific growth rate (SGR) in trout was 0.05% for the ED and 0.85% for the CD, and in turbot was -0.15% for the ED, since turbot even lost weight, compared to 0.6% for the CD. For sea bream, SGR was 0.29% for both diets.

The high ash content of starfish meal and its high hygroscopic capacity make it difficult to use it as a raw material in fish feeds. It would be possible to reduce the ash content through a hydrolyzing process of the meal, which would lead to an increase in the percentages of the remaining components, also eliminating the moisture problem. The solution to the palatability problem (detected in turbot and trout, but not in sea bream) lies in the inclusion of aromas and flavorings in the diets. According to our preliminary data, the efficiency of starfish meal *A. rubens* as a raw material for aquaculture feeds depends on the target species.

References

Bligh E.G. & Dyer W.J. (1959). doi:10.1139/o59-099.
Christie W.W. (1982). ISBN: 978-0-9552512-4-5.
Findlay J.A. *et al*. (1987). doi:10.1139/v87-234.
Singh P.A. & Ort M. (2020). doi:10.1007/978-3-030-20389-4_3.
de Blas C. *et al.* (2019). ISBN: 978-8409156887. (http://www.fundacionfedna.org).
Directive 2010/63/EU; Regulation (EC) No 853/2004 and RD 465/2003.

Acknowledgments

The authors wish to acknowledge the support of the Fundación Biodiversidad, of the Ministry for the Ecological Transition and the Demographic Challenge, through the Pleamar Program, co-financed by FEMP.

ASSESSMENT OF THE EFFECT OF TIO2 AND AG NANOPARTICLES IN CELL VIABILITY IN SEA BREAM (*Sparus aurata*) AND SEA BASS (*Dicentrarchus labrax*)

N. Mallo[1], M. Vázquez[1], B. Budiño[1*], L. Rodríguez-Lorenzo[3], I. Pinheiro[3], M. Quarato[3], B. Espiña[3], A. Moreda[2], P. Bermejo[2], S. Cabaleiro[1]

[1] Centro Tecnológico del Cluster de la Acuicultura, Punta Couso S-N, Ribeira 15965, Spain
[2] Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain
[3] Water Quality Group, International Iberian Nanotechnology Laboratory-INL, Av. Mestre José Veiga s/n 4715-330 Braga, Portugal

*budino@cetga.org

Introduction

Engineered Nanoparticles (ENPs) are an emerging technology widely used, currently applied in several fields as electronics or pharmaceutics. The rising of ENPs used in modern technologies leads to their release into aquatic environment. Potential effects in marine products is a current object of study and consequences are presently being tested by different by *in vivo* and *in vitro* model approaches (Torrealba *et al.,* 2019).

Innate immunity has been described as a biomarker to environmental pollutants. Leukocytes are one of the main components of the innate immune defences in the cellular response and have a relevant role in clearing of foreign elements. In fish species, kidney is the main immune and detoxification related organ, as well as an important source of leukocytes. Taking this into account, kidney leukocytes were obtained to expose them to the different types of NPs to assess cell viability. In this study, sea bream and sea bass were chosen as models for *in vitro* tests for being species of wide interest in aquaculture field for which the offshore culture is used, being directly exposed to marine pollutants.

Material and Methods

Animal research and ethical considerations: Sea bream (*Dicentrarchus labrax)* and sea bass (*Sparus aurata*) individuals were obtained from CETGA culture facilities. Fish were sacrificed following European regulations by using MS-222 anaesthetic overdose.

Cell extraction and leukocyte purification: anterior kidney was split off, rinsed and maintained in Leivobitz-15 (L-15) medium containing heparin (10 units/mL) and foetal bovine serum (FBS, 2%). To separate de cells, kidney pieces were passed through a 100uM mesh and stored on ice. For cell type separation, a Percoll gradient was performed and the band of interest was collected (band enriched in leukocytes). Finally, cells were re suspended at the desired concentration in L-15 without phenol red, containing 10% of FBS and antibiotics (penicillin/streptomycin), dispensed into 6-well plates and cultured for 24h to let macrophages to adhere. Non-adherent cells (mostly leukocytes) were collected.

Nanoparticles: Titanium dioxide NPs [TiO_2-5nm (TiO_2 13.3g/L, 1:1.5 TiO_2:citrate ratio), TiO_2-45nm (TiO_2 15.5g/L, 1:1.5 TiO_2:citrate ratio) and TiO_2-P25 (21nm aprox, TiO_2 13.3g/L, 1:0.8 TiO_2:citrate ratio)]; Silver NPs [PVP-Ag 15nm (Ag 6.2g/L, 1:3 Ag:PVP ratio) and PVP-Ag 100nm (Ag 4.83g/L, 1:3 Ag:PVP ratio)] as well their respective stabilizers, sodium citrate (20g/L) and PVP (30g/L) were provided from INL, that carried out the characterisation in seawater (DLS, UVvis, TEM/SEM).

Cell viability tests: Cell viability was assessed by using MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphe-nyl-2H-tetrazolium bromide) assay (modified from Tada *et al*., 1989). For such purpose, kidney cell suspension (10^7 cells/mL) were seed into a 96 well plate (100uL per well). NPs and stabilizer stocks, were diluted 1/10, 1/100, 1/500, 1/1000 and 1/10000 and each dilution (100uL) was added to the cells (n=3). Controls of the NPs alone, to check photo degradation were included as well as cell controls without NPs. Moreover, cells with different dilutions of the NPs stabilizer (citrate and PVP) were also tested. Cell culture media was supplemented with 4g/L of glucose in order to activate cellular metabolism and cells were incubated for 24h. Following, MTT tetrazolium salt solution at a 5mg/mL concentration in PBS, pH 7.4 and sterilize it by filtration (0.2um) was prepared. 40uL of MTT solution was added into each well and cells were cultured at 20ºC for 5h. Immediately after, 150uL of the supernatant were carefully removed and 100uL of a 10% Sodium dodecyl sulphate (SDS) solution were added to dissolve formazan crystals. Plates were incubated overnight at 20ºC. Finally, absorbance at 570 and 690 nm was read in a Multisky Scanner Spectrophotometer (ThermoFisher).

(Continued on next page)

Data calculation and statistical analysis: data was analysed after subtracting the background absorbance at 690nm and the absorbance of the nanoparticles alone for each case. Results were represented as viability percentage respect to the control group without NPs, including calculations for the average and the standard error. Statistical analysis was performed by t-test of each concentration respect to the previous one to infer the concentration at which NPs became toxic for the cell, by using Graph Pad Prism software. LD_{50} was calculated by using Quest Graph™ LD50 Calculator tool.

Results and Discussion

Effect of NPs depends on their properties as size, shape, material, surface, aspect-ratio, dispersion and stability among others. NPs of 2 different materials (Ag and TiO_2) and different sizes were tested. Regarding TiO_2 NPs, the loss in cell viability at 24h was higher in the bigger NPs (45nm). Nevertheless, this effect is not correlated to size as the medium size NPs (P25) didn´t have any effect on cell viability while the smallest ones (5nm) presented a similar effect to 45nm ones. Citrate, the TiO_2 nanoparticles stabilizer didn´t cause any interference in cell viability. TiO2 NPs were described to cause effects from the surface of the cells (Picchietti *et al.,* 2017). A potential explanation may be related to the NPs aggregation, avoiding being internalised by the cell but this hypothesis needs to be further studied.

Concerning Ag NPs, a decrease in cell viability was observed for both types, being as well an independent effect from NP size. Nevertheless, the stabilizer of Ag NPs (PVP) showed a slight negative effect at the higher doses in sea bream. Moreover, a higher background signal was observed in Ag NPs by naked eye, under the microscope and by spectrophotometric results in comparison to TiO_2 NPs. NPs effects were very similar in both fish species. Nevertheless, some interspecific differences can be appreciated, as for the case of 5nm TiO_2 NPs, which affects sea bream cells viability in a more pronounced way.

Ag NPs caused a reduction in cell viability at lower concentrations than TiO_2 NPs. All the effects observed for the 2 NP types showed significant differences to the control at the higher dosages (g/L range), which are far from their predicted environmental concentrations (µg/L – ng/L).

The results of this study served as the basis for designing more comprehensive toxicity studies deepen in other aspects as microscopy, spectrometry or molecular biology.

References

Tada H *et al.,* 1986. DOI: 10.1016/0022-1759(86)90183-3.
Torrealba D *et al* 2019. DOI: 10.3389/fimmu.2018.03074.
Picchietti S *et al.,* 2017. DOI: 10.1016/j.fsi.2017.01.044.
"Quest Graph™ LD50 Calculator." AAT Bioquest, Inc, 24 Aug. 2021, https://www.aatbio.com/tools/ld50-calculator.

Acknowledgments

The authors wish to acknowledge the financial support of the European Union (Interreg POCTEP, project ACUINANO, reference 07-12-ACUINANO_1_E).

MULTIDIMENSIONAL AQUACULTURE INVESTOR INDEX: BLACK SEA RIPARIAN COUNTRIES

Yuliia Buhlak[1,2], Patrice Guillotreau[1], Thomas Vallee[1], Veronique le Bihan[1], J.A. Theodorou[2]

[1]Institut d'Economie et de Management de Nantes, LEMNA, Université de Nantes, Chemin de la Censive du Tertre, 44322 Nantes, Cedex 3, France
[2]Department of Animal Production, Fisheries & Aquaculture, University of Patras, Nea Ktiria Gr 30200, Mesolonghi, Greece
E-mail: buglak@aq-ua.info

Introduction

The demand for fish products in Black Sea riparian countries has been intensively growing annually by 4% since 2000. This demand was predominantly covered by importation and/or capture fisheries. To reduce pressure on the wild fish stocks of the shared sea basin, regional and national development plans are aiming to stimulate aquaculture production. However, there is a scarcity of instruments that allow assessment of the situation on the regional level to capture the multidimensional nature of such activity. A Composite Indicator or so-called Index has been recognized as one of the best ways to investigate the complex system statement and reveal areas of concern. This study was aiming to investigate whether some indicators have greater impact on industry development. Furthermore, the index can be used by investors as a quick guide to fish farming bottlenecks and strengths of each country in the Black Sea region and facilitate investments to the industry.

Materials and Methods

The Index was constructed on the basis of best practices carefully selected by the joint collaboration of Organisation for Economic Co-operation and Development and Joint Research Centre (OECD, 2008). The conceptual foundation of the current research was drawn from the Horizon 2020 Aquaspace project deliverable D2.4: Smartphone 'Investor Appeal' application (Ferreira, et al., 2017; Ferreira, et al., 2020) with region-specific adjustments and improvements suggested by the authors. All indicators and analytical methods were selected considering the nature of the data in the given region. Moreover, 20 experts were invited to participate to the selection and validation of indicators. In addition to that, they were asked to assign weights to each indicator. Principle Component Analysis were also used for weights allocation as a counterweight for comparison of two methods. Finally, with the help of innovative approach all final indicators were tested for correlation with the main output variable such as aquaculture production per capita. This approach helped to equalize all countries in terms of production capacity and to see if some indicators have greater correlation with higher production performance among countries.

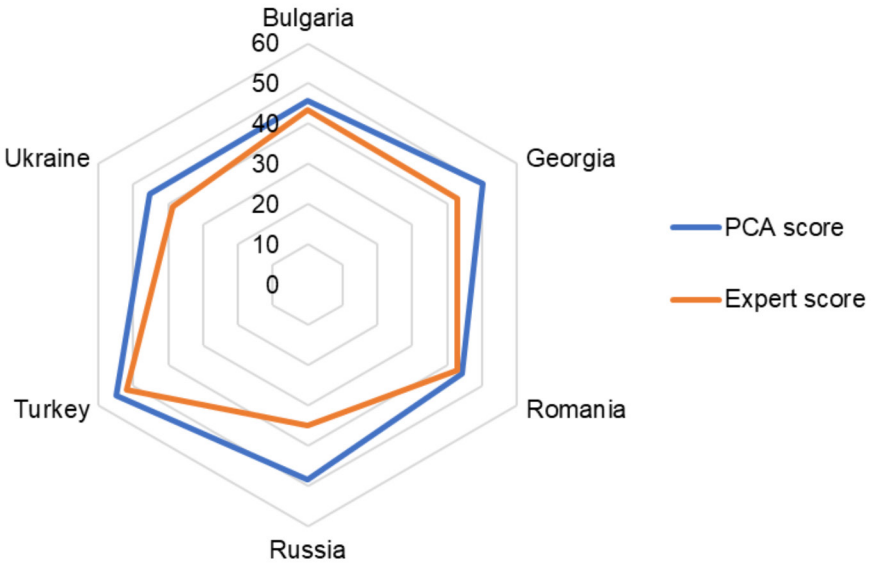


Figure 2. Comparison of final scores obtained with two weighting methods: PCA-based and Expert-based

(Continued on next page)

Results

Final scores with expert-based weighting were not largely different from PCA weighting. The main difference was observed only for Russian scores due to the low involvement level of aquaculture experts (Fig.1). It is also interesting to notice in some disparities of scores, that all experts were more concerned by the governance factors, while unbiased PCA method assigned higher scores to some market indicators which were largely underestimated by the experts. It was concluded, that further research should include experts from the market sector.

Overall market-related factors appeared to be more influencing in aquaculture development for all countries in the region (Fig.2). Environmental and Production sectors were less influencing than Market dimension; Governance and Social dimensions had the smallest impact on fish farming development in the Black Sea region.

Discussion

Multidimensional aquaculture investor index has demonstrated to be an effective method to capture the level of importance of aquaculture-relevant indicators to build successful governance strategy and to assist investors. Due to the time restrictions of the project, some indicators data couldn't be collected on time. Though, indicative results were obtained even with the reduced list of indicators. Further research with complete list of indicators will provide greater insight to the aquaculture landscape in the region and give more additional highly-influencing factors for policymakers.

References

OECD, 2008. Handbook on Constructing Composite Indicators. s.l.:OECD Publishing.

Ferreira, R. et al., 2017. EU Horizon 2020 project grant no. 633476, Deliverable D2.4, Smartphone 'Investor Appeal' application. s.l.:s.n.

Ferreira, R. G. et al., 2020. A multimetric investor index for aquaculture: Application to the European Union and Norway. Aquaculture, 516(1).

ARTIFICIAL INTELLIGENCE (AI) DRIVEN PLATFORMS TO DEVELOP NOVEL ASSAYS TO MONITOR FISH HEALTH

R. Buks[1,2,*], Y. Hu[1], A. Alnabulsi[2], A. Alnabulsi[3], C. Secombes[1], T. Wang[1], S.A.M. Martin[1]

[1]Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, UK
[2]Vertebrate Antibodies Ltd, Aberdeen, UK
[3]AiBIOLOGICS, Dublin, Ireland
*Presenting author, email: ralfs.buks@abdn.ac.uk

Introduction
Global aquaculture production more than tripled in live-weight volume in the past two decades, reaching 112 million tonnes. To ensure efficient production fish health monitoring is essential to minimise pathogen outbreaks in a continuously growing industry. Our team has developed an AI framework "EpitopePredikt" to predict B-cell immunodominant epitopes. The immunodominance is an immunological phenomenon in which immune responses are dominated by only a few peptides of the antigenic nature. The identification of antigenic peptides will facilitate the development of accurate serodiagnostic tests, more effective vaccine and adjuvants as well as improve efficiency of antibody production. The second innovative platform is a non-reactive bioengineered scaffolds "EpitoGen" whereby epitope/s can be assembled to create chimeric antigens suitable for serology-testing, vaccine delivery and efficacy testing, and immunoassay validation. Combined, the two technologies will revolutionize the health-related tools for the aquaculture sector. We currently have generated ~50 antibodies to key markers, and >100 recombinant proteins including flagellins, potent immunostimulants. Employing AI precision-based platform with innovative bioengineered scaffold will offer ground-breaking solutions to persistent problems in the sector.

Aims
1) Develop a panel of antibodies for research in fish.
2) Develop sensitive and affordable high throughput antibody and peptide-based serology assays to monitor fish health in aquaculture, i.e., enzyme linked immunosorbent assays (ELISAs).
3) Develop the next generation of bio-active immunomodulators to boost fish robustness.

Materials and Methods
We performed fish biomarker search using published based on published literature in combination with gene expression and sequence data. We identified a panel of key health markers in need of immunoassay to chart their levels. We predicted 3D structures of candidate biomarkers to identify optimal accessible and surface exposed regions suitable for antibody production. EpitopePredikt was utilised to identify "hot spots" suitable for antibody design. Short peptides were used as immunogens to generate mono and polyclonal antibodies against the fish candidates in mice and rabbits respectively. We are also developing bio-active molecules to potential immunostimulants such as interferons, antimicrobial peptides to enhance ongoing research.

Results
We have generated a range of fish antibodies and bio-active recombinants that can be used as tools for academic research, as well as prospective immunoassays to monitor fish health in aquaculture. We are currently finalising our study and validating specificity for a panel of generated antibodies and to test bioactivity. Our products will be produced to support the unmet demands of the aquaculture sector for affordable, accurate and high throughput platforms.

OXYGENATION EFFECTS ON TEMPERATURE AND OXYGEN AT A COMMERCIAL ATLANTIC SALMON (*SALMO SALAR*) FARM

M. Burke[a]*, J. Grant[a], R. Filgueira[b], T. Weire[c]

[a]Department of Oceanography, Dalhousie University, Halifax, NS, Canada, B3H 4R2
[b]Marine Affairs Program, Dalhousie University, Halifax, NS, Canada, B3H 4R2
[c]Cooke Aquaculture, St. George, NB, Canada, E5C 3E2
E-mail: Meredith.burke@dal.ca

Introduction

Climate change has threatened coastal ecosystems through elevated sea temperatures while contending 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). Adequate dissolved oxygen (DO) concentrations through finfish farms are paramount as low oxygen can directly impact the health and growth of fish. In the short-term, low oxygen can impact feed intake and feed conversion rates, and if prolonged, can result in fish stress, vulnerability to diseases, and mortality (Oldham et al. 2019). Open net-pen farms that contain high stocking densities or exhibit low oxygen during warmer months, may use oxygenation devices to increase DO (Berillis et al. 2016). However, the transport and distribution of oxygen can be costly in ocean environments thus it is only occasionally used and the viability of these systems have yet to be adequately examined. This study explores the effectiveness of oxygenation in an Atlantic salmon net-pen farm to inform farm managers on its use as climate change threatens the industry.

Materials and methods

This study was conducted at a Cooke Aquaculture Atlantic salmon farm consisting of 6 circular cages (48 m in diameter, 10 m deep), located within a bay in southwest Nova Scotia. The study period was between June 1 and November 30 2019, with the deployment of oxygenation within each of the cages between August 4 and November 6. The oxygen system was the NetOx Net (OxyVision AS, Norway), which releases micro-bubbles of oxygen as a curtain at 8m depth. The cage analyzed in this study contained 2 DO and temperature sensors (AquaMeasure, InnovaSea Systems, Inc., Bedford, Nova Scotia), one at 2m and one at 7m. Additionally, there was a reference sensor located ~75 m outside the cage. Satellite sea surface temperature (SST; 4km AVHRR Pathfinder Version 5.3) produced by NOAA National Centers for Environmental Information, was also used as a far field reference for cage temperature. Physical (e.g. tide, wind stress) and biological factors (e.g. theoretical fish consumption), which typically affect oxygen dynamics within a farm, were examined to determine how oxygenation may interact with those processes.

Results

Through June 1 – August 26, there was a strong stratification between 2m and 7m for both temperature and oxygen. The activation of oxygenation (Aug. 4) coincided with a sharp drop in temperature at both depths. There was a significant difference in temperature when comparing 3 weeks before/after the onset of oxygenation, with average values dropping from 16.7 to 15.4°C and from 14.3 to 13.2°C for 2m ($p<0.001$) and 7m ($p<0.001$), respectively (Fig. 1A). However, this drop was not observed in the satellite SST. On August 26th, autumn mixing began and stratification broke down resulting in a negligible difference between depths. While the temperatures declined at the onset of oxygenation, there was a concurrent increase in oxygen from an average value of 7.3 to 7.8 mg L^{-1} and from 8.3 to 8.9 mg L^{-1} at 2m and 7m, respectively. This resulted in an overall significant increase in oxygen on average 3 weeks after oxygenation compared to 3 weeks before ($p<0.001$; Fig. 1B).

Discussion

The results of this study suggest that although SST was similar before and after oxygenation, the system may have caused an upwelling of cooler waters which decreased temperatures throughout the cage. Similar findings were described in studies by Endo et al. (2018) and Bergheim et al. (2006). This decrease in temperature increased solubility while decreasing fish metabolism, furthering an increase in oxygen concentrations. Overall, the results imply that the oxygenation system increased upwelling and DO at the cage level until autumn mixing became the dominant source of re-aeration. This suggests that the system was effective promoting an increase in DO, but it does not need to run for the entire duration of the warm season, it may only be useful until de-stratification, which would save the farm associated economic costs.

(Continued on next page)

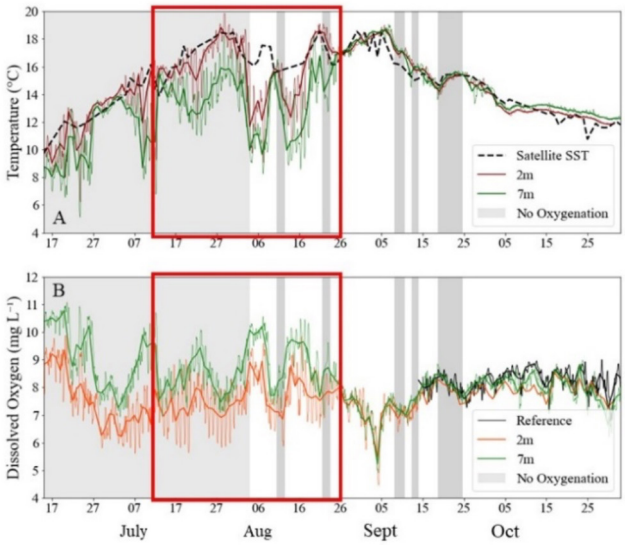


Figure 1. (A) Hourly (lighter lines) and daily (darker lines) average temperature (A) and DO (B) within cage 5 at 2m (maroon/red) and 7m (green) in 2019, along with the sea surface temperature (SST) derived from satellite data (dashed line) and at the reference (black). The grey bars indicate approximate times when the oxygenation system was off, and the red box indicates the period of analysis.

References

Bergheim, A., Gausen, M., Naess, A., Holland, P.M., Krogedal,. P., Crampton, V., 2006. A newly developed oxygen injection system for cage farms. Aquacultural Engineering 34, 40-46.

Berillis, P., Mente, E., Nikouli, E., Makridis, P., Grundvig, H., Bergheim, A., Gausen, M., 2016. Improving aeration for efficient oxygenation in sea bass sea cages. Blood, brain and gill histology. Open Life Sciences 11, 270–279.

Breitburg, D., L.A. Levin, A. Oschlies, M. Gregoire, F.P. Chavez, D.J. Conley, V. Garcon, D. Gilbert, D. Gutierrez, K. Isensee, G.S. Jacinto, K.E. Limburg, I. Montes, S.W.A. Naqvi, G.C. Pitcher, N.N. Rabalais, M.R. Roman, K.A. Rose, B.A. Seibel, M. Telszewski, M., Yasuhara, Zhang J., 2018. Declining oxygen in the global ocean and coastal waters. Science 359, eaam7240.

Endo, A., Srithongouthai, S., Nashiki, H., Teshiba, I., Iwasaki, T., Hama, D., Tsutsumi, H., 2008. DO-increasing effects of a microscopic bubble generating system in a fish farm. Marine Pollution Bulletin, EMECS 7/ECSA 40, Caen (France), May 2006 57, 78–85.

Oldham, T., Nowak, B., Hvas, M., Oppedal, F., 2019. Metabolic and functional impacts of hypoxia vary with size in Atlantic salmon. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 231, 30-38.

EFFECTS OF DIFFERENT INCLUSION LEVEL OF *Hermetia illucens* LARVAE MEAL ON GROWTH, PLASMA BIOCHEMISTRY AND FILLET QUALITY OF GILTHEAD SEA BREAM (*Sparus aurata*)

S. Busti[1]*, L. Parma[1], F. Dondi[1], M.C. Sabetti[1], E. Brini[1] L. Morsiani[1], F. Brambilla[2], A. Badiani[1], M. Magnani[1], M. Petracci[3], G. Baldi[3], F. Soglia[3], P. P. Gatta[1], A. Bonaldo[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna, Italy
[2]VRM S.r.l., Via Sommacampagna 63/d – 37137, Verona, Italy
[3] Department of Agricultural and Food Sciences - DISTAL, University of Bologna, Piazza Goidanich 60 - 47521 Cesena (FC)
E-mail: serena.busti2@unibo.it

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 insects. There are many positive aspects of insect use as sustainable aquafeed ingredient especially for their high nutritional value and being a source of macronutrients and bioactive compounds[1]. Most studies have explored the effect of insect meal as fishmeal (FM) replacement on growth and health of Mediterranean fish species, while less attention has been paid on the evaluation of nutritional value as well as technological and sensorial properties of the final fish product. Thus, this study was performed in order to assess the effects of different inclusion levels of *Hermetia illucens* (HI) larvae meal on growth performances, plasma biochemistry, as well as gilthead sea breams' nutritional, technological and sensorial quality.

Materials and methods
One trial with gilthead sea bream was conducted in a closed recirculation aquaculture system (RAS). Fish (initial weight: 98.6 ± 0.6g), were fed over 113 days with four experimental diets containing different inclusion levels of HI meal (0% CTRL, 5% HI5, 10% HI10, and 15% HI15) in substitution to FM. At the end of the trial, growth, and feed efficiency parameters (Specific Growth Rate, SGR, Feed Intake, FI, Feed Conversion Rate, FCR and survival), and somatometric indexes (Viscerosomatic Index, VSI, Hepatosomatic Index, HSI, Condition Factor, CF, Fat Index, FaI), were assessed. Blood was collected from the caudal vein of five fish per tank to perform plasma biochemistry analysis. Also, a total of sixteen fish per tank were sacrificed and the dorsal-left skinned fillets collected to carry out nutritional composition analysis, to assess the main technological properties of meat, with a special reference to its ability to absorb a marinade solution and to investigate if a sensory (panel test) difference could be detected between the control diet and each one of the three experimental diets. Data were analysed by a one-way ANOVA followed by a Tukey's multiple comparison test.

Results
At the end of the trial, no significant differences ($p > 0.05$) ascribable to the different diets were observed in terms of final body weight, SGR, FI, and FCR and survival for all treatments (Figure 1). Also, no significant differences ($p > 0.05$) were found in somatometric indexes (i.e., VSI, HIS, CF, FaI).

Discussion and Conclusion
The results showed equivalent growth performances regardless of the different diets showing that HI larvae meal could replace 5%, 10% and 15% of FM without compromising the growth and feed utilization. This is in line with previous studies on sparids in which IM was tested, stating that a replacement of FM up to 25% do not negatively affect fish performances[2]. The investigation on the technological and sensorial aspects of the fillets will boost the current knowledge on insect meal properties as valid alternative protein source to FM for aquafeed in Mediterranean fish species.

(Continued on next page)

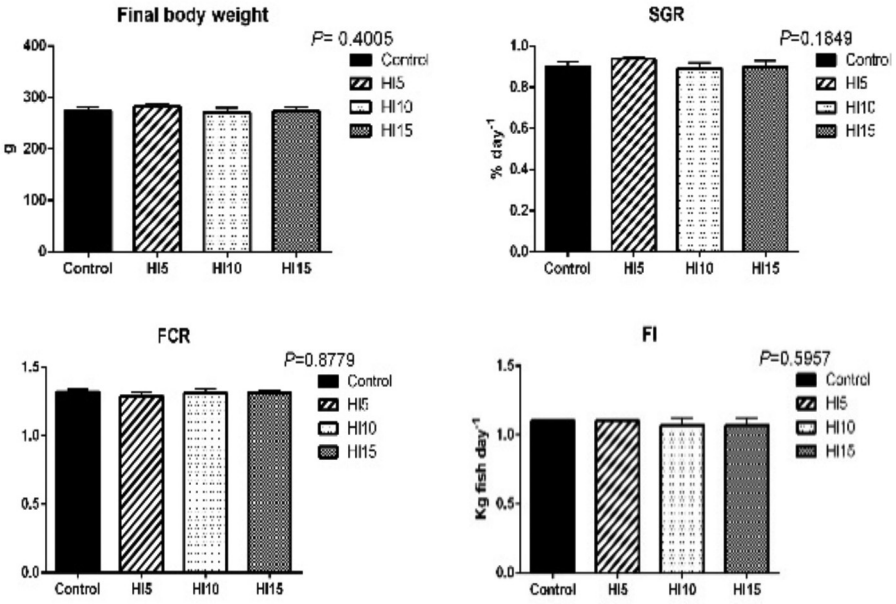


Fig. 1 Final body weight, SGR, FI and FCR obtained at the end of the trial under different treatments. No significant differences ($p > 0.05$) were observed.

References

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P.P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., Roncarati, A., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Italian Journal of Animal Science, 19:1, 360-372

Piccolo, G., Iaconisi, V., Maronoa, S., et al. 2017. Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (Sparus aurata). Animal Feed Science and Technology 226, 12-20.

Acknowledgement

This research was undertaken under the NextGenProteins (Next Generation Proteins for food and feed) project, funded by European Union's Horizon 2020, research and innovation programme. Gran Agreement number 862704, 48 months.

AQUACULTURE BUSINESSES DURING COVID-19: A UK CASE STUDY

S.C. Franco *[1], S.L. Billing[1], G. Charalambides[1]

* corresponding author: sofia.franco@sams.ac.uk
[1] Scottish Association for Marine Science, Oban, Scotland PA37 1QA, UK

Introduction

The seafood industry has been under unprecedented pressure to deliver on national food security during COVID-19/ SARS-CoV-2 pandemic, while adapting to remain viable. However, limited literature exists on the systemic impacts to the seafood industry in European countries, adaptation actions employed by businesses or its associated environmental and socio-economic consequences. This hinders both the timely adoption of measures that address ongoing challenges, and the implementation of strategic actions aimed at increasing the industry and food system resilience to future disruptive events.

This study, part of the RiseUp project (www.sams.ac.uk/science/projects/riseup/), collected evidence on the early impacts of COVID-19 disruption across the UK seafood industry, how these have been managed by businesses and how impacts propagated trough the supply network. We focus herein only on the impacts and responses as experienced by aquaculture businesses in the UK, placing these in context of the wider systemic effects across the supply network and in consideration of concurrent changes, notably the change in trade and cooperation with the EU (shown in the e-poster only).

Material and Methods

This study adopted a constructivist qualitative approach which enabled in-depth exploration of the research questions with the study participants, through 40 interviews across the UK seafood supply chain. Semi-structured interviews (av. 42 min/interview) were conducted virtually or by phone between October 2020 and February 2021 (of which 17.5% were done in 2021). Participants comprised aquaculture, fisheries, processing, wholesale, retail, food service, other supply chain, government and regulators, including associations and trade bodies; which enabled a cross-cutting representation of seafood system actors. The interview guide (see Franco et al.) explored (i) COVID-19 impacts and context of business responses, (ii) representation and interaction with government responses, (iii) pathways forward and the 'new normal'.

Interviews were transcribed and qualitatively coded using an inductive approach within a flexible thematic framework, from high-level themes based on literature and grey reports on COVID-19 impacts. This considered Seafish reporting areas (market volatility, supply-chain disruption, labour pressures, fish production, food safety and health, global business climate, sector support), the thematic areas in the interview guide and the timeline of COVID-19. Coding was conducted using a thematic approach, following from open, to focused coding and final consolidation of codes, continually referring to the interview transcripts to ensure that the final coding and emergent themes (i.e., 'communication', 'BREXIT') were representative of the raw data. We herein present the results relevant to aquaculture respondents, mostly addressing the research questions on (i) 'How were businesses in the UK seafood industry impacted by COVID-19 pandemic?'; (ii)' Which actions did businesses take in response?', and to a lesser extent on (iii) 'What helped businesses respond to change?'.

Results and Discussion

The main early challenges of the COVID-19 pandemic to UK shellfish and finfish aquaculture producers related to the added costs and operational constraints in maintaining ongoing production and keeping animal welfare standards, whilst coping with significant disruptions to trade, from the collapse of export markets and food service closures (e.g., from lockdowns) to transportation disruption (e.g., decline in passenger flights, challenges to road haulage, temporary border closure). With a high volume of UK production intended for export, respondents reported a depression in trade volume from market volatility and supply chain disruptions. Though businesses sought to identify alternatives export routes, these proved challenging (e.g., using air freight was deemed an uneconomical alternative to passenger flights), with attempts made to raise awareness and address logistical efficiency challenges (e.g., delays to product transportation; lack of chilled warehousing infrastructure). Some businesses further responded to market volatility and supply chain disruptions by shifting markets/outlets (e.g., in between export countries, from hospitality to farmers markets) and working with their supply chain to change the product offering to fit new market demands (e.g., change in range or format), with varying degrees of success. Some noted the challenge of contesting the market share with international competitors or across-segments.

(Continued on next page)

Finfish and shellfish producers continued operating with the introduction of COVID-secure measures (e.g., social distancing, etc.), despite reporting impacts from reduced staff levels at times and pressure on the workforce. The benefits of being designated as 'key workers' and support mechanisms such as the furlough scheme or various support grants were noted across the sector, despite reported differences between developed nation differences and suitability, across sub-sectors or company size. Improved communication was generally considered a key positive change. Performance across businesses/sub-sectors varied, with some recovery noted by respondents in the second half of 2020, upon the re-opening of hospitality/food service and various export markets. Various businesses reported experiencing very high losses in the first half of 2020, from the impacts to export markets and closures in hospitality, which were partially mitigated in some cases by gains in the UK market, derived from higher sales in UK retail or direct-to-consumer sales. Shellfish farmers further mentioned a shift to direct-to-consumer sales during market closures, though this was noted by businesses as not being economically viable in the long term. Some shellfish businesses reported developing seafood box schemes, diversifying product lines or increasing online presence and marketing. Others reported halting expansion plans and using up contingency funds (i.e, planned for disease outbreaks). Further specific responses from fish farmers included consulting more regularly with regulators and government on animal welfare matters (e.g., stocking biomass, medication). Seaweed producers also increased efforts in business-to-business relations to presell stock, with impacts felt to specific product lines (e.g., grab & go), further highlighting issues of lack of representation as a relatively new sector, with one mentioning the difficulty of start-ups to access government support.

The impacts of market volatility and supply chain disruptions to 'business-as-usual' were reflected in a number of adaptations by aquaculture businesses, which focused in one hand on ensuring continuity of operations and avoid market oversupply, and in the other in re-establishing routes to market and build new income streams. Whilst impacts-to-responses varied by sub-sector, vulnerabilities such as the high reliance on given export markets, hospitality sector, locked-in transportation routes, limitations from price-benefit positioning, importance of the chilled segment and of short time to costumers, were exacerbated by structural limitations such as poor chilled infrastructure or export pathways, limitations in seafood uptake and price-competitiveness in the domestic market, and the lack of recognition of sub-sector-specific challenges in some of relevant government departments. Major strengths related to focus on business-to-business relations, benefits of sector representation in engaging with policy-makers, flexibility within the regulatory system, as well as individual financial contingency mechanisms or eligibility for funding, and internal capacity to diversify and/or evolve business models.

Acknowledgments
RiseUp 'Resilience of the UK Seafood industry to COVID-19' is supported by the Economic and Social Research Council grant ref.ES/V009907/1 to Franco. The authors would like to recognise Seafish's (www.seafish.org) continued support and express their gratitude to the businesses that took part in the interviews and shared their experience.

VACCINATION OF GILTHEAD SEABREAM (*Sparus aurata*) AFTER ESTROGENIC ORAL MODULATION ALTERS THE GUT ENDOBOLOME AND IMMUNE STATUS VIA GPER1

Isabel Cabas[1,†,*], P. Castejón[1,†], Victoria Gómez[1], Elena Chaves Pozo[2], Isabel Cerezo-Ortega[3], Miguel Ángel Moriñigo[3], Eduardo Martínez Manzanares[3], Alfonsa García-Ayala[1,‡], Jorge Galindo-Villegas[4,‡]

[1] Department of Cell Biology and Histology, Regional Campus of International Excellence "Campus Mare Nostrum," University of Murcia, IMIB, CIBERER, 30100 Murcia, Spain
[2] Oceanographic Center of Murcia (IEO), Mazarrón, 30860 Murcia, Spain
[3] Department of Microbiology, Faculty of Sciences, University of Malaga, Spain
[4] Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway
[†,‡] These authors contributed equally to this work.
[*] Email: icabas@um.es

Introduction

In fish culture setting grounds, exogenous steroids input is a matter of concern. Recently, we unveiled that in the gilthead seabream (*Sparus aurata*), the G-protein-coupled estrogen receptor agonist G-1 (G1) and the endocrine disruptor 17α-ethinylestradiol (EE_2) are potent modulators in polyreactive natural antibodies production. However, the integral role of the microbiota upon antibody production under the effect of EE_2 remains largely unexplored.

Material and Methods

Here, 240 juvenile seabreams continuously exposed for 84 days to oral G1 or EE_2 at a fixed dose (5 μg/g food) were i.p. vaccinated on day 42 with the model antigen keyhole lymphet hemocyanin (KLH). A critical panel of systemic and mucosal immune markers, serum vitellogenin, humoral enzymatic, and bacteriolytic activities were recorded and correlated with the gut bacterial microbiome 16S rRNA status one day post priming (dpp). Besides, 15 dpp animals received a boost to explore the systemic- and mucosal-specific anti-KLH titers production by the end of the trial.

Results and Conclusion

EE_2 but not G1 induced a significant shift in the serum vitellogenin level one dpp. Simultaneously, in the serum and gut mucus of the EE_2 treated group, we recorded significant changes in some immune enzymatic activities. While we only inferred an attenuated profile in the immunized groups. The gut genes qPCR analysis exhibited a related pattern only emphasized by the significant shift on the EE_2 group *il1b* expression. The gut bacterial microbiome undergoes dynamic changes in alpha diversity indices, but only with exposure to dietary G1, supporting functional alterations on cellular processes, signaling, and lipid metabolism. By the same token, the immunization in both treated groups decreased the relative abundance of Fusobacteria and remarkably promoted changes in the estrogen-associated bacterial genera. Besides, systemic and mucosal KLH-specific IgM/T titers observed significant changes after 84 days of estrogenic oral administration.

In summary, these are the first results highlighting the intrinsic relationship between estrogens and their associated receptors in the ubiquitous fish immune regulation and the subtle but significant crosstalk with the gut endobolome.

Acknowledgements

This study was funded by Fundación Séneca, Coordination Center for Research, CARM (04538/GERM/06). The Spanish Ministry of Science and Innovation (AGL2008-04575-C02-01 co-funded with Fondos Europeos de Desarrollo Regional/ European Regional Development Funds). Ministerio de Ciencia e Innovación and FEDER (AGL2017-85978-C2-1-R). The Fundación Séneca (CARM) (19883/GERM/15).

DEPLOYMENT OF MICROBIOMES FOR SUSTAINABLE FISH FARMING IN THE SIMBA PROJECT

J. Cabello*, I. Folgueira, I. Iglesias, E.-j. Malta

Centro Tecnológico de Acuicultura de Andalucía (CTAQUA), Muelle Comercial S/N, 11500, El Puerto de Santa María, Spain
Presenter: j.cabello@ctaqua.es

Introduction
SIMBA is a €10.5 million European Union Horizon 2020-funded Innovation Action project that has the vision to create a better EU Agro-Aqua-Food system using microbiomes that is resource efficient, climate resilient, sustainable and consumer centred. To do this, over the four-year project, SIMBA will explore and utilise microbiomes from the land and sea to develop numerous innovative solutions to address key challenges identified.

Work Package 3 of the project I dedicated to marine microbiomes. In this WP, one of the tasks is the optimisation of fish feed for sustainable fish farming. On the one hand, 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, work carried out by the Danish partner Fermentation Experts. On the other hand, the role of the microbiome in the production of phytoplankton species that can serve as feed additives is evaluated by the Portuguese partners NECTON and ALLMICROALGAE. Potential new feed mixes made with these ingredients are evaluated for sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) at CTAQUA (Spain) and for freshwater and seawater grown Atlantic salmon (*Salmo salar*) at LUKE (Finland) and NIVA (Norway) respectively.

Material and Methods
Effects of dietary administration (2% inclusion) of microalgae (*Chlorella vulgaris*, *Nannochloropsis* spp, *Tisochrysis lutea*), 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 (to be analysed by SIMBA partner MATIS, Iceland), water quality, main non-specific immune 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.

Results and Discussion
Fish performance as measured by growth rate and feed conversion was significantly 10 to 15% lower for the EP199 diet compared to the control diet for sea bream. In sea bass, the other diet containing fermented compounds (EP299) also showed a slight but significant reduction in performance compared to the control. Diets containing phytoplankton did not differ significantly from the control (Figs. 1A and B).

Analyses of most relevant non-specific immune indicators are currently in progress. First results of the non-specific immune indicator lysozyme activity, showed higher values for the diets containing fermented compounds and the microalga *Chlorella* in both fish species, however the differences were not significant compared to the control diet. A consistent significant decrease of lysozyme activity compared to the control diet was observed in diets containing *Nannochloropsis* sp. and *Tisochrysis lutea* (Fig. 2).

Additional analyses of non-specific immune indicators, fillet composition and composition of the intestinal flora are currently being carried out. Current results indicate that addition of phytoplankton to fish feeds does not impact nor improve general performance, whereas effects on immune indicators might be species specific. Partial substitution (50%) of fish meal protein by fermented compounds, in particular compound EP299 had no negative impact in the case of sea bream and a slight reduction in performance in the more exclusively carnivorous sea bass, suggesting that perhaps at lower substitution percentages this might be a sustainable alternative for fish meal protein.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431 (SIMBA). This output reflects only the author's view and the Research Executive Agency (REA) cannot be held responsible for any use that may be made of the information contained therein.

(Continued on next page)

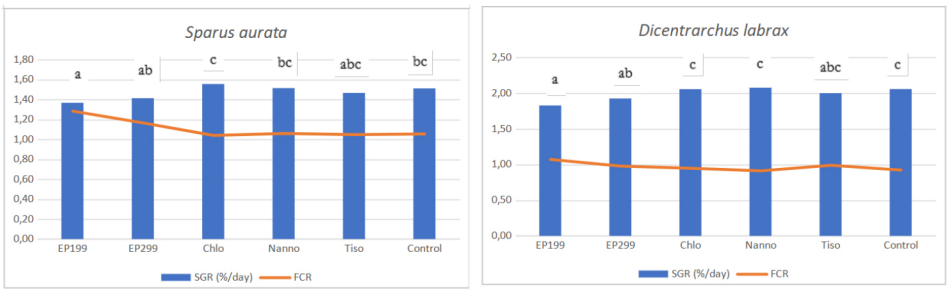


Fig. 1 A and B: Fish performance (growth rate and feed conversion rate) in five experimental diets containing fermented compounds or phytoplankton compared to a standard control diet in *Sparus aurata* (A) and *Dicentrarchus labrax* (B).

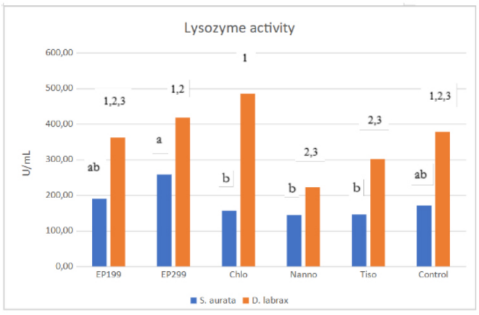


Fig. 2: Lysozyme activity in *Sparus aurata* (blue bars) and *Dicentrarchus labrax* (orange bars) fed five experimental diets containing fermented compounds or phytoplankton compared to a standard control diet.

FUNCTIONAL GENOMIC ANALYSIS OF LANDLOCKED ATLANTIC SALMON (*Salmo salar*) POPULATIONS

Ross Cairnduff [1] *, Erik Kjærner-Semb[1], Rolf B. Edvardsen[1], Per Gunnar Fjelldal[1], Tom Hansen[1], Tom Ole Nilsen[2], Anna Wargelius[1]

[1] Institute of Marine Research, Bergen, Norway
[2] University of Bergen, Bergen, Norway
E-mail: ross.fisher.cairnduff@hi.no

Introduction

Given both the economic and societal impact of the Atlantic Salmon *(Salmo salar*) farming industry in Norway, increased knowledge around heritable traits, which may translate into welfare gains in both wild and farmed salmon, is of high demand. This may include traits associated with disease resistance, growth and sea-water adaption. Genetic potential may reside in landlocked populations which have undergone different selection pressures compared to their anadromous counterparts for the last 10,000 years, due to their lack of sea water transfer, lack of marine pathogen pressures and different feed availability. We have in previous studies identified genes and genomic regions diverging between anadromous and landlocked Atlantic salmon populations from a widespread geographical distribution. However, we do not yet understand the biological significance of these diverged genomic regions, spanning about 250 genes distributed on several chromosomes. Hence, the objective of this study was to increase the biological understanding of the genetic differences between anadromous and landlocked populations. From the 250 genes present in diverging regions, we picked 22 candidate genes previously associated with either disease resistance, growth and sea-water adaption in other species. Gene expression was assayed in several tissues before and after the predicted smoltification season in a common garden smoltification experiment including one-year old anadromous farmed salmon (Mowi) and landlocked salmon (Gullspång).

Results

From the 22 genes we identified differential expression in 4 genes; *ncor1*, encoding a thyroid hormone mediator in the gill was shown to be down-regulated in Gullspång post smoltification, while remaining fixed in the Mowi counterpart. *spcs3* encodes a protein involved in microsomal peptide signalling in the head kidney and was shown to be up-regulated in Mowi, while remaining fixed in Gullspång. *csf2rb2* encodes an activator in the JAK-STAT pathway and was down-regulated in Gullspång while being fixed in Mowi. *bcl2l13,* linked to adipocyte biogenesis, was significantly higher Gullspång than it was in Mowi. To further investigate whether the identified genomic regions contain genetic variation relevant for understanding and improving traits in farmed salmon, we are currently screening farmed salmon strains for potential landlocked alleles using a genotype-by-sequencing (GT-seq) approach. The plan is to perform genotyping of 28 regions in the salmon genome in a vast material of farmed fish in which traits of interest have been quantified, such as growth and sea-water adaption. This study provides a basis for further functional studies of candidate genes through the creation of knockout models and subsequent functional studies including growth, disease and feeding trials.

TRACEABILITY TOOLS TO VERIFY THE GEOGRAPHIC ORIGIN OF AQUACULTURE PRODUCTS: FOSTERING ORIGIN CERTIFICATION AND FIGHTING FRAUDULENT PRACTICES

R. Calado*, R. Mamede, A. Santos, D. Gonçalves, M.R. Domingues, A.I. Lillebø, P. Domingues, S. Cruz, F. Rey, P. Cartaxana, E. da Costa, B. Marques, C. Patinha, E. Ferreira da Silva and F. Ricardo

ECOMARE, CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Santiago University Campus, 3810-193, Aveiro, Portugal
E-mail: rjcalado@ua.pt

Seafood trade has been steadily growing worldwide over the last decade, with aquaculture production playing a key role. Food safety is increasingly important to consumers that want to know the origin and mode of production of their seafood. Traceability in seafood supply chains is increasingly becoming a requirement in importing countries to safeguard public health and ensure good practices. The concept of traceability has been defined by the EU as the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution. Implementation of seafood traceability regulations is especially challenging on origin certification, although EU legislation has specific requirements targeting seafood origin. It is worth emphasizing article 58 of EC 1224/2009 that states the need of "all lots of fisheries and aquaculture products shall be traceable at all stages of production, processing and distribution, from catching or harvesting to retail stage".

DNA barcoding tools are commonly employed to reveal species mislabeling and adulteration but fail to resolve issues on the geographic origin of aquaculture products. These constraints can be overcome using elemental fingerprints of mineralized structures and biochemical approaches surveying the fatty acid profile and the lipidome of specific tissues. Both approaches are cost-effective, relatively fast and rely on well-established techniques. Both hold the potential to accurately discriminate cultured specimens originating from production sites less than 1 Km apart, with biochemical fingerprints also allowing to infer the time elapsed post-after harvesting.

Elemental and lipidomic tools coupled with feature selection methods allow speeding-up biogeochemical and biochemical analysis (respectively) by determining which chemical elements and/or lipids best predict geographic origin. These approaches have already been successfully employed to discriminate the geographic origin of multiple farmed fish, shellfish and seaweed species, thus confirming their potential for origin certification and prevention of fraudulent practices.

Overall, these traceability tools can contribute to add-value to aquaculture products by safeguarding that trade chains are increasingly more transparent, by maximizing food safety and enhancing the trust of consumers on aquaculture products.

This work was financially supported by project TraSeafood (Tracing the geographic origin of seafood as a pathway towards the smart valorization of endogenous marine resources) (PTDC/BIA-BMA/29491/2017), funded by FEDER, PT2020 Partnership Agreement and Compete 2020 and by national funds (OE), through FCT/MEC. We also acknowledge FCT/MEC for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020) through national funds and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020.

ADVANCE SENSING OF OCEANIC AQUACULTURE SYSTEMS

Rui Caldeira*[1], João Monteiro[2], Carlos Lucas[1], Jesus Reis[1], Patrício Ramalhosa[2], Sílvia Almeida[2], Sahar Chebaene[2], Pedro Diniz[3], Natacha Nogueira[4], João Canning-Clode[2], Carlos Andrade[1,4]

1 - Oceanic Observatory of Madeira, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, Funchal, Madeira, Portugal
*rui.caldeira@oom.arditi.pt
2 - MARE - Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI). Edifício Madeira Tecnopolo, Caminho da Penteada, 9020-105 Funchal, Madeira, Portugal
3 – Marismar, Aquicultura Marinha, Lda, Portugal
4 – Maricultura Centre of Calheta, Calheta, Madeira, Portugal

The growing global demand for seafood has prompted an increase in offshore aquaculture facilities and farms worldwide. However, despite the crucial role that aquaculture plays in reducing marine natural food resources exploration, concerns on the potential impact of offshore activities warrant close monitoring of cages, infrastructures, and surrounding areas. As such, industry standards and good practices require offshore systems to regularly monitor how their activity may affect surrounding fish communities, benthos, water quality, and sediments. Comprehensive monitoring of offshore aquaculture infrastructures, cages, moorings, and surrounding areas can contribute to the early detection of potential negative impacts on surrounding habitats and marine diversity and provide valuable information for operational optimization, infrastructure maintenance, and damage prevention. In this context, remote sensing, remotely operated vehicles, and autonomous monitoring technologies can provide significant operational advantages to aquaculture activities. While remote sensing, autonomous apparatus, robotic systems, and automation technology require a relevant initial investment, over time, it can effectively contribute to optimizing operations, maximizing yields, reduce maintenance costs and prevent critical damage. Additionally, the operational flexibility and range of available sensors of these systems and technology make them suitable for various applications and scenarios. In this context, we developed a case study in Madeira Island (NE Atlantic) to explore how open source and commercially off-the-shelf unmanned and autonomous technologies can be combined to monitor multiple variables in offshore aquaculture infrastructures.

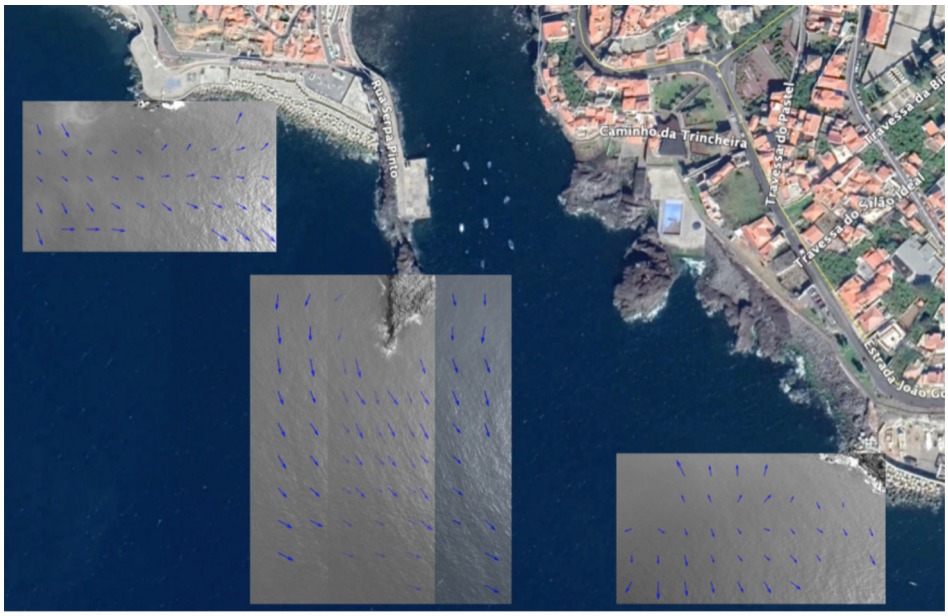


Figure 1 – Sea surface currents calculated from sequential quadcopter images

Unmanned Aerial Systems (UAS) remote sensing were used to measure temperature and currents at the sea surface. Aerial imagery and Structure from Motion photogrammetry were used to assess cages' response to tidal flow and deformation in floating structures. The evolution of a plume of food and/or detritus can also be monitored with UAS videos. Acoustic Doppler Current Profiles (ADCP) and temperature sensors provided a time series of ocean currents through the water column. Ocean circulation impacts the surface dispersion of food and sedimentation of residues on the seafloor. Moreover, timing the feeding window with the ocean circulation is of utmost importance for optimizing the operation. Aerial systems were used to monitor temperature and currents, Remotely Operated Vehicles equipped with multiple cameras and sensors to provide valuable information on the sea bottom and benthos, assess sedimentation, and enable visual inspection anchoring and mooring systems. Finally, the use of Baited Remote Underwater Video Systems was tested to monitor pelagic and benthic fish diversity and communities in surrounding areas, replacing the need for the underwater visual census. Video annotation, automated image analysis, and artificial intelligence-assisted image classification streamline aerial and underwater image processing and data compilation.

SUSTAINABLE AQUACULTURE: FROM NUTRIENT RECYCLING TO IMPROVED HATCHERY DIETS TECHNOLOGY

C. Campanati*[1,2], L. Aranzamendi[3], I. Zorita[3], D. Willer[1], J. Schubert[4], D.C. Aldridge[1]

[1]Department of Zoology, Cambridge University, UK
[2]Present address: ARDITI-MARE Madeira, Funchal, Portugal
[3]AZTI Foundazioa, Pasaia-Gipuzkoa, Spain
[4]RethinkResource, Zurich, Switzerland

*presenting author: camilla.campanati@gmail.com

Introduction

As aquaculture intensifies, there is increasing pressure to find more sustainable practices that save resources and reduce waste. Combined with increases in resource use efficiency across the aquaculture sector, from feeding methodologies to product storage, nutrient recycling can enable aquaculture to contribute sustainably towards the nutritional requirements of billions of people over the next century. The marine aquaculture sector is expanding towards lower trophic level cultures such as macroalgae and bivalve molluscs, which offer numerous sustainability and human health benefits, with a low environmental footprint and a rich source of essential fatty acids and micronutrient. Despite these clear benefits, the growth of bivalve industry has failed to keep pace with fish aquaculture due to a number of challenges including suboptimal and unreliable diets, yield loss due to disease, and unpredictable quality of product. Indeed, conventional hatchery systems rely on the cultivation of lipid-rich algal diets, which are highly vulnerable to contamination and can occupy half the hatchery footprint. In this project we selected and encapsulated algae as alternative more cost-effective substitutes to conventional microalgal diet. Nutritional requirements of mussel *Mytilus galloprovincialis* spat and conditioning broodstock were researched and growth, gametes development and tissue fatty acid contents were assessed. The aim of this study was to determine the substitution level of microalgae by microencapsulated feeds (BioBullets) in hatchery feeds which could uplift mussel production through a reduction of costs.

Material and method

Optimal nutrients for encapsulation were identified from a wide range of aquaculture side streams, with the marine algae *Undaria pinnatifida* and *Schizochytrium* offering complementary nutritional profiles with collectively high EPA and DHA levels. Diets were produced commercially and characterised in the laboratory, showing a spherical shape, a 40-50% loading of nutrients by mass, near-neutral buoyancy and a size of 20-140 μm. Various nutritional feeding studies were designed to identify the optimum level of commercial microalgal substitution (partial or complete) with microencapsulated feeds to support high growth and fast gamete development of *M. galloprovincialis* at commercial scale. In particular, we firstly conducted feeding trials using blended BioBullets (BB), containing *U. pinnatifida* and *Schizochytrium* sp., for mussel spats and compared hatchery performances with spat fed commercial algal diets (A), the combination of conventional diets and BioBullets (ABB) and unsupplemented spat in the laboratory (NC) and in natural conditions (Mutriku). We further conducted experiments on *M. galloprovincialis* spat and broodstock using BioBullets containing *Schizochytrium sp*. at different level of commercial microalgal diet substitution (0 % (A); 60 % (ABL); 80 % (ABM); 100 % (B)).

Results

A circular economy, which allows side streams and by-products to be recycled within the aquaculture sector, will certainly play a key role in increasing production output (Fig. 1A). BioBullets containing *Schizochytrium* sp. and *Undaria pinnatifida* supplied to mussel spat at 100 % substitution of commercial microalgae, provided a balanced nutritional diet which supported survival and growth rates similar to mussels fed conventional diets (Fig. 1B). BioBullets containing only *Schizochytrium* sp. were able to sustain mussel spat growth at 60 % inclusion in the diet (Fig. 1C). At the end of the 6 weeks conditioning, adult mussels fed algae + BioBullets at low and medium inclusion (ABL and ABM) presented 40-60 % of individuals already in resting stages, similarly to A-fed mussels. (Fig.1 C).

Discussion and conclusion

Hatchery diets with microencapsulated feeds can provide dependable, contaminant-free diets that reduce costs of seed production and, thus, can sustainably support mussel farming. Additionally, by sourcing the encapsulated algae from side streams of the aquaculture industry, microencapsulated feeds can promote more sustainable practices and circular economies within the sector. Further tailoring of the nutritional composition of microcapsules to specific bivalve species or growth stages could allow microcapsules to replace a greater proportion of or even completely replace algal diets. Inclusion into microcapsules of therapeutics or even flavourings open up additional potential benefits to this important and growing industry.

(Continued on next page)

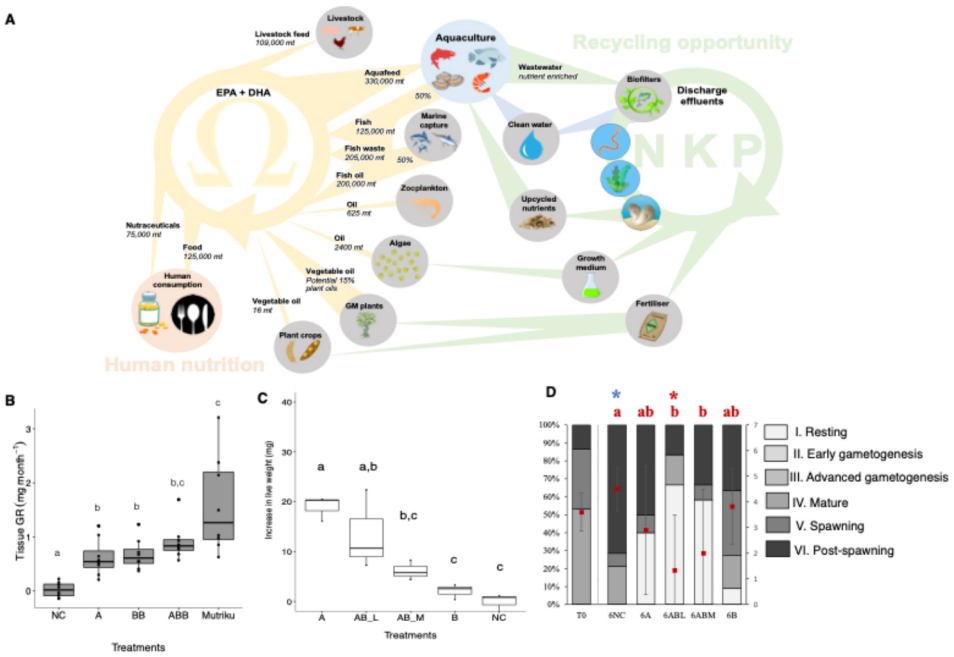


Fig. 1. A) Supplies and demands of omega-3 fatty acids (EPA and DHA). Major demand for omega-3 is from aquaculture, which can then substantially contribute to the market supply, through recycling of nutrients from farm wastewaters. Nutrients can be upcycled into biomass through the food web and/or to fertilize omega-3 producing crops. The use of biofilters in the recycling of nutrients from farm effluents not only provides potential for increased biomass yield that can be used as aquafeeds, but also clears water that can then be reused for farmed species, promoting a circular economy within aquaculture. The vertex of each triangle represents the direction of the arrows and the size is proportional to the volume (in MT). B) Spat tissue growth in the first feeding trial being fed for 6 weeks with different diets; C) Spat increase in live weight in the second feeding trial being fed for 8 weeks with different diets; D) Percentages of gamete developmental stages (I-VI) in adult mussels before (t0) and after 6 weeks of experiment being fed with different diets. NC=No food; A=conventional microalgae; BB=blended Biobullets containing *U. pinnatifida* and *Schizochytrium* sp.; ABB= conventional microalgae + blended BioBullets; Mutriku=natural field condition; ABL= 40 % microalgae + 60 % BioBullets containing *Schizochytrium* sp.; ABM= 20 % microalgae + 80 % BioBullets containing *Schizochytrium* sp.; B=100 % BioBullets containing *Schizochytrium* sp.; Different letters represent significant differences among treatment groups.

@AQUAGIS: A G.I.S. WEB APPLICATION FOR MARINE SPATIAL PLANNING AND AQUACULTURE IN ITALY

M.P. Campolunghi*, S. De Corso, C. Cipolloni, F. Cardia, T. Petochi, A. Bruschi, G. Calise, G. Marino

ISPRA - Italian Institute for Environmental Protection and Research, Rome, Italy
Email: mariapaola.campolunghi@isprambiente.it

Introduction

The key feature of EU Directive for Maritime Spatial Planning (2014/89/EU) is the integration of various sectors and societal needs to achieve a sustainable development of blue economies. In this framework marine aquaculture moves from traditional single sector planning to a more integrated approach, where Geographic Information Systems (G.I.S.) are essential analytical tools (Gimpel et al., 2018).

The INSPIRE Directive (2007/2/EC) established the access to spatial data sets and services between public authorities of EU Member States and the Open Data Directive (2019/1024/EU) encouraged public sector bodies to make data available and recognizing geospatial, earth observations and environmental data as high-value dataset.

@AquaGIS is a WebApp, published on the ISPRA's Portal, which provides services and tools to visualize, query and analyse heterogeneous data for marine spatial planning and aquaculture supporting the identification of AZAs (*Allocated Zones for Aquaculture*) in coastal and offshore marine areas and performing site suitability assessment (Marino et al. 2020).

Materials and Methods

The '@AquaGIS' WebApp is an application tool that is part of an Integrate Information System for cartographic services and web applications that provides aquaculture data. The Aquaculture Information System is integrated into the ISPRA's Spatial Data Infrastructure SINACloud, a complete system for the acquisition, validation, management and publication of data, metadata and network services, working according to INSPIRE Technical guidelines. It consists of two main frontend components: i) an application server for publishing the data and delivering them as cartographic API and OGC/INSPIRE web services (WMS, WFS, WCS) and ii) a geoportal for cartographic content management. The platform is supported by an SDI architecture composed farmhouse of ArcGIS and GeoServer web servers that allow the publication of network services. The data is incorporated in an Enterprise-type multi-user Geodatase integrated as part of SDI and managed by the GIS Client applications.

The '@AquaGIS' WebApp is the consultation application of the aforementioned cartographic network services. In addition to the visualization component, @AquaGIS provides users with a system performing complex queries using standard and customized widgets.

Results

@AquaGIS currently stores 111 layers that can be displayed and queried, including feature classes, tables and raster data, most of them with a national geographic extension (Fig.1). The table of contents allows the navigation trough different groups of layers related to administrative boundaries, aquaculture constraints, environmental conditions and site suitability, including:

- National and regional waters boundaries within 12 NM
- Marine uses (e.g. aquaculture licenses, ports, marine traffic, fishing effort, tourism, industries, military area, pipelines and cables, wind farms)
- Environmental constraints (e.g. MPAs, protected species/habitats, contaminated sites, river mouths, wastewater treatment plants)
- Other constraints (e.g. wrecks, dumping sites, unexploded ordnances)
- Physic and biogeochemical marine data (e.g. bathymetry, currents, waves, SST, Chl-a) from satellite and numerical models
- National marine monitoring network (e.g. MSFD, WFD)
- Maps of non-compatible or restricted areas for aquaculture activities
- National, regional and local maps of suitability for fish and shellfish farming

(Continued on next page)

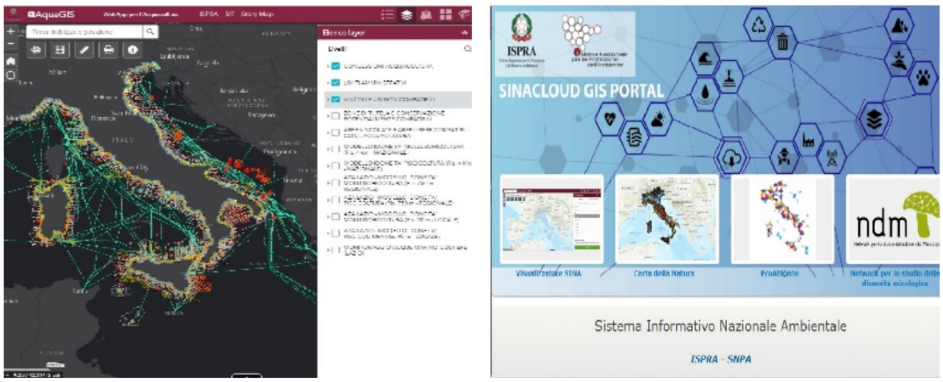


Fig.1 – @AcquaGIS WebApp and the SINACloud GIS Portal

For the process of AZA identification, the data analysis is carried out on a national scale with a resolution of 4 km. In some marine areas, analysis are provided with a regional resolution of 750 m. Site suitability for new fish and shellfish aquaculture installations is performed with a local resolution of 90 m in some key case studies. A section of @AquaGIS is dedicated to the national environmental monitoring network (MSFD, WFD), making available *in situ* marine data collected and processed by the National System for Environmental Protection (ISPRA-SNPA).

Discussion and conclusion

@AquaGIS is a multifunctional decision-making tool that, through an iterative and interactive process, permits the visualization and interrogation of the factors contributing to the aquaculture planning and suitability assessment. It can also be a node for exchanging information from different institutional sources as a "*mirror station"* of their services. @AcquaGIS also provided information on aquaculture and the planning process for AZAs through a "*Story Map*". @ AquaGIS is a key tool for national, regional and local authorities, aquaculture stakeholders and other marine users involved in the maritime spatial planning in Italy.

Acknowledgments

This project has been developed also using data from CMEMS, EMODnet, the Italian Navy Hydrographic Office and the Ministry of Ecological Transition website.

References

Gimpel, A., Stelzenmüller, V., Töpsch, S., Galparsoro, I., Gubbins, M., Miller, D., Murillas, A., Murray, A.G., Pınarbaşı, K.,Roca G., Watret R. (2018). A G.I.S.-based tool for an integrated assessment of spatial planning tradeoffs with aquaculture. Science of the Total Environment 627, pp. 1644-1655.

Marino, G., Petochi, T., Cardia, F. (2020). Assegnazione di Zone Marine per l'Acquacoltura (AZA). Guida Tecnica, 214 p., Documenti Tecnici ISPRA, ISBN: 978-88-448-1014-6.

YTIDE - METEOROLOGICAL AND OCEANOGRAPHIC SERVICE FOR AQUACULTURE

Francisco Campuzano[1,*], Nuno Ferreira[2], Tiago Garcia[1], Nuno Loureiro[1], Caio Fonteles[1], Sónia Romão[1], Sofia Aguiar[1], Artur Costa[1], Nuno Lourenço[1]

1 +ATLANTIC, Molhe Leste, 2520-620 Peniche, Portugal
francisco.campuzano@colabatlantic.com
2 ExporSado, Parque Logístico Tensai, Pavilhão D1, Sala 4, Rua Principal de Praias do Sado, 2910-857 SETÚBAL, Portugal

Introduction

The bivalve mariculture sector is an important food-producing sector since is one of the five sectors listed in the EU's Blue Growth Agenda. Careful management practices need to be implemented to ensure its sustainable development. Major challenges include the competition for space, production and ecological carrying capacity and food safety (e.g., harmful algal blooms). Management and development of this sector is highly dependent on the information on the physical conditions: current speeds, temperature, and wave conditions.

The H2020 FORCOAST project (https://forcoast.eu) aims to offer information services co-designed with stakeholders, which provide high-resolution data of water quality and metocean variables at coastal zone and nearshore that are used to give focused answers to specific questions from the targeted wild fisheries, bivalve mariculture, and oysterground restoration sectors.

These services allow improving operation, planning, and management of different marine activities in these sectors. Their value depends on the efficiency and effectiveness in the operations and productivity while decreasing the pressures on the marine and coastal environment. The increasing availability of data and technological advancements will contribute to a better and deeper understanding of the surrounding environment. The demand for such services is increasing as the marine related business has been observed to be growing in economic value.

The Ytide service

Despite the proliferation of means to extract and utilise environmental data (observed and modelled), various potential users are still not using them to optimise their daily outdoor activities. In other words, many ocean-related businesses can further improve their efficiency, reduce costs, and maximise profits should they start using data science tools. At the same time, the users' contribution to the development of data interface/delivery systems is paramount to tailor data services to their specific needs.

The Ytide service aims to provide easy access to tailored information for the aquaculture sector through existing mobile messaging applications. It has been co-designed by the +ATLANTIC CoLAB and the ExporSado company to meet the requirements of professional bivalve producers in intertidal areas and to reduce their operational environmental constraints, considering that:

- Producers need to reach the production areas in time, following local tides,
- Bivalve producers can only work during low tide conditions,
- Production sites are frequently located distant from tidal gauges,
- Local meteorology and hydrography that influence water levels are often disregarded in existing solutions, and
- The location-specific information is needed with a few days in advance for planning.

The current version of Ytide provides access to various environmental parameters that are key for the oyster production activity: daylight period, high and low tide heights and times, and optimum operation time window. Other environmental limiting factors, such as wind and rain, are also included (Figure 1).

The main innovation of the Ytide service is that the information is formatted and distributed automatically through existing messaging smartphone apps, such as Telegram. The main advantages of this approach include:

- Ease of access: faster and easier to access a smartphone app than a website,
- Interaction with colleagues: sharing information and interacting is quick and easy, and
- Productivity: enables better performance, time optimisation and more efficient work

To configure a new service, the end-user must provide geographic coordinates of the aquaculture production areas, optimal water hight for their activities, thresholds of environmental limiting factors and a list of contacts that will receive the information. After that, the end-users simply need to install the Telegram application to start receiving the information.

(Continued on next page)

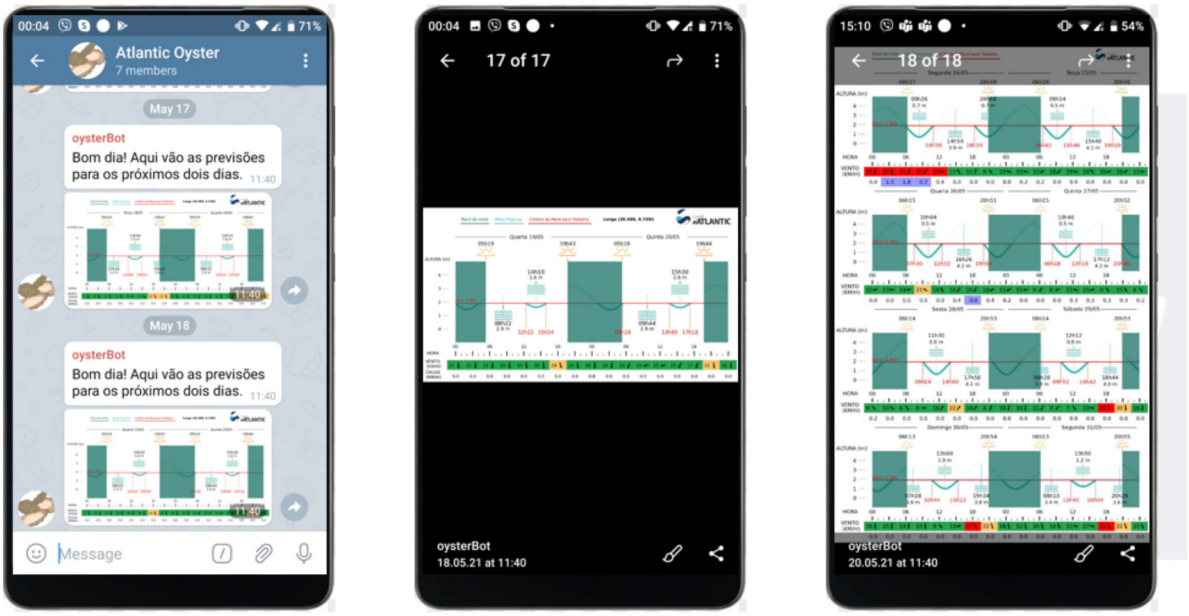


Figure 1: Notification messages shared through Telegram for the ExporSado production area

The Sado estuary pilot service

Based on the identified needs of local oyster producers, the Sado estuary aquaculture community is being used as a pilot application. The service is being executed automatically in two formats:

- Daily: information for the two following days with the best operational forecast, and
- Weekly: every Thursday, with information on the conditions for the next week.

Conclusions

The FORCOAST project aims to improve the quality, reliability, and customization of the currently offered operational marine products. By making these products more accessible and usable to the European wild fisheries, oystergrounds restoration, and bivalve mariculture, it benefits these industries' know-how, expertise, and competitiveness, increasing their potential to seize market opportunities.

The Ytide service is based on the harmonisation of different types of data, resulting in a location-specific and user-customisable tool that adds value to the end-users. Their involvement in the customisation and testing phases is key for the quality of the provided forecasts. The Ytide service can be easily customised to other geographical areas and to various other ocean-based economic activities and communities.

Acknowledgments

The FORCOAST project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 870465.

CAGED GILTHEAD SEABREAM LIKES BITING THE NETS. SO WHAT?

Paula Canada [1,2] *, Benjamin Costas [2,3], Raquel Xavier [4], Miguel Santos [2,5], Ana Couto [2,5], Marco Cerqueira [6], Sérgio Fernández-Boo [2], Marlene Pinheiro [2], Teresa Neuparth [2], Lydia Png-Gonzalez [1,7], Ana Garcia [2,3], Ana Pereira [4], Carlos Andrade [1,2,8], Natacha Nogueira [1,2,8]

[1] OOM – Oceanic Observatory of Madeira, ARDITI – Regional Agency for the Development of Research Technology and Innovation, Ed. Madeira Tecnopolo, 9020-105, Funchal, PT
[2] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, 4450-208, Matosinhos, PT
[3] ICBAS – Instituto de Ciências Biomédicas de Abel Salazar School of Medicine and Biomedical Sciences, University of Porto, 4050-313 Porto, PT
[4] CIBIO- Research Centre in Biodiversity and Genetic Resources, Univ. of Porto, 4485-661, Vairão, PT
[5] FCUP – Department of Biology, Faculty of Sciences, University of Porto, 4169-007 Porto, PT
[6] CCMAR – Centro de Ciências do Mar, Universidade do Algarve, 8005-139 Faro, PT
[7] Instituto Español de Oceanografía Centro Oceanográfico de Baleares – Palma de Mallorca, ES
[8] Mariculture Center of Calheta, Regional Directorate for the Sea, Av. D Manuel I, Nº7, 9370-133, Calheta, PT
* Email: paula.canada@oom.arditi.pt

Introduction

Traditionally the nets used in sea cage farming are made of nylon, a synthetic thermoplastic polymer widely used in fabrics production. As a robust polymer, nylon is not easily degraded (Lewis et al., 2004), but it can be broken into small sized particles as a result of aging and UV exposure. In the particular case of fishing nets and sea cage nets, biofouling establishment is likely to further potentiate the aging and breakability of nylon.

Net biting is a gilthead seabream specific behavior long reported by farmers and suggested to be related with feeding ratio and the presence of micro-fouling (Glaropoulos et al., 2012; 2013). Whether seabream actually ingests net pen debris has not been documented. But if it does, it can be hypothesized whether net pen debris may induce intestinal alterations, directly or indirectly as possible vehicles of toxic chemicals (Oliveira et al., 2013; Peda et al., 2016).

As an alternative to the traditional netting, a copper-alloy mesh conceived for sea-cages has been recently introduced in the market. Being made of copper, its exposure to micro-fouling (including pathogens) and its susceptibility to predators' attack is said to be minimized. Although it seems to be promising as an alternative to nylon, it remains to be known whether the mesh aging could lead to copper leaching and whether that could affect fish health.

This study aimed to evaluate the risks coming from the interaction of gilthead seabream with net pens of different materials and aging status by studying: 1) the bacterial community established on the nets with an emphasis on potentially pathogenic genera; 2) the biting behaviour and net erosion in relation to biofouling establishment and 3) the impact of net exposure on fish health status.

Materials and methods

Fish were grown out from 100 to 270g (up to 6Kg/m^3, with 12 fish/tank) in a flow-through system, where 12 fiber glass indoor tanks were supplied with unfiltered and untreated seawater withdrawn at 130m from the coastline. The control group was grown in clear tanks, with no net; the NNy group was exposed to a <1-year-old nylon net; the ONy group was exposed to a >5-year-old nylon net; the Cu group was exposed to a new copper net. Nets were placed in the tanks as panels (40x20cm) immersed within the first 35 cm of the water column. Fish were fed once a day, at 12.00, except for Mondays. In each tank, the area surrounding the net panel was videotaped on Mondays (starving day), Tuesdays and Thursdays, in the morning, during the meal-time and in the afternoon, for 10 min, to evaluate the fish-net interaction behavior.

After 125 days, each net panel was hanged up off the water and two pieces of each net were sampled/swabbed for further microbiome analysis. The net panel was then removed off the tank and photographed for damage evaluation and a 10x10cm square was cut off for mobile epifauna counting e identification. Five fish per tank were sampled for blood collection for hematological profile characterization, the plasma was snap-frozen in liquid nitrogen and stored for further analysis of immune status parameters. The liver was excised for further analysis on oxidative status markers. The skin and distal gut were sampled for microbiome analysis. Distal gut sections were sampled also for histopathological qualitative analysis and for the quantification of transcript levels of inflammation-related genes by RT-PCR.

(Continued on next page)

Results and Discussion

The bacterial communities associated with copper and nylon nets used in sea cages were compared using a metataxonomic approach through the high-throughput sequencing of the 16S rRNA V4 hypervariable region. Most differences were found between both nylon nets and copper nets, with less bacterial diversity in copper nets, which confirms its bactericidal effect. However, Tenacibaculum known to harbor potentially pathogenic species, was more abundant in copper nets than in nylon nets. These results bring up the need for monitoring the bacterial communities established on aquaculture gear, particularly on nets and including copper nets, to evaluate whether they may serve as reservoirs for pathogenic bacterial strains.

No mobile epifauna was observed on copper nets. Mobile epifauna communities were different between old and new nylon nets, being more abundant in the new nylon. Although the behaviour analysis does not suggest clear differences between the 3 net types, the new nylon nets were clearly more damaged at the end of 4 months than the old nylon nets, which is probably related to epifauna abundance that should attract seabream towards the nets.

Concerning possible effects on fish health, although no signs of inflammation were found in gut histological analysis and no differences were found in gut microbiota, NNy fish displayed a higher expression of the anti-inflammatory cytokine IL-10 in the distal gut and increased bactericidal activity in plasma when compared to the control fish, as if exposed to a foreign agent. On the other hand, the distal gut of ONy fish displayed higher expression of CD8 which regulates cytotoxic T-cells activity. This group also displayed increased activities of catalase and glutathione S-transferase in their livers, when compared to the control group, while the NNy and Cu groups displayed intermediate levels. Further analysis are being conducted to check for nylon debris detection on fish gills and gut.

Overall, these results highlight the importance of sea-cage net material and aging status on the fish-net interactions and associated welfare risks, either due to the biofouling communities that could harbor fish pathogens, but also possibly due to toxicological effects of the net material.

References

Glaropoulos, A., Papadakis, V.M., Papadakis, I.E., Kentouri, M., 2012. Escape-related behavior and coping ability of sea bream due to food supply. Aquaculture International. 20, 965-979.

Glaropoulos, A., Papadakis, V.M., Papadakis, I.E., Georgara, A., Kentouri, M., 2013. Sea bream interactions toward the aquaculture net due to the presence of micro-fouling. Aquaculture International. 22, 1203-1214.

Lewis, P.R., Reynolds, K., Gagg, C., 2004. Forensic Materials Engineering: Case studies. . CRC Press.

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecological Indicators. 34, 641-647.

Peda, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256.

UNDERSTANDING THE PUBLIC IMAGE OF AQUACULTURE AND ITS PRODUCTS: DOES THE INFLUENCE OF POSITIVE OR NEGATIVE WORDING MATTER? DO THE OPINIONS VARY AMONGST DIFFERENT SEGMENTS? – AN APPLICATION TO THE ISLAND OF GRAN CANARIA

Javier Cantillo*, Juan Carlos Martín, Concepción Román

Universidad de Las Palmas de Gran Canaria, Institute of Tourism and Sustainable Economic Development. 35017. Las Palmas de Gran Canaria (Spain)
*E-mail: javier.cantillo101@alu.ulpgc.es

Introduction

The research on aquaculture perceptions has been mainly focused on the attitudes of consumers towards aquaculture products, opinions of the general public on the aquaculture industry and the perceptions of aquaculture key stakeholder groups (Bacher, 2015). A great part of these studies have used Likert scales to measure the level of importance/agreement for certain characteristics or statements for aquaculture's image and its products. From these, some investigations have mixed negatively and positively worded survey items (e.g. López-Mas et al. (2021); Murray and D'Anna (2015)making aquaculture the most suitable alternative to support increase in fish consumption. However, farmed fish have a less positive image among consumers than their respective wild-caught equivalents. Food product images can be affected by consumers' beliefs, which are useful to infer the quality of the food product and the consumers' food choices. This paper investigates European consumers' beliefs regarding farmed versus wild fish. The goal is to understand not only what hinders farmed fish consumption but also provide guidelines for producers and governments to improve the image of farmed fish. An online questionnaire reaching 2511 consumers in five European Union (EU), looking to decrease the potential acquiescence bias (to agree with questionnaire statements irrespective of the content). However, Chyung et al. (2018) found that this option can be problematic, as the negatively worded items, which are usually reverse recoded, might not measure exactly the same as the positively worded statement counterparts. In addition, negatively worded items may appear as separate factors (method effect) or might be misunderstood by unwise respondents, causing erroneous data. To our knowledge, the effects of using a mix of positively and negatively worded statements have not been evaluated in the context of aquaculture perceptions. Following this, the objectives of this investigation are twofold: (1) to understand the influence of positive and negative wording on the public image of aquaculture and its products and (2) to identify whether the public image of aquaculture differs amongst segments in Gran Canaria (Spain).

Materials and methods

The study is based on a subset of a larger online survey analysing the preferences of consumers in Gran Canaria, Spain, which was conducted between April and June of 2020. In this section, respondents were asked to rate their level of agreement for 16 statements related to the image of aquaculture and its products on a scale from 1 (completely disagree) to 5 (completely agree). These statements included information about consumers' opinions about aspects such as pesticides and fish illnesses, pollution, crowded conditions of fish, cleanliness and healthiness of the environment, the naturalness of the fish farming process, comparisons with other types of farming and fisheries, the affordability of farmed fish, sustainability, the diet of the fish and the social and economic benefits.

Moreover, to understand the influence of positive and negative wording, two different survey blocks were distributed: one with the statements written in a positive way towards aquaculture, and the other one with the same statements but written in a negative perspective towards aquaculture. For example, a statement was written like this in each survey: (1) aquaculture farming is a natural process and (2) aquaculture farming is not a natural process. In the end, 167 respondents answered the negatively worded survey block and 184 the positively worded survey block.

For the analysis of the data, we used a methodology using a hybrid approach based on Fuzzy Set Theory (FST) and TOPSIS (Techniques for order preference by similarity of the ideal solution). TOPSIS is particularly useful when respondents make choices with multiple attributes, as these techniques prioritize and rank the items for easy managerial interpretation, which leads to the appropriate policy and strategy implementation. We established synthetic indicators to measure the public acceptance of aquaculture and its products, considering three different treatments (mixing positive and negative statements, just positive statements and just negative statements) and with respect to different segments, in which the sample was divided. In addition, fuzzy methods can capture the essence of human ambiguity judgement when respondents deal with multidimensional attributes. Also, we used fuzzy clustering techniques with a three-cluster solution to segment the consumers with a more realistic multidimensional description.

(Continued on next page)

Results and discussion

Results show that it is not a good idea to include negative statements on Likert-scale instruments assessing the acceptance of aquaculture and its products, as when the information of these statements is reversed for statistical analysis, the reliability and validity of the data are compromised, and the variability in the responses increases. The data with positive statements, on the other hand, retained their validity and reliability even when reversed, so it is recommended that all instruments are worded in this manner. This might be due to consumers understanding better positively worded statements, while those negatively worded might be confusing. This also indicates that it is not the same to ask questions in a positively worded manner than to ask them in a negatively worded manner.

Moreover, we obtained the findings of the perceptions of aquaculture image in Gran Canaria (Spain), based on the results of treatment 2 (positively worded items), which is the most reliable based on what was previously discussed. According to the crisp values, the three most valued items are as follows: the fish that come from aquaculture are a healthy food option (52.83), the fish that come from aquaculture are consistent and affordable (54.99), and the fish that come from aquaculture contribute to fish mass sustainability (55.37). Meanwhile, on the other hand, the three least valued items for aquaculture image are related to: fish farming is a natural process (38.41), the crowded conditions of fish farms are adequate for fish (40.01) and the captured fish have a natural diet, and this is not better for them in comparison with farmed fish (40.38). Interestingly, the three least valued items are related to fish conditions and this result can be considered strategically in order to improve the aquaculture image.

The results indicate that there is a better acceptance of aquaculture and its products for the respondents belonging to the following segments: consider salmon as one of their favourite species, prefer farmed fish over wild fish, prefer products of EU origin, live in a big household, earn lower than the national average, and are part of the youngest (below 25 years) and oldest generation (above 66 years). On the contrary, there is a lower acceptance of aquaculture and its products for respondents belonging to the following segments: consider seabream as one of their favourite species, prefer wild fish over farmed fish, prefer local products, do not read the information accompanying the seafood products, have a very low educational level, live in a small household, earn greater than the national average, and are part of the generation between 26 and 35 years of age.

According to the fuzzy clusters, it can be said that the profile of an extreme Pro-Aquaculture consumer is characterized by a consumer who perceives all of the attributes at the maximum or second-maximum values. Meanwhile, extreme Anti-Aquaculture consumers are characterized for perceiving all statements on the scale as having the lowest value. The intermediate cluster is characterized by (1) seven low valued attributes with a value of 2, (2) three intermediate valued attributes with a value of 3, and (3) six high valued attributes showing values of 4. In summary, the average probability of belonging to the “Pro-Aquaculture” consumer segment is 37.26%, to the “Anti-Aquaculture” consumer segment is 35.88%, and to the “intermediates” consumer segment is 41.63%.

References

Bacher, K., 2015. Perceptions and misconceptions of aquaculture: a global overview. GLOBEFISH Research Programme 120, I.

Chyung, S.Y. (Yonnie), Barkin, J.R., Shamsy, J.A., 2018. Evidence-Based Survey Design: The Use of Negatively Worded Items in Surveys. Performance Improvement 57, 16–25.

López-Mas, L., Claret, A., Reinders, M.J., Banovic, M., Krystallis, A., Guerrero, L., 2021. Farmed or wild fish? Segmenting European consumers based on their beliefs. Aquaculture 532, 735992.

Murray, G., D’Anna, L., 2015. Seeing shellfish from the seashore: The importance of values and place in perceptions of aquaculture and marine social–ecological system interactions. Marine Policy 62, 125–133.

SUITABILITY ANALYSIS FOR THE IDENTIFICATION OF ALLOCATED ZONES FOR AQUACULTURE (AZAs) IN THE TYRRHENIAN SEA, ITALY

F. Cardia[1]*, M.P. Campolunghi[1], T. Petochi[1], G. Calise[1], A. Bruschi[1], S. Querin[2], G. Cossarini[2], G. Bellotti[3], A. Grillo[4], A. Bolignano[4], G. Marino[1]

[1] ISPRA - Italian Institute for Environmental Protection and Research, Rome, Italy
[2] OGS - National Institute of Oceanography and Applied Geophysics, Sgonico (TS), Italy
[3] University of Roma TRE, Dept. of Engineering, Rome, Italy
[4] ARPA Lazio - Regional Agency of Environmental Protection, Rome, Italy
Email: francesco.cardia@isprambiente.it

Introduction

The lack of suitable sites for aquaculture is one of the main limiting factors for the expansion of the Mediterranean marine fish farming sector (COM 2021/236 final). In the framework of the EU Directive for Maritime Spatial Planning (2014/89/EU), Italy is planning the use of marine space units for different blue economy sectors, including aquaculture. This study presents a new suitability model based on Earth Observation (EO), numerical models and in situ data for 12 criteria, to identify Allocated Zones for Aquaculture (AZAs) and suitable farming sites in coastal and offshore marine areas in the Tyrrhenian Sea.

Materials and Methods

A Spatial Multi-Criteria Evaluation (SMCE) analysis (Dapueto et al., 2015) has been applied to assess the suitability for fish and shellfish aquaculture of around 9300 Km^2 of marine waters in the Lazio Region (from the coastline to 12 NM). The SMCE was divided into two phases: i) mapping of administrative, infrastructural and environmental constraints, including buffer zones, to identify areas not available for aquaculture (Boolean overlay); ii) identification of criteria (Tab.1) to map the most suitable sites for fish and shellfish farming. EO data and numerical models from different sources were compiled in the SMCE. Each layer was remapped on a regular grid with a horizontal resolution of 750 m, while each criterion was reclassified on a suitability scale from 1 (low) to 10 (high), according to the suitability criteria classification available in Marino et al. (2020). A Weighted Linear Combination (WLC) analysis was then applied following the attribution of a relative weight to each criterion (weighted overlay).

Results

The resulting outputs calculated with the WLC are the suitability maps for shellfish and fish aquaculture along the marine waters of the Lazio Region (Fig. 1, top). The marine areas with constraints were then subtracted from the two maps. The bathymetric ranges considered suitable for shellfish (8-40 m) and fish (25-80 m) farming were also applied to further refine the suitability maps (Fig. 1, bottom).

An additional query was carried out for selecting those areas characterized by suitability indices higher than 8. The analytical process finally resulted in the identification of areas with high suitability along the Lazio coasts, for a total of about 80 km^2 for shellfish aquaculture and about 190 km^2 for fish farming (Fig. 2).

Discussion and conclusion

The use of WLC, which entails the attribution of different weights for each criterion, is a very powerful tool in the decision-making process and it allows the definition of different scenarios depending on the relative importance assigned to each layer. The results obtained have been successfully used within the activities of the funded EMFF project AZA-Lazio: the maps of highly suitable areas for shellfish and fish aquaculture where used to support the consultation process with relevant stakeholders and coastal municipalities, in order to identify, among these areas, those to be allocated as zones for aquaculture (AZAs). The outputs of the process for the identification of suitable areas as well as all the spatial information used to implement the study have been integrated in a GIS WebApp specific for aquaculture spatial planning (@AquaGIS), available on the ISPRA website.

(Continued on next page)

Criteria	*Sources*
Bathymetry; Type of seabed	EMODnet
Seabed slope	Calculated from bathymetry
Currents; SST; DO	MITgcm-BFM coupled model (OGS)
Wave height	SWAN Numerical model (Roma TRE Univ.)
Chl-a concentration	CMEMS
Vessel density	EMODnet
Fishing effort	Global Fishing Watch repository
Distance from ports	Calculated using Euclidean distance analysis
Visibility	Calculated using QGIS Viewshed analysis plugin

Table 1. Suitability criteria used in the WLC and data source

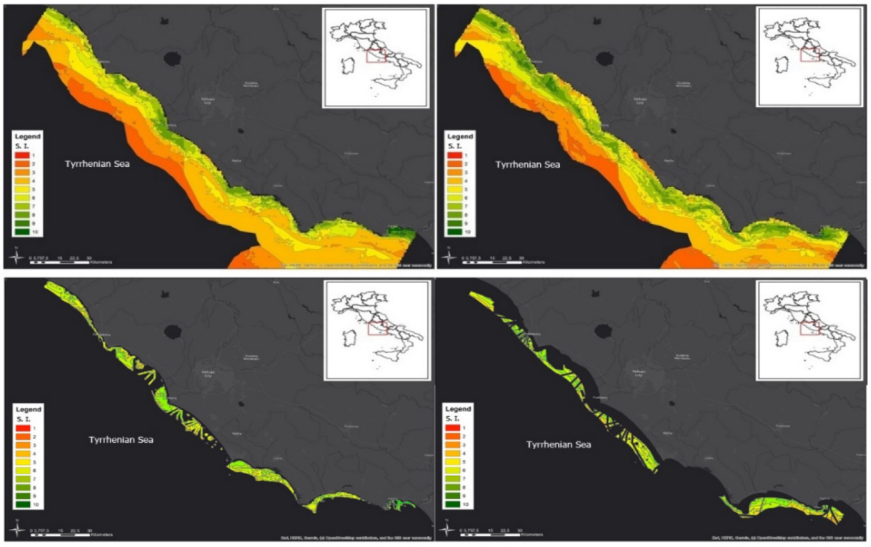


Fig. 1. Suitability maps for shellfish (left) and fish (right) aquaculture based on suitability indices from low (red) to high (green), before (top) and after (bottom) constraints are subtracted.

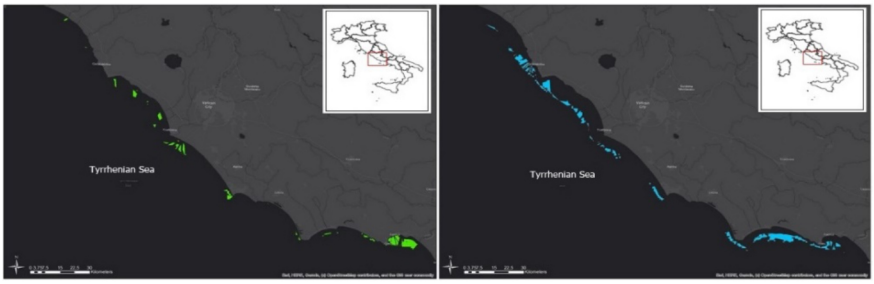


Fig. 2. Highly suitable areas for shellfish (left) and fish aquaculture (right)

Acknowledgments

The study has been funded by the Lazio Region through the EMFF program. CMEMS and EMODnet data have been used.

References

Dapueto, G, Massa, F., Costa, S., Cimoli, l., Olivari, E., Chiantore, M., Federici, B. and Povero, P. (2015). A spatial multi-criteria evaluation for site selection of offshore marine fish farm in the Ligurian Sea, Italy. Ocean and Coastal Management 116 (2015), 64-77.

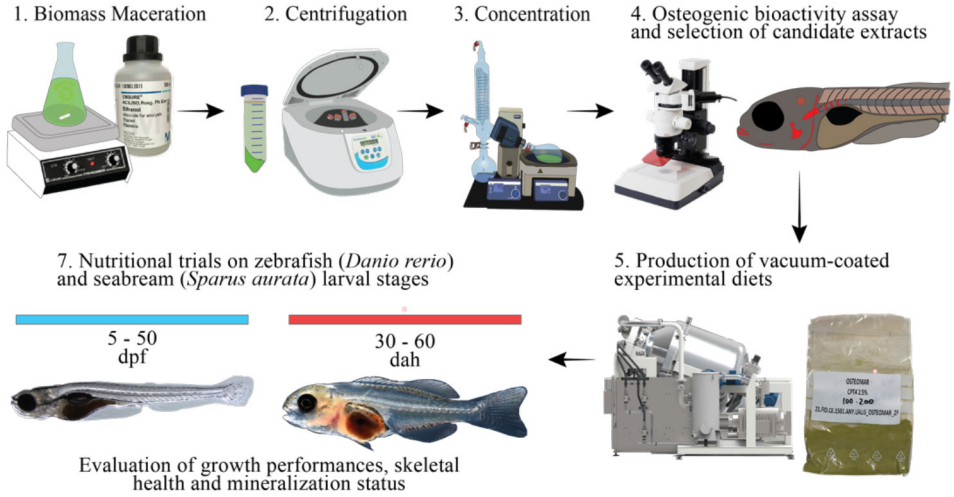
Marino, G., Petochi, T., Cardia, F. (2020). Assegnazione di Zone Marine per l'Acquacoltura (AZA). Guida Tecnica, 214 p., Documenti Tecnici ISPRA, ISBN: 978-88-448-1014-6

EFFECT OF DIETARY SUPPLEMENTATION OF MICROALGAE EXTRACTS ON THE SKELETAL HEALTH OF ZEBRAFISH *Danio rerio* AND GILTHEAD SEABREAM *Sparus aurata* LARVAL STAGES: A COMPARATIVE STUDY

A. Carletti[1,*], J.T. Rosa[1], K. Pes[1], S. Engrola[1], V. Serra[1], R. Colen[1], M.L. Cancela[1,2], V. Laizé[1], P.J. Gavaia[1]

[1]Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal
[2]Algarve Biomedical Center (ABC), University of Algarve, Faro, Portugal
*E-mail: acarletti@ualg.pt

Graphical abstract



Introduction

Skeletal anomalies are omnipresent in most farmed fishes worldwide, raising concerns for animal welfare and causing economic losses for the aquaculture industry [1, 2]. Larval nutrition has been recognized as one of the main factors leading to skeletal anomalies [3, 4], thus the development of feeds supplemented with osteo-active compounds is seen as an important strategy to improve the skeletal status of farmed fish. In this context, extracts from marine organisms, a well-established and valuable source of nutrients, have recently been found to contain bioactive compounds with osteogenic and mineralogenic activities that could be highly relevant to improve skeletal health in larvae of commercial species. Here, we produced inert diets enriched with different concentrations of the ethanolic extracts from Skeletonema sp. and Tetraselmis sp. and tested them on larvae of the model species zebrafish (Danio rerio) and the commercial species gilthead seabream (Sparus aurata), evaluating their effect on growth, survival and development of skeletal anomalies.

Materials and Methods

Extracts were prepared through the maceration of freeze-dried biomass of *Skeletonema* sp. and *Tetraselmis* sp. (Necton S.A.) with 96% ethanol. Ethanol extracts were coated on a commercial zebrafish diet (Sparos Lda) at 0.5% and 2.5% and a commercial seabream diet at 0.5%. For zebrafish feeding trials, 5 days post-fertilization (dpf) larvae were maintained in 2.5 L tanks in static conditions at 60 larvae/L until 20 dpf, then moved to 3 L tanks in recirculating system at a density of 23 larvae/L. Experiments were conducted in triplicates for each of the 5 diets. From 5 to 16 dpf, zebrafish larvae were co-fed with the supplemented inert diet and rotifers, gradually reducing the concentration of rotifers to address the weaning from live feeds. From 17 dpf until the end of the trials, larvae were fed only supplemented inert diets. At 50 dpf, juvenile fish were given a lethal anaesthesia and final survival, total length and dry weight were assessed. Fish were sampled to asses calcium and phosphorus content and for double staining with alizarin red S and alcian blue for bony and cartilaginous structures to evaluate skeletal anomalies. The expression of marker genes of bone development, matrix mineralization and oxidative stress were assessed by qPCR.

(Continued on next page)

For the seabream trials, larvae of 30 days after hatching (dah) were fed for 30 days with control diet or diets supplemented with 0.5% of *Tetraselmis* sp. or *Skeletonema* sp. ethanolic extracts. Microdiets were formulated and processed to be isonitrogenous, and isoenergetic. Larvae were maintained in cylindroconical 100 L tanks in a semi-closed RAS with an initial density of 52 larvae/L and a photoperiod of 10h light:14h dark. Environmental parameters and mortality were monitored daily. At the end of the trial larvae were euthanized, sampled and growth parameters assessed - dry weight, length and condition factor. Samples for oxidative status, digestive capacity, total mineral content, calcium and phosphorous ratio, mRNA levels of marker genes for bone formation, matrix mineralization, and oxidative stress were collected. Some fish per condition were stained to reveal bone and cartilaginous structures and asses incidence of skeletal anomalies

Results and Discussion

In the past years, our laboratory has screened a large number of extracts from microalgae for bone anabolic activity using zebrafish in vivo tools and identified ethanolic extracts of Skeletonema sp. and Tetraselmis sp. as promising source of osteoactive compounds. Although the use of zebrafish as model organism in aquaculture is a controversial topic due to physiological differences and evolutionary distances between this small freshwater cyprinid and commercial teleost species, its use in nutritional research has been recognized as a great opportunity and results obtained in zebrafish were shown to have predictive power of the effect on stress, immunological response and dietary toxicity towards species reared in aquaculture [5, 6]we define the characteristics that a fish species should have to serve as a model for finfish aquaculture research and argue that the zebrafish fulfils essentially most of them. We first describe several aspects of the biology of the zebrafish including phylogenetic relationships, development and growth and reproduction, both in the wild and under laboratory conditions. Next, we review the work already carried out in zebrafish that is related to different aspects of aquaculture research (reproduction, stress, pathology, toxicology nutrition and growth. Aiming at providing a comparative view on the potential application of microalgae extracts in finfish nutrition, we produced inert diets enriched with different concentrations of two promising osteogenic extracts, tested them on larval stages of zebrafish and the gilthead seabream, evaluating their effect on growth, survival and the occurrence of skeletal anomalies. Regarding the feeding trials with seabream, results showed that dietary treatments had no impact on performance indicators like dry weight, length, or condition factor. For both species, the impact of the supplementation of the extracts on bone mineral content, expression of marker genes of bone growth and mineralization and incidence of skeletal anomalies revealed the advantages of microalgae extracts supplemented diets, and their potential for aquaculture applications.

Funding

Work financed by the European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 (grant 16-02-01-FMP-0057/OSTEOMAR), by the European Regional Development Fund (ERDF/FEDER) through the Transnational Cooperation Programme Atlantic Area (grant EAPA/151/2016/BLUEHUMAN), by the Marie Skłodowska-Curie innovative training network BIOMEDAQU (grant H2020-MSCA-ITN/766347) and by National funds through the Portuguese Foundation for Science and Technology (grant UIDB/04326/2020).

Bibliography

1. Boglione, C., Gavaia, P., Koumoundouros, G., Gisbert, E., Moren, M., Fontagné, S., and Witten, P. E. (2013) Skeletal anomalies in reared European fish larvae and juveniles. Part 1: Normal and anomalous skeletogenic processes. *Rev. Aquac.* **5**, 99–120
2. Boglione, C., Gisbert, E., Gavaia, P., Witten, P. E., Moren, M., Fontagné, S., and Koumoundouros, G. (2013) Skeletal anomalies in reared European fish larvae and juveniles. Part 2: Main typologies, occurrences and causative factors. *Rev. Aquac.* **5**, 121–167
3. Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., and Boglione, C. (2013) Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. *Rev. Aquac.* **5**, S59-S98.
4. Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L. E. C., and Izquierdo, M. (2013) Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. *Rev. Aquac.* **5**, S26-S58.
5. Ribas, L., and Piferrer, F. (2014) The zebrafish (Danio rerio) as a model organism, with emphasis on applications for finfish aquaculture research. *Rev. Aquac.* **6**, 209–240
6. Ulloa, P. E., Medrano, J. F., and Feijo, C. G. (2014) Zebrafish as animal model for aquaculture nutrition research. *Front. Genet.* **5**, 1–6

THERMAL AND NUTRITIONAL STRATEGIES AS ALTERNATIVES TO ANTIBIOTICS IN AQUACULTURE

R. Carrilho*, P. Rodrigues and M. Cerqueira

Centre of Marine Sciences, CCMAR, Universidade Do Algarve, Campus de Gambelas, Edifício 7, 8005-139 Faro, Portugal
Email: rvcarrilho@ualg.pt

Introduction

Aquaculture is one of the major sources of human food production, and its intensification, due to the increasing demand for healthy protein, can lead to environmental issues and poor fish welfare.

Farmed fish are often subjected to diverse rearing practices and environmental changes that can be stressful and affect the fish's immunity, contributing to disease outbreaks. When these occur, antibiotics are commonly used to combat the pathogens, however with the continuous use, antibiotic-resistant bacteria can develop affecting the outcome. Furthermore, the use of antibiotics can pose risks to human health and harm the environment since they can accumulate in fish and residues are often discarded in aquatic environments. Hereupon, research on antibiotic alternatives or preventive strategies is imperative.

To this end, this work proposes alternative environmentally friendly methods to boost the fish's immune system and prevent pathogenic infections. A nutritional strategy including diets supplemented with immunostimulants, and a thermal strategy to increase fish's infection coping ability, will be developed to reduce the use of antibiotics and mitigate their consequences. Overall, such alternatives will boost fish's welfare and aquaculture biosecurity, and advance the industry's and for the environment sustainability.

Material and Methods

In a first approach, gilthead seabream will be infected with the bacteria *Tenacibaculus Maritimum* to characterize the bacterial infection through Label-free shotgun proteomic (LC-MS/MS) analysis and identify possible disease biomarkers. In this experiment, the fish behavior will be observed through cameras displayed in the tanks and the optimal temperature of non-infected seabream will be assessed in thermal gradient tanks. Furthermore, hematologic, histological, and microbiological analyses will be performed.
In the second experiment, seabream will be subjected to infection and allowed to swim along a thermal gradient or being exposed to a constant temperature (obtained previously). Post-infection fish behavior and thermal preference will be evaluated through video recording.

At last, seabream will be subjected to a nutritional therapy where will be fed with two experimental diets with natural immunostimulants against a commercial diet. After this, fish will be infected with the bacteria to assess the diet's impact on fish disease behavioral phenotype and proteome. The biochemical parameters of the plasma (cortisol, lactate, and glucose) will be assessed and the liver glycogen.

THE EFFECT OF CONVENTIONAL AND NOVEL OILS ON BRAIN FATTY ACID INCORPORATION, BEHAVIOUR AND NEURAL FUNCTION OF GILTHEAD SEA BREAM (*Sparus aurata)* JUVENILES

M. Carvalho[a*], D.Montero[a], P. Domenici[b], J. M. Afonso[a], M. Izquierdo[a]

[a]Grupo de Investigación en Acuicultura (GIA), Instituto Universitario ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[b]CNR- IAS, Istituto per lo studio degli impatti Antropici e Sostenibilità in ambiente marino Torregrande, 09072 Torregrande, Oristano, Italy.
E-mail: marta.ribeiro101@alu.ulpgc.es

Introduction

N-3 long-chain polyunsaturated fatty acids (LC-PUFA) are involved in several metabolic pathways, including neural development and function (Tocher, 2015). Indeed, composition of neural cells can direct influenced brain activity that, in turn, modulates fish behaviour, which is closely related with well-being and health status and of pivotal importance in fish production. Docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) have great biological roles and are essential fatty acids (EFA) for marine species, due a limited activity of fatty acid desaturase (FADS) and elongases (ELOVL), necessary for their endogenous synthesis.

Although work has been carried out on larvae, very few studies have been conducted to understand the impact of n-3 LC-PUFA in brains and behaviour responses of fish in later stages of development. This topic deserves further attention on more species for a number of reasons: (1) With the increasing replacement of fish oil (FO) in aquafeeds, mostly with terrestrial plant or animal ingredients, n-3 LC-PUFA dietary contents could be significantly reduced. This is reflected in fish tissues and could affect fish cell responses. (2) Fish behaviour and neural function are good indicators of fish health and development, thus being of pivotal importance their study and monitorisation for an effective production of fish.

Therefore, using a long-term feeding protocol for sea bream juveniles, the aim of the present study was to investigate the effect of different conventional and novel oils with different dietary EPA and/or DHA contents on (1) brain fatty acid incorporation, (2) fish behavioural response to an external mechano-sensory stimulus and (3) expression of neurogenesis and neural activity-related markers.

Material and methods

A nutritional trial of 5 months was conducted in triplicated groups of gilthead sea bream (*Sparus aurat*a) juveniles (initial body weight of 2.50±0.01 g). Two experimental diets were formulated to present high EPA and DHA contents, one containing similar EPA and DHA levels and based on fish oil (FO diet) and the other containing 5 times more DHA than EPA using a blend of microalgae and poultry oil (DD diet). A third diet was formulated based on poultry oil (PO diet) and containing low EPA and DHA levels.

After the feeding period, 9 fish per treatment were randomly selected for testing behaviour response to an external mechano-sensory stimulus. Fish were tested in three groups per treatment, of three fish each, using a white bottom-tank of 100 L. Fish were exposed to a mechano-sensorial vibrational stimulus, which consisted in an iron pendulum of 600 g that was dropped without any additional force against the tank wall from a distance of 71 cm. Fish behaviour were monitored by video using a video-camera (Xiaomi Mijia 4k) and the following behaviour responses were analysed: responsiveness, type of escape response, basal and after stimulation activity levels, escape latency, escape distance and escape turning rate. Fish brains were collected for fatty acid composition analyses and gene expression of neurogenesis and neural activity-related markers.

Results

There was an increase in fish escape latency in response to the external stimulus with the decrease of dietary EPA and n-6 DPA, suggesting a slower reactivity to the stimulus in these fish, which might be related to a lower velocity in the translation of the neural signal to activate escape response. No significant effects were recorded for any other behavioural variable, contrary to what seen in fish larvae in previous studies (Benítez-Santa, 2012, 2014), suggesting that brains of juveniles' fish, that are fully developed, are less sensitive to dietary modification of FA composition, possibly due to the capacity of retention of n-3 LC-PUFA, mostly DHA in neural tissues for preserving vital neural functions. Indeed, DHA was highly retained in fish fed the low dietary DHA (PO diet).

Furthermore, an increase in neurogenesis and neural activity markers (*neurod1* and *bdnf*) as well as in *fads2* in fish fed PO diet was observed, suggesting a higher neurogenesis and neuroactivity as well as an activation of PUFA synthesis in those fish.

(Continued on next page)

References

Benítez-Santana, T., Atalah, E., Betancor, M. B., Caballero, M. J., Hernández-Cruz, C. M., & Izquierdo, M. (2014). DHA but not EPA, enhances sound induced escape behavior and Mauthner cells activity in Sparus aurata. *Physiology & behavior*, *124*, 65-71

Benítez-Santana, T., Juárez-Carrillo, E., Betancor, M. B., Torrecillas, S., Caballero, M. J., & Izquierdo, M. S. (2012). Increased Mauthner cell activity and escaping behaviour in seabream fed long-chain PUFA. *British journal of nutrition*, *107*(2), 295-301.

Izquierdo, M. (2005). Essential fatty acid requirements in Mediterranean fish species. *Cahiers Options Méditerranéennes*.

Tocher, D. R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture*, *449*, 94-107.

EFFECTS OF DIETARY HISTIDINE AND PLANT LIPIDS ON THE ATLANTIC SALMON (*Salmo salar*) HEALTH STATUS FOLLOWING TRANSFER TO SEAWATER

I. Carvalho[1,2*], S. C. Remø[3], D. Peixoto[1,2], N. H. Sissener[3], P. G. Fjelldal[4], R. Waagbø[3], B. Costas[1,2]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal
[3]Institute of Marine Research (IMR), 5817 Bergen, Norway
[4]Matre Research Station, Institute of Marine Research (IMR), N-5984 Matredal, Norway

E-mail: mariainesrcarvalho97@gmail.com

Introduction

With the aquaculture expansion, sustainability challenges have been emerging. Among them, there is great urgency in reducing the industry's dependence on finite-marine ingredients. Plant lipid sources have been commonly used to replace fish oils in feed formulations. However, vegetable oils-based diets might markedly deviate from fish natural feeding habits and negatively impact their health. In the particular case of Atlantic salmon (*Salmo salar*), this issue acquires major relevance during the seawater transfer (SWT), a stressful event and challenging production moment often associated with great losses. To improve the fish overall health, supplementing diets with immuno-modulators, such as certain amino acids, is becoming a common approach (Andersen *et al*., 2015). Among the amino acids recognized to cover functional roles that may improve fish health, histidine (His) and its derivatives (Remø *et al*., 2014) were found to behave as buffer and antioxidant and to have anti-inflammatory properties in the tissues they are present (Andersen *et al*., 2015). In this study, we have investigated whether dietary lipid source and His supplementation could influence the non-specific immune response and antioxidant system in Atlantic salmon after SWT.

Materials and methods

The fish were given four experimental diets differing in lipid source (100% fish oil or blend of vegetable oils) and His content (10 or 14 mg His/g diet) in freshwater (FW), and a cross-over was done after SWT to investigate the effect of supplying His supplementation in FW vs SW (**Figure 1**). At the end of the FW phase and 2 and 4 weeks after SWT, fish were sampled for blood plasma and liver to assess immune parameters and oxidative stress biomarkers, respectively. Gut samples were also collected for gene expression measurements.

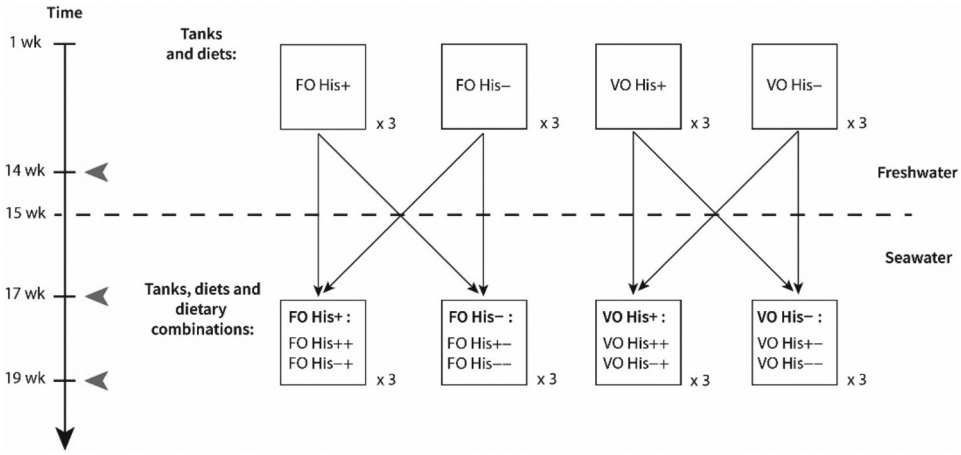


Figure 1. Schematic representation of the feeding trial design, displaying the dietary histidine (His) treatments cross-over at the seawater transfer moment (dashed line). Red arrows identify the 3 sampling points. FO His+, fish oil and high His content diet; FO His−, fish oil and low His content diet; VO His+, vegetable oil and high His content diet; VO His−, vegetable oil and low His content diet.

(Continued on next page)

Results and discussion

Regardless of dietary treatment, post-smolts appeared to be under oxidative stress two weeks after SWT, as indicated by the lower antioxidant enzymes activity. Even so, they seemed to manage to protect themselves from oxidative damage, since no increase in hepatic lipid peroxidation (LPO) levels were observed between the two times. Additionally, the increased alternative complement pathway activity in plasma found at the end of the feeding trial further suggests that all groups recover well from the transfer.

Post-smolts fed fish oil-based diets displayed higher plasma bactericidal and alternative complement activities than those fed vegetable oil feeds. Increased LPO levels were also observed in fish fed fish oil diets, possibly due to the greater susceptibility of marine lipids to oxidation. Apart from a stimulating effect of His supplementation on plasma IgM levels four weeks after SWT, it did not seem to significantly influence the post-smolt Atlantic salmon immunity and oxidative status, independently of the dietary lipid source.

Conclusion

All dietary groups seemed to recover well from the stressful SWT moment. There were, however, some minor signs of improved immunity of fish fed fish oil-based diets compared to those fed plant lipids. The small differences in both plasma innate immune parameters and liver oxidative stress biomarkers between the dietary His groups suggest that it did not significantly influence the post-smolts health status. Further analyses on gut samples are currently underway to provide more insight into the fish health condition.

Acknowledgements

This work was partially supported by UIDB/04423/2020, UIDP/04423/2020, IF/00197/2015 and INFLAMMAA (reference PTDC/CVT-CVT/32349/2017), financed by Portugal and the European Union through FEDER and COMPETE 2020, and through the COMPETE and Operational Human Potential Programmes and national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal). The feeding experiment was funded by the Institute of Marine Research (Bergen, Norway) and the feeds were produced by BioMar AS.

References

Andersen, S., Waagbø, R., & Espe, M. (2015). Functional amino acids in fish nutrition, health and welfare. *Frontiers in bioscience (Elite edition), 8*, 143-169.

Remø, S. C., Hevrøy, E. M., Olsvik, P. A., Fontanillas, R., Breck, O., & Waagbø, R. (2014). Dietary histidine requirement to reduce the risk and severity of cataracts is higher than the requirement for growth in Atlantic salmon smolts, independently of the dietary lipid source. *British Journal of Nutrition, 111*(10), 1759-1772.

INSECT MEAL AND SINGLE-CELL PROTEIN AS REPLACERS OF FISH MEAL IN DIETS FOR GILTHEAD SEA BREAM (*Sparus aurata*)

M. Carvalho[a*], A. Sanmartín[a], D.Montero[a], Ramon Fontanillas[b], Grethe Rosenlund[b], M. Izquierdo[a]

[a]Grupo de Investigación en Acuicultura (GIA), Instituto Universitario ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[b] Skretting Aquaculture Research Centre, Stavanger, Norway
E-mail: marta.ribeiro101@alu.ulpgc.es

Introduction

The expansion of aquaculture industry along with the limited marine resources available, including fish meals (FM), an emergent need to find alternative protein sources for use in aquaculture feeds had arisen in the last decades. A wide number of researches have been carried out studying plant alternatives, which are nowadays successfully incorporated in modern aquafeeds at elevated percentages. However, despite the potential to increase environmental and economical sustainability of aquaculture feeds, plant proteins often present insufficient protein content to meet fish requirements, unbalanced amino acid profile and reduced digestibility, particularly for carnivorous species. Consequently, without additional supplementation, diets with high contents of plant proteins could negatively affect fish productivity and/or health, and composition of fish flesh, reducing the benefits for the consumer. To solve these issues, a new era of research has been recently open with the purpose to find novel alternative protein sources for aquafeeds, reducing the dependency not only on marine ingredients (FM) but also on plant sources, for maximizing aquaculture productivity. From the novel raw materials studied, insect meal and single-cell proteins are possibly the most promising due to their high protein content and their balanced amino acid profile. Therefore, the main objective of this study was to test the potential of insect and single cell protein meals as fish meal replacers to reduce dependency on marine and plant ingredients, on gilthead sea bream growth performance, feed utilization and health parameters.

Material and methods

A nutritional trial of 112 days was conducted in triplicated groups of gilthead sea bream (*Sparus aurat*a) juveniles (initial body weight of ±65 g). A control diet was formulated with 15% FM and 63% of plant protein sources (CTRL). Insect meal (*Hermetia illucens*) was used to replace only FM at 5% (5-INS diet) or 10% (10-INS diet); and single-cell protein (a by-product generated by M*ethylococcus capsulatus'* fermentation) was used to replace only FM at 5% (5-SCP) or FM at 5% plus 5% of plant proteins (10-SCP). After the feeding period, fish were weighed for growth and feed utilization parameters estimation, and some of them were sampled for biochemical composition analysis. Additionally, health-related genes expressions were determined in posterior gut as well as plasma lysozyme.

Results

Sea bream fed 5% INS and SCP diets showed similar growth performance and feed utilization than those fed CTRL diet. However, 10% INS diet led to a reduced body weight than those fed CTRL and SCP diets, as well as a lower feed intake when compared to 5% SCP. Despite no significant differences were observed for specific growth rate and feed conversion ratio, the same tendency as body weight was observed. The dietary composition did not affect whole-body and flesh composition, neither plasma lysozyme. Similarly, no significant differences were observed in the relative gene expression of health-related parameters (*hsp90*, *hsp70*, *cox2*, *mhcII* and *tnf*α), although in general SCP-fed fish presented more similar expression values than those fed CTRL diet, whereas those fed INS presented tendentially lower expression values.

In conclusion, the results of the present study indicate the suitability of single-cell protein in replacing FM and reducing plant proteins dependency in diets for sea bream juveniles. Furthermore, insect meal showed also potential to replace FM in moderate quantities, whereas the high replacement levels affected growth performance and feed intake, possibly related to the chitin content of this meal.

TURN-UP THE SHELL: ADVANCES ON THE PATELLID LIMPETS' AQUACULTURE

Diego Castejón[1]*, Natacha Nogueira[1,2] and Carlos A. P. Andrade[1,2]

1. Centro de Maricultura da Calheta. Av. D. Manuel I, n.º 7. 9370-135 Calheta, Madeira (Portugal)
2. CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Universidade do Porto, s/n 4450-208 Matosinhos (Portugal)
Email: diego.castejon.dcb@gmail.com

Introduction

The true limpets (Patellogastropoda) are highly specialized marine gastropods commonly found colonizing rocky seashores. The limpets have been a fishery resource since the Neolithic period. Currently several limpet species are threatened by the overexploitation, which is leading to smaller and male biased populations, which individuals have less reproductive capacity and higher inbreeding ratio. Consequently, several limpets populations are endangered being taken protective measures focused on harvesting restrictions (Faria et al., 2018; Sousa et al., 2020). As alternative measure for the exploitation of the natural stocks, the limpets' aquaculture could be a sustainable effort able to develop different programs, either for human consumption or for the reproduction and reintroduction of different limpet species.

Limpets' aquaculture is a recent research field which efforts were mainly focused on the larvae production with variable success, while juvenile production has been mostly unsuccessful thus far. Recently, this state of the art changed thanks to advances done in Madeira Island and focused on the native species *Patella aspera* and *P. candei* (Castejón et al., 2021), it is now possible to obtain juveniles from an already settled larvae production.

Material and Methods

The adults were captured during the breeding period (mid autumn to mid spring in both species) and kept using an open system with high aeration, at 20 ± 1 °C and 36 ± 1 psu. The adults showed higher survival when no lesions occurred in the soft body during capture and management in captivity. Gametes were obtained dissecting adult specimens to expose the gonads. The sperm was gathered using a Pasteur's pipette and diluted in 100 ml filtered seawater. The sperm of 4 males was pooled and kept in the fridge until required. The oocytes were released breaking a female gonad placed inside a crystal beaker with filtered seawater using a Pasteur's pipette. Then, the oocytes were carefully washed using nylon meshes (200 and 55 μm). The oocytes of 4-5 females were pooled and matured artificially using NaOH alkalinized seawater baths during 3h (pH 9.0 in *P. aspera* and 9.5 in *P. candei*). The oocytes should be carefully washed after maturation since longer baths could damage the oocytes. The matured oocytes were fertilized at 100 oocytes ml^{-1} (or less) and 10^5 sperm cells ml^{-1} for a better larval production. Fecundation was realized in 500 ml crystal beakers. The sperm was kept with the oocytes during the incubation time, which lasted 24h.

Larvae were observed 24h post-fecundation as trocophora, 2/3 of the water column was siphoned using a 55 μm filter to avoid collecting debris, then the larvae were re-distributed in new beakers with clean filtered seawater at 10-20 larvae ml^{-1}, and cultured during additional 48h until pediveliger stage was reached. Then, the larvae were siphoned again using a 55 μm filter and re-distributed in culture cell plates at 5-10 larvae ml^{-1} for experimental purposes. Several substrates plus control were tested: two diatom species (*Halamphora* and *Navicula*), haptophyte *Pavlova*, and limpet shells covered by different communities of crustose coralline algae (CCA) as natural substrate. The timing for settlement was based on the observation of post-larvae, while success for settlement was based on the average ratio of juveniles in each treatment. The incubation, larval culture, and settlement assays were realized at 17 ± 1 °C.

Results and Discussion

Post-larvae were identified by the shedding of the velum and increased eye distance, while juveniles showed juvenile shell and active grazing behaviour. Earliest post-larvae occurred on CCA treatments in both species, while settlement success varied in both species: *P. aspera* juveniles were observed on CCA treatments (with rare exceptions), while *P. candei* juveniles were more frequent on *Navicula* biofilms and CCA treatments. The type of CCA community and their abundance influenced the settlement success, being *P. aspera* apparently more restrictive than *P. candei* on their settlement requirements. The larval development was completed in absence of food suggesting lecithotrophy and facilitating the larvae management. On the contrary, juvenile were active feeders. Grazing was concomitant with movement, which suggest that limpet juveniles are opportunistic consumers that needs continuous feeding. The development of a juvenile aquafeed is a mandatory step for further studies on the limpets' aquaculture. In this sense, the observation of *P. candei* juveniles grazing upon *Navicula* biofilms marks a starting point for the culture of the limpet juveniles.

(Continued on next page)

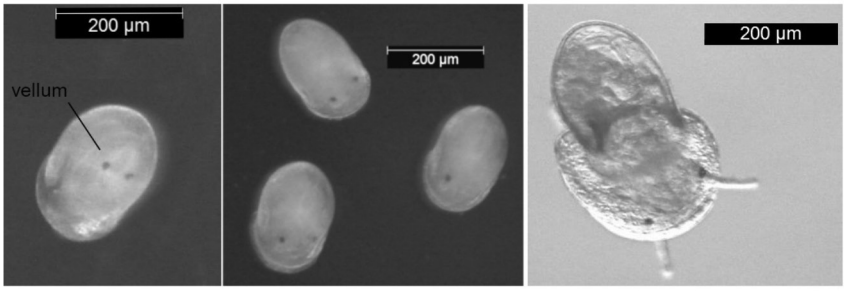


Fig. 1. Different stages of *Patella candei* (left to right): pediveliger larva, early post-larvae, juvenile

Bibliography

Castejón, D., Cañizares, J.M., Nogueira, N., Andrade, C.A.P., 2021. Artificial maturation and larval production of the limpet *Patella aspera* Röding, 1798 (Patellogastropoda, Mollusca): Enhancing fertilization success of oocytes using NaOH-alkalinized seawater. Aquac. Res. 52, 1904– 1914.

Faria, J., Pita, A., Martins, G.M., Ribeiro, P.A., Hawkins, S.J., Presa, P., Neto, A.I., 2018. Inbreeding in the exploited limpet *Patella aspera* across the Macaronesia archipelagos (NE Atlantic): Implications for conservation. Fisheries Research. 198, 180-188.

Sousa, R., Pinto, A., Vasconcelos, J., Riera, R., 2020. Does harvesting affect the relative growth in *Patella aspera* Röding, 1798? The European Zoological Journal. 87, 395-401.

FINE-TUNED PROTOCOLS FOR SOPHISTICATED INTENSIVE HATCHERY PRODUCTION OF TURBOT (*Scophthalmus maximus*)

Carolina Castro[1*], Newton Gomes[2], António Louvado[2], Verónica Lede[1], Eduardo Matos[1], Igor Bernardo[1], Andreia Silva[1], Sofia Engrola[3], Luís Conceição[4], Renata Serradeiro[1]

[1]ACUINOVA-Actividades piscícolas, SA- Praia de Mira,Portugal
[2] CESAM - Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro (UA) Aveiro, Portugal
[3]CCMAR-Centre of Marine Sciences, University of Algarve, Faro, Portugal
[4] Sparos Lda, Olhão, Portugal

*ccastro@acuinova.pt

Turbot (*Scophthalmus maximus*) is a flatfish with high-quality flesh and market value. In the European Union (EU) the vast majority of turbot production takes place in Spain (8258 tonnes in 2018, 76.9 % of total EU production) and Portugal (2350 tonnes in 2018, 21.6% of total EU production). Mass production of high-quality juveniles is still a major bottleneck in turbot aquaculture production and is due to highly variable larval survival rates (survival rates of 10-20% during the larval stage). Inadequate rearing techniques, unknown nutritional requeriments and detrimental microbiological conditions in the rearing system (e.g. the presence of pathogenic organisms) are pointed as the main causes for the losses during the early life stages.

Optimization of larviculture protocols, through the assessment of larvae nutritional needs and development of efficient rearing techniques and microbial management strategies, is essential to improve larvae and juvenile quantity and quality. Such development will contribute to enhance the profitability of turbot aquaculture production. Maximus.pt project (2021-2023) aims to create a technological basis to establish fine-tuned protocols that will allow maximum growth and survival of hatchery-reared turbot at early life stages. For this goal the project aims to 1) optimize live prey enrichment for turbot larvae; 2) improve the the weaning protocols for turbot larvae and post-larvae; 3) define optimum turbot cultivation densities and hydrodynamic conditions in rearing tanks at first feeding, weaning and pre-fattening stages; 4) characterize bacterial communities and identify taxonomic groups and/or functions that lead the community to disease or health states in turbot larvae and postlarvae.

A holistic and innovative approach will be applied in the project through the application of nutrient flux techniques and microbiome studies combined with a careful analysis and selection of nutritional strategies (algae, enrichments and microdiets). The Maximus.pt brings together a multi-disciplinary consortium of a Portuguese turbot company (ACUINOVA) and R&D institutions (CCMAR, UA) with established expertise in rearing techniques of marine flatfish larvae and juveniles (ACUINOVA and CCMAR); larvae and juvenile feeding and nutrition (CCMAR); and aquaculture microbiome diversity and function (UA).

A deep knowledge will be obtained on the interaction between rearing conditions, microbiome and feeding strategies at the larval stage, which will lead to significant improvements in the system microbial ecology and larvae performance. As a result, the Maximus.pt will leverage the supply of high volumes of premium larvae and juveniles, which will meet the current market demand. Furthermore, with project implementation, the turbot company of the consortium, ACUINOVA, will be able to expand its own robust juvenile production, which will increase the company's competitiveness.

Acknowledgements: This work is part of MAXIMUS.PT project (ref. 69769) supported by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020; and co-financed by the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education, Portugal) through project UIDB/04326/2020 to CCMAR.

OPTIMIZATION OF VACUUM COATING CONDITIONS TO IMPROVE OIL RETENTION IN TROUT FEED

Asma Chaabani[12*], Laurent Labonne[1], Vanessa Durrieu[1], Antoine Rouilly[1], Fabien Skiba[2] and Philippe Evon[1]

[1]Laboratoire de Chimie Agro-industrielle (LCA), INP-ENSIACET, Toulouse, France
[2]Nutricia, Haut-Mauco, France
E-mail: asma.chaabani@toulouse-inp.fr

Introduction

Fish are the most efficient farmed animals for converting feed nutrients into edible meat. The quality of such fish feed is crucial to ensure this goal. Fish feed is presented in the form of extruded pellets obtained through twin-screw extrusion. It is an agro-material whose composition, nutritional value, density, and size variations have to suit the development stages, behaviour and therefore the nutritional requirements of various fish species. For carnivorous species, their diets must have high lipid content to provide a source of easily available energy. In our case, for large Trout (i.e. weight of about 2.5 kg per specimen), the formulation has to contain up to 30 % of fat. This operation is allowed by injecting a mixture of vegetable and animal oils (*i.e.*, rapeseed oil, and fish oils) using a vacuum coating operation. When reaching these nutritional requirements, the obtained pellets may present a major defect, which is the oil leakage over time. This work describes a process approach to study this phenomenon in Trout pellets.

Materials and methods

To study the influence of the coating parameters on oil leakage rate (OLR), sixteen coating experiments were conducted with the help of an experimental design having the form of a three-variable Doelhlert's matrix. For those experiments, the coating parameters varied as follows: a stirring speed from 45 to 85 Hz, a pressure in the coater from 140 to 310 mbars, and a filling rate of the coater from 35 to 55 % in volume. For the experimental design, the time to restore the atmospheric pressure after coating was set at 120 s. Then, it was also studied at five different values (i.e. 60 s, 75 s, 90 s, 105 s, and 120 s). Pellets OLR was evaluated through the determination of the oil quantity lost and expressed in proportion to the total weight of pellets before leakage. Two temperature conditions were tested: T1 (climatic chamber, 60 % relative humidity (RH), 20 °C), and T3 (water bath, 60 °C).

Results

Coating conditions showed a real ability to influence the OLR value. The pressure in the coater was the most influential parameter on OLR. The latter contributed to a reduction of more than 50 % in the oil leakage rate at 20 °C (T1 incubation condition) (Fig. 1). This result underlines the importance of the coater pressure on OLR variation, with a progressive reduction in the oil leakage phenomenon as the coater pressure was reduced. This condition allowed deeper penetration of oil in the pellet core during coating, and thus lower oil leakage.

Lastly, the time to restore the atmospheric pressure was also influential in the reduction of the oil leakage phenomenon. **Fig. 2** shows a decreasing trend of OLR when this time is increased from 60 s to 120 s, either at 20 °C (T1 incubation condition) or at 60 °C (T3 incubation condition). In other words, better oil penetration into the core of pellets was ensured when the time to restore the atmospheric pressure was longer (i.e. 120 s). When the oil addition sequence was completed under vacuum, the latter was slowly released up to the atmospheric pressure (1.015 bars). This phenomenon created a pressure differential that forced the oil into the voids of the pellets.

To conclude, the most important finding in this work is that the control of pressure at coating is the major factor to decrease OLR, and 140 mbars pressure in the vacuum coater was identified as the optimal condition for an efficient coating and therefore for a greater reduction in OLR.

(Continued on next page)

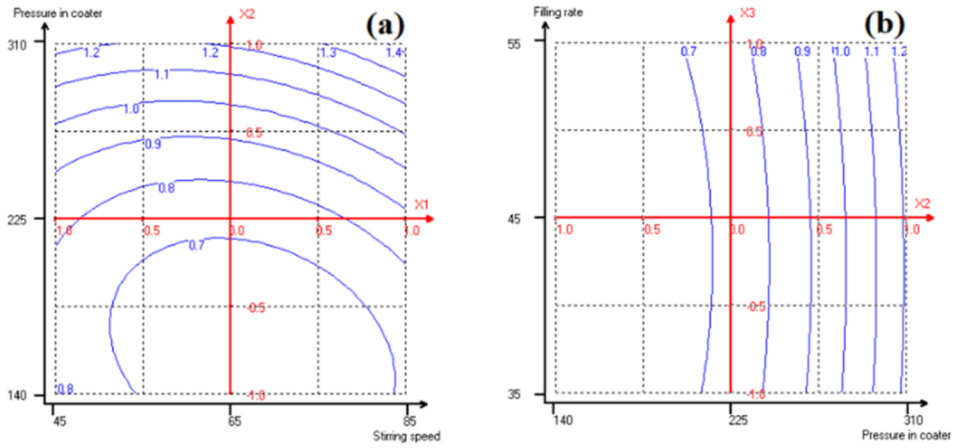


Fig. 1. Isoresponse curves for OLR at 60 % RH and 20 °C in a climatic chamber (T1 incubation condition), at a 45 % filling rate of the coater (a), at a 65 Hz stirring speed (b).

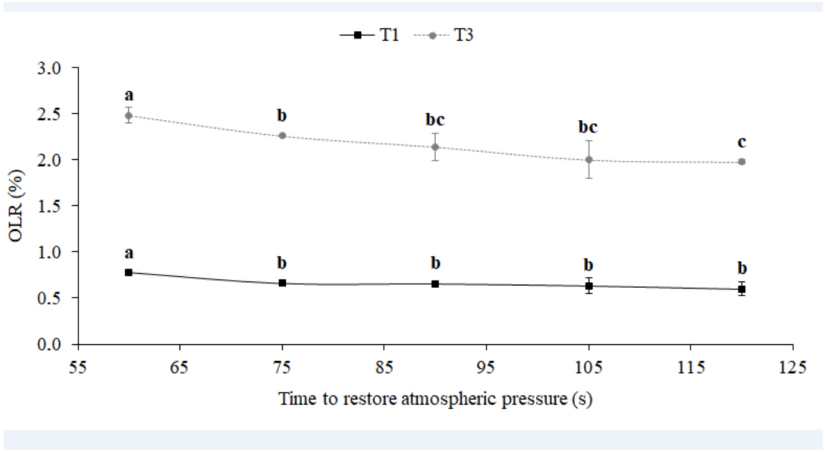


Fig. 2. Effect of time to restore the atmospheric pressure on OLR at 60 % RH and 20 °C in a climatic chamber (T1 incubation condition), and at 60 °C in a water bath (T3 incubation condition), for the next coating conditions: 65 Hz stirring speed, 140 mbars pressure in the coater, and 45 % in volume filling rate (means in the same curve with the same superscript letter (a-c) are not significantly different at $P < 0.10$).

SCREENING OF CAPTURED WILD ATLANTIC COD *Gadus morhua L.* FOR VHS VIRUS AND EVALUATE THE RISK OF TRASMITTING VHS VIRUS FROM ATLANTIC COD TO ATLANTIC SALMON *Salmo salar*

Uthpala Chandrarathna*[1], Kjetil Korsnes[1,3], Hilde Sindre[2], Mette Sørensen[1] and Ioannis N. Vatsos[1]

[1] Faculty of Biosciences and Aquaculture, Nord University, Universitetsalléen 11, 8026 Bodø, Norway
[2]Norwegian Veterinary Institute, Oslo, Norway
[3]BioVivo Tech AS. Stobjørnen 17, 8029 Bodø, Norway
E-mail: uthpala.chandrarathna@nord.no

Introduction

With the expansion of mariculture industry, Atlantic cod (*Gadus morhua L.*), which is previously mainly acquired by wild fisheries, has been identified as a possible candidate for Capture Based Aquaculture (CBA). In Norway, 3 to 5 years old wild Atlantic cod, is captured, graded and transferred to sea cages. Then, after a weaning period, which consists of starvation and wet feeding, the fish are allowed to adapt to captivity. They are usually cultured for 6-8 months with feeding, before marketed as fresh cod products (Dreyer et al, 2008). However, introducing wild caught cod to aquaculture stations of Norway may lead to adverse consequences with the possibility of disease spreading specially to Atlantic salmon (*Salmo salar*), which is the main mariculture fish species farmed in Norway. Therefore, appropriate screening of wild caught cod for the prevalence of notifiable fish diseases is necessary. Viral Hemorrhagic Septicemia (VHS) is a notifiable list 2 disease according to OIE (OIE, 2019), and has led to mass mortalities in both freshwater and marine water aquaculture species causing serious economic losses. In the present study, we focused on screening wild cod population in Norwegian waters to estimate the prevalence of VHSV. Furthermore, to evaluate pathogenicity and the transmission risk of VHSV from Atlantic cod to Atlantic salmon, artificial infection trial was performed.

Materials and methods

For VHSV screening, about 2800 wild Atlantic cod from 3 main locations along the Norwegian coastline namely Vesterålen (at small community Myre), Båtsfjordand in Finnmark and Alesund were sampled to collect brain, heart and head kidney tissues, throughout the main cod fishing seasons from 2019 to 2021. RNA was extracted from 2200 brain tissues so far and screening for VHS virus was done using Taqman-based real-time PCR assay, which was previously validated and published (Jonstrup et al, 2013).

Artificial infection trial started in April in 2021 and is currently ongoing for 8 weeks, using 2 VHS virus isolates belonging to 2 main genotypes of VHSV, namely VHSV genogroup III (Storfjorden, 2007) and VHSV 1b (Baltic Sea, 2016). In individual challenge experiments, naïve Atlantic Cod and Atlantic Salmon juveniles (~80-100 g) were infected with the 2 virus strains by immersion and intra-peritoneal injection. In the cohabitation challenge experiment, infected Atlantic cod juveniles are cohabited with naïve Atlantic salmon juveniles to analyze the transmission risk of the tested virus strains from Atlantic cod to Atlantic salmon. Tissue samples will be collected at different time points and analyzed by cell culture (Ex: IFAT staining), histopathology, molecular (Ex: RT-PCR) and genetic (Ex: transcriptome) methods. Water samples from each tank will also be collected, to analyze the amount and rate of virus shedding.

Results and discussion

There was no VHSV positive sample detected, out of 2200 cod brain tissue samples which were screened so far. Therefore, according to current results, we conclude that prevalence of VHSV in the main Atlantic cod populations in Norwegian waters is very low which is consistent with previous screening studies as only two positive cases of VHSV from 8395 different species of wild marine fish have been reported (Brudeseth and Evensen, 2002).

We expect to graphically visualize mortality rates and tissue distribution of virus, and also include microscopic images of histopathological changes inflicted by VHSV in main organs of both Atlantic cod and Atlantic salmon. Furthermore, transcriptome analysis will be focused on differential gene expression to understand host specificity of VHS virus and immunological responses in two fish species, and will be analyzed statistically. Considering disease transmission, European VHSV isolates in genotype Ib, II or III which often originated from marine environment generally has shown low pathogenicity to freshwater fish (Skall et al, 2004). However, VHSV isolate from recent VHSV outbreaks in marine cultured rainbow trout in Storfjorden, Norway (Dale et al, 2009) was discovered as genetically closer to VHSV III. Therefore, we expect ongoing challenge trials which includes a co-habitation element will give us new insight to understand the transmissibility of marine originated European VHSV isolates.

(Continued on next page)

References

B.M.Dreyer, B.H.Nøstvold, K.Ø.Midling, Ø.Hermansen, Capture-based aquaculture of cod. In A. Lovatelli and P.F. Holthus (eds). Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No. 508. Rome, FAO (2008) pp. 183–198.

OIE, 2019. Manual of Diagnostic Tests for Aquatic Animals.

S.P.Jonstrup, S.Kahns, H.F.Skall, T.S.Boutrup, N.J.Olesen, Development and validation of a novel Taqman-based real-time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus. J Fish Dis 36 (2013) 9-23.

B.E.Brudeseth, O.Evensen, Occurrence of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish species in the coastal regions of Norway. Dis Aquat Organ 52 (2002) 21-28.

H.F.Skall, W.J.Slierendrecht, J.A.King, N.J.Olesen, Experimental infection of rainbow trout Oncorhynchus mykiss with viral haemorrhagic septicaemia virus isolates from European marine and farmed fishes. Dis Aquat Organ 58 (2004) 99–110.

O.B.Dale, I.Orpetveit, T.M.Lyngstad, S.Kahns, H.F.Skall, N.J.Olesen, B.H.Dannevig, Outbreak of viral haemorrhagic septicaemia (VHS) in seawater-farmed rainbow trout in Norway caused by VHS virus Genotype III. Dis Aquat Organ 85 (2009) 93–103.

THE USE OF COMPLETE mtDNA OF THE RED PORGY *Pagrus pagrus* AS A MOLECULAR TOOL TOWARDS FISH AUTHENTICITY

E. Chatzoglou*[1], N. Tsaousi[1], G. Triantaphyllidis[1], E. Malandrakis[1], H. Miliou[1]

[1]Agricultural University of Athens, Laboratory of Applied Hydrobiology, Iera Odos 75, 11855 Athens, Greece
E-mail: echatzoglou@aua.gr

Introduction

The red porgy is a benthopelagic species of high commercial value, widely traded in the Greek market. Its wild populations are located the Mediterranean Sea, but also in the Atlantic coasts of America, South Europe and Africa (Fishbase). Wild *Pagrus pagrus* from fisheries in Greece, shows similar morphological features with the corresponding imported, and closely-related species of Sparidae, as *Pagellus erythrinus,* and *Dentex gibbosus*. *Pagrus* sp. is reared in Greek fish farms, with its production conquering the third place among reared species in Greece. *Pagrus* fish farming products sold in the Greek market are found under various names, with the majority reported as *Pagrus major*. The trading of wild, reared, or imported representatives in the market, raises the question of authenticity and proper labeling of *Pagrus* products. Although fish can be classified by their morphological characteristics, molecular techniques based on genetic material (DNA), are currently being used for accurate and robust species identification. Having the ability to cross-check the sequences with databanks, the genetic authenticity and origin of each sample can be assessed, thus contributing to the detection of mislabeling and fraud. The mitochondrial DNA (mtDNA) is a useful tool for investigating molecular mechanisms and evolutionary relations between different fish species (Ceruso et al. 2019). The gene region used as DNA barcode in species identification (Ratnasingham 2007), is part of the cytochromic oxidase subunit I gene (COI). However, for the discrimination between closely related species or populations within species, data could also be drawn from other mtDNA regions (*cytb*, 16sRNA, D-loop etc.). Up to date, the complete mitochondrial genome of *P. major*, *P. auriga* and *P. caeruleostictus* have been determined. In this study, the complete mtDNA of *Pagrus pagrus*, captured in the central Aegean Sea, was sequenced. Data inferred from this sequence along with barcoding of samples collected in the Greek market (locally fished, reared, imported) were used in different molecular techniques, in order to establish a fast and reliable method for species identification within *Pagrus* genus and for estimation of population differentiation within wild *Pagrus pagrus*.

Materials and Methods

The specimens included wild *P. pagrus* (70 samples) caught in the Greek seas, FAO subareas 37.2 (Ionian Sea division 37.2.2) and 37.3 (Aegean and Cretan Seas), farmed *Pagrus* (15 samples from various Greek fish farms) and imported (labelled as *P. pagrus*), purchased from fish markets and supermarkets (10 samples). Fish species were identified according to their morphological characteristics and categorized in groups by their origin. Total DNA was extracted from the skeletal muscle or liver using NucleoSpin® Tissue Macherey-Nagel, according to the manufacturer's instructions. For the determination of total mtDNA sequence, a specimen caught at Central Aegean FAO division 37.3.1 was selected. Primers were either designed or selected from the literature (Miya and Nishida 2000), for the amplification of total mtDNA in 42 overlapping fragments ranging from 300-4.500 bp. Both mtDNA strands were completely sequenced. For barcoding, a fragment of 648 bp of COI was amplified using universal primers (Ward et al., 2005). In order to distinguish *P. pagrus* populations, Forensically Informative Nucleotide Sequencing (FINS) was carried out for *cytochrome b* gene. A 583 bp fragment was amplified using primers designed in this study. Sequences were identified with Blast search tool, compared and aligned with sequences from BOLD database and aligned with clustalW2 (Sievers et al. 2011). After in silico analysis, 3 fragments were chosen for Restriction Fragment Length Polymorphism analysis (PCR-RFLP) using a set of 10 restriction enzymes. Smaller fragments of COI and *cytb* (100-300 bp) were used in Real-time PCR (qPCR) and High-Resolution Melting (HRM) analysis.

Results

The complete mtDNA of *Pagrus pagrus* has a size of approximately 17 kb and revealed the same structure as all mtDNAs of teleosts, containing 13 protein, 22 tRNA and 2 rRNA genes and two non-coding regions (D-loop and L-origin of replication). Gene arrangement was typical of Sparidae species, with most of the genes encoded by the heavy strand, except for the NADH dehydrogenase subunit 6 (ND6) and eight tRNAs that are encoded by the light strand. The results showed that there is a clear distinction between wild specimens, imported and reared ones. According to homology in FishBol, all specimens from fisheries or imported were identified as *P. pagrus* and all specimens from Greek fish farms as *P. major*. Sequences of the same samples that were analyzed for *cytb* (FINS), confirmed these findings, being more indicative for differences between populations. Results from RFLP showed a pattern that could distinguish between *P. pagrus* and *P. major* but not between Greek and imported *P. pagrus*. Preliminary results of qPCR and HRM analyses from regions of COI, and *cytb*, are indicative and promising for fish species and population identification.

(Continued on next page)

Conclusions

Fisheries plays an important socioeconomic role in Greece, providing the market with products of high nutritional value. Due the high price of red porgies, it is important to conserve and validate the value of these products. The detection of *P. major* as a common species in Greek porgy aquaculture today raises the questions about the environmental impact from the rearing of an alien species (escapes, mixes with wild populations) in the Mediterranean and authenticity issues as described in Regulation (EU) No 1379/2013. In order to enable consumers to make informed choices, it is necessary to provide clear and comprehensive information on, inter alia, the origin and the method of production of the products. *P. pagrus*, fished in Greece, show similar morphological features with the corresponding imported, farmed fish of the same genus and closely-related species of Sparidae. Furthermore, in case the fish has been processed (exfoliated, fillet or cooked), even classification experts are unable to identify the sample. Data of this study, will ensure the identity, originality and authenticity of Greek fishery products. Moreover, extended sequence data are already being used for the development of a methodology (qPCR, HRM) that will provide a rapid tool against fish mislabeling and phenomena of fish fraud.

Acknowledgements

This work is supported by the Greek Operational Programme Maritime & Fisheries 2014-2020 (MIS 5033599), which is co-financed by the EU- European Maritime and Fisheries Fund and the Hellenic Republic Ministry of Rural Development and Food.

References

Ceruso M., Mascolo C., Anastasio A., Pepe T. & Sordino P. 2019. Frauds and fish species authentication: Study of the complete mitochondrial genome of some Sparidae to provide specific barcode markers. Food Control 103, 36–47.

Miya M. & Nishida M. 2000. Use of Mitogenomic Information in Teleostean molecular Phylogenetics: A Tree-Based Exploration under the Maximum-Parsimony Optimality Criterion. Molecular Phylogenetics and Evolution 17, 437-455

Ratnasingham, S. & Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7, 355-364.

Sievers F., et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology 7, 539.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R. & Hebert P.D.N. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 360, 1847–1857.

EFFECT OF DIETARY ELECTROLYTE BALANCE AND MOISTURE CONTENT ON THE INTERPLAY BETWEEN WATER BALANCE AND DIGESTIVE FUNCTIONING ALONG THE GASTROINTESTINAL TRACT OF FRESHWATER RAINBOW TROUT (*Oncorhynchus mykiss*)

E. Ciavoni[1]*, M. Koppelaars[1], R. Maas[1], P. Antony Jesu Prabhu[2], J. W. Schrama[1]

[1]Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands
[2]Feed and Nutrition research group, Institute of Marine Research, Bergen 5817, Norway
E-mail: elisa.ciavoni@wur.nl

Introduction

Feed formulation in aquaculture is undergoing a transition towards alternative ingredients (*i.e.*, plant based) due to the reduced sustainability and availability of fish meal. Yet, reduced fish meal in a diet and increased use of alternative ingredients implies reduced quality of the diet (less nutritious and digestible ingredients) together with altered mineral profile and availability. Minerals have different functions and are important for osmoregulation and acid-base balances in fish. Fish osmoregulation has been mainly studied using non-fed animals where the effects of digestion on homeostatic mechanisms in the gastrointestinal tract (GIT) were not considered. However, previous works have revealed that osmoregulation can be challenged when fish are fed (Wood *et al*., 2005; Bucking and Wood, 2006; Taylor and Grosell, 2006). During digestion, water and ion secretion disturbs fish homeostasis both in freshwater and saltwater medium. In this study, we aimed at investigate the effect of dietary moisture content and dietary electrolyte balance (dEB = $K^+ + Na^+ - Cl^-$ meq/kg) on chyme characteristics (dry matter, pH and osmolality) along the GIT of freshwater rainbow trout (*Oncorhynchus mykiss*). Moreover, the relation between chyme characteristics and digestibility in different segments of the GIT is studied.

Material and methods

This trial was performed using freshwater rainbow trout (*Oncorhynchus mykiss*) as the test animal. Four experimental diets were formulated to have contrasting dEB (- 98 and 600 meq/kg of dry matter) and moisture content (0 and 0.4 - 0.9 g water g feed). These two dietary factors were tested in a 2x2 factorial design resulting in 4 test diets. Fish performance, nutrient utilization, osmotic balance and digestive functioning along the gastrointestinal tract were measured. Chyme, blood and tissue samples were collected at 2 time points (3 and 7 h) to test the effect of time after feeding on chyme and blood characteristics according to a 2x2x2 experimental design. The diets were assigned to 24 tanks with 23 fish each, and therefore each treatment was tested with 3 replicates. Chyme was collected quantitatively from four segments of the GIT namely stomach, proximal, middle and distal intestine and was analysed for pH, osmolality and dry matter (DM) content. Blood was collected from caudal vein and heart to measure blood pH and osmolality. Faecal material was collected daily to measure apparent digestibility coefficient (ADC) of nutrients using the indicator method with yttrium oxide (Y_2O_3) as a marker.

Results and Discussion

Contrasting dEB led to differences in chyme pH and osmolality in the stomach but no significant difference was observed in the intestine. A lower ($p < 0.001$) stomach chyme pH (4.12) was measured at low dEB compared to high dEB (5.71), whereas higher ($p < 0.006$) stomach chyme osmolality (398 mOsm) was recorded at low dEB compared to high dEB (358 mOsm). Chyme DM in the proximal intestine was lower ($p < 0.004$) when fish were fed high dEB diet (13.6%) compared to those fed low dEB diet (15%). The same trend was observed in the middle intestine ($p < 0.002$) where lower chyme DM content (15%) was measured in the high dEB diet group compared to the low dEB diet (16.2%).

Dietary moisture content altered ($p < 0.034$) stomach chyme osmolality, being highest in the fish fed high moisture diet (392 vs. 363 mOsm). Dietary moisture content did not affect pH and DM in stomach and had no impact on chyme characteristics in the intestine.

A time effect on chyme DM, pH and osmolality was detected in the stomach and on chyme DM in the middle intestine. Stomach chyme DM decreased ($p < 0.032$) from 26.6% at 3 hours to 22.9% at 7 hours postprandially. In contrast, chyme DM in the middle intestine increased ($p < 0.002$) from 15.2 to 16% at 3 and 7 hours after feeding, respectively. In the stomach, both chyme pH and osmolality decreased with time (3h to 7h) from 5.34 to 4.50 ($p < 0.002$) and from 399 to 353 mOsm ($p < 0.003$) respectively.

(Continued on next page)

Blood pH altered with time postprandial, but was unaffected by the dietary treatments. The caudal blood pH decreased ($p < 0.014$) with time after feeding from 7.22 to 7.18 at 3h to 7h, respectively. Similarly, the heart blood pH was higher ($p < 0.004$) at 3h (7.20) compared to at 7h (7.16) postprandially.

Blood osmolality of the caudal vein was unaffected by differences in dietary moisture and dEB content and time after feeding. In contrast, blood osmolality sampled at the heart was affected by dietary moisture content ($p < 0.020$). It decreased from 322 to 317 mOsm when fish were fed high and low moisture diets, respectively.

ADCs, plasma electrolyte and mineral are currently being analysed and will be related to chyme characteristics.

These preliminary results suggest that most of the processes of transformation of the chyme and digestion occur in the stomach and decrease towards the intestine. One of the more significant findings to emerge from this study is that dEB has an impact on the digestive process by affecting the pH and liquefaction of the chyme in the gut and, as a consequence, on postprandial blood chemistry. Further analyses should assess whether these changes observed in chyme characteristics also have an impact on digestion.

References

Bucking, C., & Wood, C. M. (2006). Gastrointestinal processing of Na^+, Cl^-, and K^+ during digestion: implications for homeostatic balance in freshwater rainbow trout. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *291*(6), R1764-R1772.

Taylor, J. R., & Grosell, M. (2006). Feeding and osmoregulation: dual function of the marine teleost intestine. *Journal of Experimental Biology*, *209*(15), 2939-2951.

Wood, C. M., Kajimura, M., Mommsen, T. P., & Walsh, P. J. (2005). Alkaline tide and nitrogen conservation after feeding in an elasmobranch (*Squalus acanthias*). *Journal of Experimental Biology*, *208*(14), 2693-2705.

AQUACULTURE EUROPE 2021, Funchal, Madeira, October 4 -7, 2021

PHYTOCHEMICAL PROFILING OF *Fucus virsoides* J. Agardh: VOLATILE ORGANIC COMPOUNDS, FATTY ACIDS AND FUCOXANTHIN

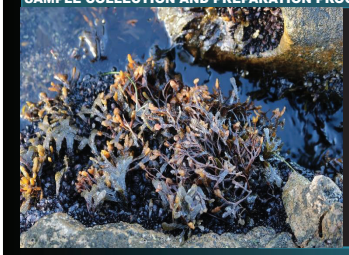
Ana-Marija Cikoš [1]*, Stela Jokić [1], Krunoslav Aladić [1], Rozelindra Čož-Rakovac [2], Drago Šubarić [1], Jurislav Babić [1], Igor Jerković [3]

[1] Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek, Croatia
[2] Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia
[3]Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21000 Split, Croatia
*acikos@ptfos.hr

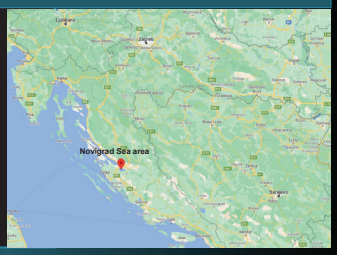
INTRODUCTION

Fucus virsoides J. Agardh (family Fucaceae, order Fucales) is known as an endemic brown macroalga distributed in the mediolittoral zones on rocky sheltered or moderately exposed shores only in the Adriatic Sea .(In the previous reports it has been stated that this alga can be a potential source of polyunsaturated fatty acids and fucoxanthin that are known compounds with certain biological activities. This study encompasses the analysis of volatilome profile of fresh samples of *F. virsoides* obtained by headspace solid-phase microextraction (HS-SPME), along with the determination of fatty acid composition and fucoxanthin content.

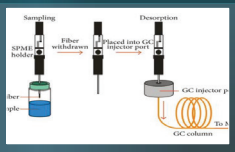
SAMPLE COLLECTION AND PREPARATION PROCEDURE



Fucus virsoides J. Agardh, 1868, was collected in the middle part of the Adriatic Sea coast, Novigrad sea area, in February 2021. Single point sample collection provided a representative sample. *F. virsoides* was collected from a depth of 0.5 m at the sea temperature of 12°C. The sample was collected and placed in an airtight plastic bag with surrounding seawater and was immediately transported to the laboratory.
Before HS-SPME, the sample was held at 4°C in the dark and the extraction was performed within 48 h of the sampling. The sample of *F. virsoides* was cut into small pieces, and the excess seawater was removed by the filter paper layers.
For the extraction of fatty acids and less polar non-volatiles, fresh *F. virsoides* was freeze-dried. Before the freeze-drying, the sample was washed five times in water and twice in deionized water, then it was cut in slices (5–10 mm) and frozen at -60°C for 24 h in an ultra-low freezer. Five trays of frozen samples were placed in a laboratory freeze dryer. The freeze-drying was performed under a high vacuum (0.13–0.55 hPa) for 24 h with -30°C and 20°C as the primary and secondary drying temperatures. Freeze-dried samples were further used for the analysis of fatty acids and fucoxanthin content.



ISOLATION AND DETERMINATION OF VOLATILE COMPOUNDS

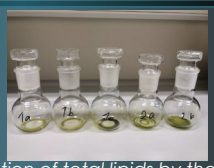


Headspace solid-phase microextraction (HS-SPME)

Gas Chromatography and Mass Spectrometry (GC-MS)

Injection temperature	250°C
Detector tempertature	300°C
Temperature gradient	70°C 2 min; 70-200°C po 3°C/min; 200 °C 15 min
Split ratio	1:50
Carrier gas	He, 1.0 mL/min

EXTRACTION OF TOTAL LIPIDS AND FATTY ACIDS PROFILE DETERMINATION



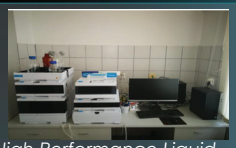
Extraction of total lipids by the Folch method

Gas Chromatography with Flame Ionization Detector (GC/FID)

Injection temperature	250°C
Detector temperature	300°C
Temperate gradient	110°C 2 min; 110-175°C po 10°C/min, 8 min; 175-210 °C po 5°C/min, 5 min; 210-230°C po 5°C/min; 230°C 7 min
Split ratio, injection volume	1:10; 2 µL
Carrier gas	He, 1.0 mL/min

FRACTIONATION AND DETERMINATION OF FUCOXANTHIN

Solid-Phase Extraction (SPE)



High Performance Liquid Chromatography (HPLC)

HPLC column	YMC-C30 (250 mm x 4,6 mm id, 5 µm)
Mobile phases	A: methanol:isopropanol:H_2O (90:7:3, v/v/v) B: methanol:isopropanol (10:90, v/v)
Gradient	0 min, 0% B; 50 min, 100% B; 60 min, 0%
Flow	0-50 min 0,6-0,8 mL/min; 50-60 min 0,8 mL/min
Injection volume	30 µL
Recorded spectra	350-660 nm

RESULTS

Table 1. The headspace VOCs of *F. virsoides* determined by HS-SPME/GC-MS (I – fresh sample extracted by DVB/CAR/PDMS fiber; II – fresh sample extracted by PDMS/DVB fiber)

No.	Compound	Rt	RI	I Av ± SD	II Av ± SD
1.	Hexanal	2.742	< 900	-	1.14 ± 0.03
2.	3-Methylbutanoic acid	3.161	< 900	-	0.25 ± 0.02
3.	Heptanal	4.162	901	-	1.41 ± 0.02
4.	(E)-Hept-2-enal	5.361	962	-	0.35 ± 0.01
5.	Benzaldehyde	5.504	968	-	1.43 ± 0.04
6.	Oct-1-en-3-one	5.896	983	-	0.18 ± 0.01
7.	Oct-1-en-3-ol	5.917	983	-	0.87 ± 0.02
8.	6-Methylhept-5-en-2-one	6.118	988	-	0.47 ± 0.02
9.	Octanal	6.537	1003	-	0.56 ± 0.01
10.	Benzyl alcohol	7.686	1044	-	19.67 ± 0.42
11.	Nonanal	9.937	1106	-	3.36 ± 0.05
12.	Tridecane	17.852	1300	-	0.89 ± 0.01
13.	Pentadec-1-ene	25.726	1493	17.98 ± 0.82	-
14.	Pentadecane	26.044	1500	61.90 ± 1.51	60.27 ± 2.00
15.	Tridecanal	26.477	1511	-	1.13 ± 0.02
16.	(E)-Pentadec-7-ene	26.644	1517	9.12 ± 0.80	-

Av – average area percentage composition obtained by GC-FID/MS of 3 replicates, SD – standard deviation of the area percentages for 3 replicates, RI - retention indices relative to C9 - C25 alkanes

Table 2. Fatty acids composition of *F. virsoides* determined by GC-FID

No.		Fatty acid	Av ± SD (%)
1.	C12:0	Lauric acid	0.06 ± 0.01
2.	C14:0	Myristic acid	10.51 ± 0.07
3.	C15:0	Pentadecyclic acid	0.18 ± 0.00
4.	C16:0	Palmitic acid	8.10 ± 0.07
5.	C18:0	Stearic acid	1.35 ± 0.02
6.	C20:0	Arachidic acid	0.43 ± 0.11
7.	C24:0	Lignoceric acid	1.42 ± 0.33
		Total saturated fatty acids (SFA)	**22.03**
8.	C14:1	Myristoleic acid	0.20 ± 0.00
9.	C16:1	Palmitoleic acid	0.99 ± 0.07
10.	C18:1n9† + C18:1n9c	cis-Oleic acid+*trans*-Oleic acid	42.28 ± 0.24
11.	C20:1n9	Eicosenoic acid	0.65 ± 0.10
12.	C22:1n9	Erucic acid	0.32 ± 0.01
		Total monounsaturated fatty acids (MUFA)	**44.43**
13.	C18:2n6c	cis-Linoleic acid	6.51 ± 0.07
14.	C18:3n3	α-linolenic acid	2.13 ± 0.93
15.	C18:3n6	γ-Linolenic acid	0.42 ± 0.02
16.	C20:2n6	Eicosadienoic acid	1.51 ± 0.08
17.	C20:3n3	Eicosatrienoic acid	0.26 ± 0.06
18.	C20:3n6 (DGLA)	Dihomo-γ-linolenic acid	2.96 ± 0.03
19.	C20:4n6	Arachidonic acid	15.00 ± 0.05
20.	C20:5n3 (EPA)	Eicosapentaenoic acid	3.71 ± 0.02
21.	C22:2	Docosadienoic acid	1.24 ± 0.04
		Total polyunsaturated fatty acids (PUFA)	**33.71**
		Total ω3 fatty acids	**6.09**
		Total ω6 fatty acids	**27.62**

* Av – average area of 3 replicates expressed in percentage (%) with standard deviation (SD)

Table 3. Fucoxanthin content of semi-purified fractions of *F. virosides* determined by HPLC

Fraction	Fucoxanthin (AV± SD)*
F3	79.30±10.29
F4	n.q.

* results are expressed in mg/100 g of dry mass of 3 replicates (mean values±SD; n=3); n.q. - not quantified)

CONCLUSION

The headspace composition of fresh *F. virsoides* revealed the dominance of pentadecane followed by pentadec-1-ene. The main fatty acid was oleic acid, followed by arachidonic acid and myristic acid. The significant amounts of ω3 and ω6 fatty acids were observed where eicosapentaenoic acid was the dominant ω3, while arachidonic acid was found as the major ω6 fatty acid. The fucoxanthin content determined in methanolic fraction F3 was 79.30±10.29 mg/100 g and it represented the main peak found in chromatographic analysis. Since macroalgae represent a valuable source of various bioactive compounds it is important to investigate their chemical profiles that will lead to the better understanding of marine algal biodiversity in the Adriatic Sea. The seasonal variations, differences in geographic locations and species-inherent characteristics can have an influence on the volatile compounds, fatty acids and fucoxanthin contents and further exploration is necessary.

ACKNOWLEDGMENT

This research was supported by the Croatian Government and the European Union through the European Regional Development Fund - the Competitiveness and Cohesion Operational Programme (KK.01.1.1.01) through the project Bioprospecting of the Adriatic Sea (KK.01.1.1.01.001) granted to The Scientific Centre of Excellence for Marine Bioprospecting –BioProCro)

BIOPROSPECTING JADRANSKOG MORA
Projekt sufinancira Europska unija iz Europskog fonda za regionalni razvoj

Republika Hrvatska Ministarstvo znanosti i obrazovanja
Europska unija Zajedno do fondova EU
Republika Hrvatska Ministarstvo regionalnoga razvoja i fondova Europske unije
EUROPSKI STRUKTURNI I INVESTICIJSKI FONDOVI
Operativni program KONKURENTNOST I KOHEZIJA

TOWARDS SUSTAINABLE AQUACULTURE FEEDS USING BREWERS´ BYPRODUCTS

M. Cidad[*1], S. Ramos[1], B. Iñarra[1], L. Padrell[2], A. Estévez[2]; D. San Martin[1]

[1]AZTI, Food Research, Basque Research and Technology Alliance (BRTA), Bizkaia, Spain
[2]IRTA, Tarragona, Spain
E-mail: mcidad@azti.es

Introduction

The FAO Committee on Fisheries stressed the increasingly key role of aquaculture in fish production for human nutrition and poverty alleviation. Its contribution to world fish production has reached 46% in 2016-2018 (FAO, 2020). Indeed, aquaculture is projected to be the prime source of seafood by 2030, as demand grows from the global middle class and wild capture fisheries approach their maximum take.

Therefore, there is a need to ensure a more sustainable aquaculture development to mitigate the environmental impacts linked to this growth. Considering that the feed production accounts for up to 60% of the total environmental impact linked to an aquaculture product (Cidad et al., 2021), efforts should be focused on reducing the impact generated on that stage. On feed production, animal feed ingredients, such as fish meal or fish oil contribute most to the impacts (up to 60%), followed by the plant-based ingredients as soybean and corn gluten (33%). Thus, more sustainable feed ingredients appear as valuable alternatives in order to reduce significantly the overall environmental impact of aquaculture products. This solution will also reduce the dependence on marine resources (Turchini et al. 2012), as 83% of fish oil and 65% of fish meal annually produced is assigned to aquafeeds (Tacon et al. 2008).

Within this framework, LIFE BREWERY project has demonstrated the environmental benefits of valorising spent grains and yeast produced by the brewery industry, as alternative ingredients for aquaculture feeds. For that purpose, a comparative Life Cycle Assessment (LCA) has been performed following the ISO 14040:2006 specifications.

Methodology

To identify, quantify and compare environmental impacts linked to different alternatives of aquaculture feeds, the LCA method appears as an internationally recognized methodology. According to the ISO 14040:2006, the method consists of 4 steps i) Goal and Scope definition; ii) Life Cycle Inventory, iii) Life Cycle Impact Assessment and iv) Interpretation. The steps are not simply sequential; LCA is an iterative technique that allows to be increased the level of detail in successive iterations.

Results and discussion

Step 1. Goal and scope definition

The goal of the study is to compare 3 different aquaculture feeds in order to assess potential environmental improvement of replacing current aquafeeds ingredients by brewers' by-products: spent grains and yeast. The functional unit of this study is 1 ton of aquafeed. The system boundary for the aquafeeds considers the collection and manufacturing of conventional and valorised feed ingredients, the transportation to the feed mill and to the final users.

Step 2. Life Cycle Inventory

The aquafeeds analysed are a conventional feed (control) and two feeds including 15% and 20% of brewers´ spent grains (BSG) and yeast (BY) meal, respectively. The data of feeds formulation and the valorisation process of brewery by-products was obtained from the demonstration trials performed during the LIFE BREWERY project (San Martin et al. 2020). The inventory data for the conventional feed ingredients has been obtained from the Ecoinvent 3.5 and Agrifootprint databases. SimaPro 9 software has been used for the assessment. It is important to highlight that the proposed aquafeed alternatives have been tested and validated with 2 aquaculture fish species: *Sparus aurata*, and *Oncorhynchus mykiss*, as models of a Mediterranean and freshwater specie, respectively (Nazzaro et al. 2021).

Step 3. Life Cycle Impact Assessment

Climate change, particulate matter, terrestrial and freshwater acidification, land use, eutrophication terrestrial and water scarcity categories are the most representative environmental impacts of the activity for the aquafeed production. In all the selected impact categories, a decrease is observed when comparing the control feed with the two alternatives. Specially, in land use (BSG:25% and BY:32%) and water scarcity (BSG:24% and BY:42%) due to the avoided animal and plant-based ingredients production. In contrast, a 6% reduction is shown in climate change in both new aquafeeds.

(Continued on next page)

Step 4. Interpretation

Environmental impact assessment determined that the proposed aquaculture feeds including brewers´ by-products appear as a sustainable alternative for reducing the impact linked to Mediterranean and freshwater aquaculture.

Conclusion

The LCA of aquafeeds have proved that replacing 15-20% of animal and plant-based ingredients with brewers´ by-products could reduce environmental impacts and obtain a more sustainable feed solution for fish aquaculture world.

Acknowledge

This project is co-funded by LIFE European Environment Programme (LIFE16 ENV/ES7000160), which is the EU's financial instrument supporting environmental, nature conservation and climate action projects throughout the EU.

References

A.G.J. Tacon, M. Metian. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and futures prospects. Aquaculture 285:146–158.

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

G. Turchini, W.K. Ng, D.R. Tocher. 2012. Fish oil replacement and alternative lipid sources in Aquaculture feeds. CRC Press, Taylor & Francis Group, 551 pp.

D. San Martin, M. Orive, B. Iñarra, J. Castelo, A. Estévez, J. Nazzaro, I. Iloro, F. Elortza, J. Zufía. 2020. Brewers' spent yeast and grain protein hydrolysates as second- generation feedstuff for aquaculture feed. Waste Biomass Valoriz. https://doi.org/101007/s12649-020-01145-8

J. Nazzaro, D. San Martin, A.M. Perez-Vendrell, L. Padrell, B. Iñarra, M. Orive, A. Estévez. 2021. Apparent digestibility coefficients of brewer's by-products used in feeds for rainbow trout (Oncorhynchus mykiss) and gilthead seabream (Sparus aurata). Aquaculture 530: 735796

M. Cidad, S. Ramos. 2020. Environmental footprint of seabass production in the Mediterranean Sea. Oral in Aquaculture Europe 2020 conference, Virtual.

EVALUATION OF NEW SUSTAINABLE DIETS FOR THE SEA URCHIN *Paracentrotus lividus* IN A RECIRCULATING AQUACULTURE SYSTEM

L. Ciriminna[1*], A. Rakaj[2], L. Grosso[2], D. Pensa[2], A. Fianchini[2], M. Scardi[2,3], A. Mazzola[1,3], S. Vizzini[1,3]

[1]Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università degli Studi di Palermo, Via Archirafi 18, 90123 Palermo, Italia
Email: laura.ciriminna@unipa.it
[2]Laboratorio di Ecologia Sperimentale ed Acquacoltura-LESA, Dipartimento di Biologia, Università di Roma "Tor Vergata" via Cracovia 1, Roma, Italia
[3]Consorzio Nazionale Interuniversitario per le Scienze del Mare, CoNISMa, P.le Flaminio 9, 00196 Roma, Italia

Introduction

The extensive use of fishmeal and fish oil-based feeds in aquaculture contrasts strongly with the blue growth principles of sustainability and protection of biodiversity in marine ecosystems (Eikeset et al., 2018). In recent years, increasing attention has been focused on the formulation of alternative feeds produced using food waste and discards (Kang et al., 2010; Vizzini et al., 2019). The objective of this study is to test the exploitation of discarded leaves of the lettuce *Lactuca sativa* as the main ingredient of alternative and sustainable diets for *Paracentrotus lividus,* the most exploited Mediterranean sea urchin.

Material and Methods

Five diets were tested, four were made mainly of industrial discards of *L. sativa* (86%) and to a much lesser extent (10%) of one of four seafood meals (fish, krill, mussels, and anchovy industrial discards). The fifth was a control diet composed of 48% of both *Ulva lactuca* and *Laminaria laminaria*. A binder composed the remnant 4% of each diet, while a source of $CaCO_3$, *Lithothamnium calcareum*, was added. 110 adults of *P. lividus* were collected at Santa Marinella, Italy (42°03'00"N, 11°49'09"E), and transported to the LESA lab. of University of Rome "Tor Vergata". From the initial pool, 15 specimens, randomly chosen, were sacrificed to assess the gonad index (GI) baseline, while other 75 specimens were pit tagged and acclimatized in a 600 L closed circulation aquarium. After one month-starvation, sea urchins were measured (test diameter: 47.58±4.26 mm; total wet weight-TW: 48.89±15.07 g) and randomly allocated into 15 tanks (3 for each diet) of a recirculating aquaculture system. Sea urchins were fed *ad libitum* six days a week, for five months. To evaluate the effectiveness of the experimental diets, we measured: ingestion rate (IR) and absorption efficiency (AE) on a daily basis for three weeks; TW before the start of the experiment (T0), after 10 weeks, (T1) and at the end of the experiment (T2); GI before (T0) and at the end of the experiment (T2).

Results

Sea urchins showed similar IR and different AE, which peaked for the control diet (Fig. 1a). TW showed a significant increase during the experiment, without differences between diets (Fig. 1b). A significant increase of GI was also observed at the end of the experiment with respect to the initial value (3.64±2.22%) for all the experimental diets. The algal diet led to the lowest GI (12.82±8.87%) followed by the fish diet (16.56±11.15%), while krill, mussels and the anchovy discard diets gave higher and similar results (21.09±10.59, 20.69±10.34, 19.73±11.04% respectively).

Discussion and Conclusion

All the experimental diets showed low ingestion rates, suggesting appropriate nutritional quality (Boudouresque and Verlaque, 2020) and the lack of large among-diet differences indicates that all diets were qualitatively comparable despite the different origin of the ingredients. By contrast, the different composition of the sustainable diets does seem to influence absorption efficiency, since the control diet proved to be the most efficient, while the new formulated diets composed mainly of lettuce processing discards showed lower values. Although sea urchins are herbivorous, it is possible that terrestrial vegetables, rich in fibres and insoluble carbohydrates (Plazzotta et al., 2017)[fruit and vegetable waste (FVW], are less effective as a food source than macroalgae. In addition, the inclusion of *L. calcareum* as a source of inorganic carbon and other minerals may have contributed to the low efficacy of the diets containing mussels and anchovy discards that are already characterized by a higher inorganic content due to the presence of shells and bones in the seafood meal. However, the increase in somatic and gonadic growth, confirmed the suitability of all the experimental diets. Gonad index values recorded for the sustainable diets were higher than for the algae control diet, confirming the greater effectiveness of diets containing a mix of vegetables and seafood – even with only low amounts of seafood meal - over those of entirely vegetal origin (Fernandez and Boudouresque, 2000; Grosso et al., 2021). In addition, values were comparable or higher than those obtained in other studies using formulated diets based on terrestrial vegetables (Sartori et al., 2014; Vizzini et al., 2019).

(Continued on next page)

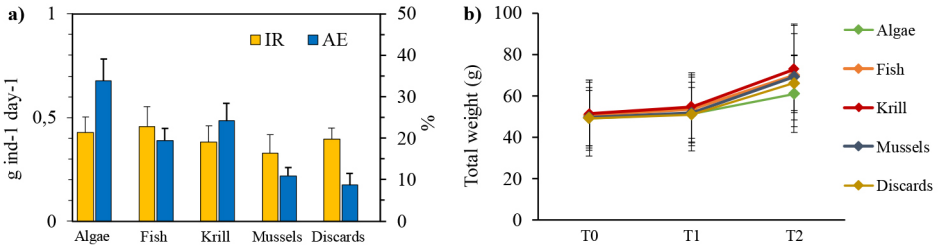


Figure1. Ingestion rate (IR), absorption efficiency (AE) **(a),** and total wet weight **(b)** of *P. lividus* for the five experimental diets, and across time (T0, T1, T2).

All the experimental diets performed well, proving to be adequate as food sources and promoting both somatic and gonadic growth. However, the krill diet provided the best performance, coupling low ingestion rate and high absorption efficiency, as well as recording the highest value for both somatic and gonadic growth.

References

Boudouresque, C.F., Verlaque, M., 2020. *Paracentrotus lividus*, in: Lawrence, J.M. (Ed.), Developments in Aquaculture and Fisheries Science. Elsevier B.V, pp. 447–485.

Eikeset, A.M., Mazzarella, A.B., Davíðsdóttir, B., Klinger, D.H., Levin, S.A., Rovenskaya, E., Stenseth, N.C., 2018. What is blue growth? The semantics of "Sustainable Development" of marine environments. Mar. Policy 87, 177–179.

Fernandez, C., Boudouresque, C., 2000. Nutrition of the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different artificial food. Mar. Ecol. 204, 131–141.

Grosso, L., Rakaj, A., Fianchini, A., Morroni, L., Cataudella, S., Scardi, M., 2021. Integrated Multi-Trophic Aquaculture (IMTA) system combining the sea urchin *Paracentrotus lividus*, as primary species, and the sea cucumber *Holothuria tubulosa* as extractive species. Aquaculture 534, 736268.

Kang, H.Y., Yang, P.Y., Dominy, W.G., Lee, C.S., 2010. Bioprocessing papaya processing waste for potential aquaculture feed supplement - Economic and nutrient analysis with shrimp feeding trial. Bioresour. Technol. 101, 7973–7979.

Plazzotta, S., Manzocco, L., Nicoli, M.C., 2017. Fruit and vegetable waste management and the challenge of fresh-cut salad. Trends Food Sci. Technol. 63, 51–59.

Sartori, D., Scuderi, A., Sansone, G., Gaion, A., 2014. Echinoculture: the rearing of *Paracentrotus lividus* This in a recirculating aquaculture system—experiments of artificial diets for the maintenance of sexual maturation. Aquac. Int. 23, 111–125.

Vizzini, S., Visconti, G., Signa, G., Romano, S., Mazzola, A., 2019. A new sustainable formulated feed based on discards from food industries for rearing the sea urchin *Paracentrotus lividus* (Lmk). Aquac. Nutr. 25, 691–701.

FISH INDIVIDUAL IDENTIFICATION – CAN WE SUBSTITUTE INVASIVE TAGGING?

Petr Cisar*, Dinara Bekkozhayeva, Oleksandr Movchan

Laboratory of Signal and Image Processing, Institute of Complex Systems, Faculty of Fisheries and Protection of Waters, CENAKVA, University of South Bohemia in Ceske Budejovice, Zámek 136, Nové Hrady 373 33, Czech Republic
Email: cisar@frov.jcu.cz

Introduction

The optimization of the fish production in intensive aquaculture can lead to the increase of the production with the respect to the fish welfare. The new technologies enable to develop the Precision Fish Farming (PFF) (Fore et al. 2017) concept whose aim is to apply control-engineering principles to fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms. Individual fish identification is one of the tasks necessary for precision fish farming.

There are many methods of individual fish identification. The widespread and popular methods of fish identification are tagging and marking (PIT, RFID or VIE tags). Most of the commonly used methods are invasive and can have adverse effects on fishes, increasing the risk of sequelae or mortality, mainly for small and sensitive fish as most of the stream fish species.

Non-invasive fish identification based on the fish appearance is cheap, less stressful for the fish and accurate. Human based identification of individual fish of the same species using the skin pattern was proved by Hirsch (Hirsch 2015). There are several different patterns which can be observed on the fish body (eye pattern, scale pattern, body texture). Even the image of the fish without any obvious pattern can be used for identification. The aim of this paper is to summarize the usability of image-based individual fish identification from the point of view of identification accuracy and pattern stability for long term identification.

Materials and methods

Five image-based individual identification studies were done for five fish species to prove the possibility of automatic computer vision fish identification.

Brook trout - 32 individuals were used for identification. Three data collections of fish out of water using the digital camera were done in 3 weeks. One image of each fish was taken for each session. The fish was automatically detected and the region of interest (ROI), containing unique dot pattern, was determined, Fig.1-A. The texture descriptor histogram of oriented gradients (HOG) was used to create the feature vector for identification. The identification was performed for all combinations of the data collection sessions.

Atlantic salmon – 328 fish individuals were used for identification. Four data collections of the fish out of water and under water were done in 6 months. Eight images per fish were taken for each individual in each session. For the first data collection all 328 individuals were used. Selected 30 fish were tagged and used for next 3 sessions. The HOG descriptor and dots position on the body were used as features for identification, Fig.1-B. The identification was done for all 328 fish for first session and for 30 fish for all sessions. Fish eye pattern was also used for the identification.

Sumatra barb – 25 fish individuals were used for identification. Two data collections were done in 2 weeks. Ten images of the fish under water were collected in each session. The fish was automatically detected in the image and the middle part of the body with two stripes was used as ROI, Fig.1-C. The identification was tested between data collections and within data collections.

Sea bass - 300 fish individuals were used for identification. Two data collections of the fish out of water were done in 2 months. Ten images per fish were taken for each individual in each session. In the first data collection all 300 individuals were used. Selected 32 fish were tagged and used for second sessions. The HOG descriptor was used to generate the features for identification, Fig.1-D. The identification was done for all 300 fish for first session and 32 fish for both sessions.

(Continued on next page)

Common carp 32 fish individuals were used for identification. Four data collections of the fish out of water were done in 4 months. Ten images per fish were taken for each individual in each session. All 32 fish were tagged and used for identification. The HOG descriptor was used to generate the features for identification, Fig.1-E.

Results

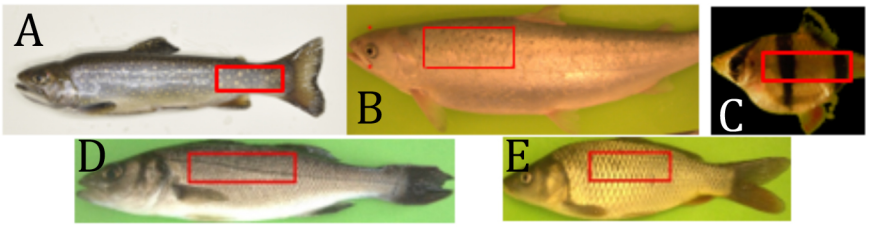


Figure 1. Region of interest used for different species identification: A – Brook trout, B – Atlantic salmon, C - Sumatra barb, D – Sea bass, E – common carp

Table 1. Identification accuracy for five species. ST – short term experiment.

	individual identification accuracy %											
	Brook trout		Atlantic salmon - eye		Atlantic Salmon - body		Sumatra barb		Sea bass		Common Carp	
	ST (32 fish)	3 weeks (32 fish)	ST (328 fish)	6 months (30 fish)	ST (328 fish)	6 months (30 fish)	ST (25 fish)	2 weeks (25 fish)	ST (330 fish)	2 months (30 fish)	ST (32 fish)	4 months (32 fish)
under water	---	---	---	---	100	100	100	100	---	---	---	---
out of water	100	100	95	40	100	100	---	---	100	100	100	90

Conclusion

Individual fish identification is one of the keystones of the emerging concept of Precision Fish Farming. The series of five studies demonstrated that the image based (fish appearance) individual identification using computer vision can be used for long-term identification. The identification was tested on three species with obvious patterns and two species with ambiguous patterns. High accuracy of the identification was reached for all species together with the long-term pattern stability. Image-based identification is non-invasive and remote approach which can substitute invasive fish identification methods.

Acknowledgement: European Union's Horizon 2020 research and innovation program under grant agreement No. 652831" (Aquauexcel2020)

Reference:

Fore, M., Frank K., Norton T., Svendsen E., Alfredsen J. A., Dempster T., Eguiraun H., Watson W., Stahl A., Sunde L.M., Schellewald C., Skøien K. R., Alver M. O., Berckmans D. 2017. Precision fish farming: A new framework to improve production in aquaculture, Biosystems Engineering, https://doi.org/10.1016/j.biosystemseng.2017.10.014

Bekkozhayeva, D., Saberioon, M. & Cisar, P. Automatic individual non-invasive photo-identification of fish (Sumatra barb *Puntigrus tetrazona*) using visible patterns on a body. *Aquacult Int* (2021).

Schraml, Rudolf & Hofbauer, Heinz & Jalilian, Ehsaneddin & Bekkozhayeva, Dinara & Saberioon, Mehdi & Cisar, Petr & Uhl, Andreas. (2020). Towards fish individuality-based aquaculture. *IEEE Transactions on Industrial Informatics*.

MARINE-DERIVED POLYSACCHARIDES FROM ADRIATIC SEA: INVESTIGATION OF DIVERSE BIOLOGICAL ACTIVITIES

L. Čižmek[a,b,*], S. Babić[a,b], K. Van Halewyck[c], K. Bojanić[a,b], M. Jurin[a,b], A. Dobrinčić[d], M. Bujak[a,b], M. Galić Perečinec[a,b], M. Roje[a,b], V. Dragović-Uzelac[d] and R. Čož-Rakovac[a,b]

[a]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
E-mail:lcizmek@irb.hr
[b]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[c]Faculty of Bioscience Engineering, University of Gent, Onderbergen 1, Belgium
[d]Faculty of Food Technology & Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia

Introduction

Among a large number of marine extracts and different isolated bioactive compounds, polysaccharides are recognized as one of the most promising sources of different biological activity (Shi et al, 2017; Gopu & Selvam, 2020). Their structure and biological activity are highly influenced by growth location and harvesting season that cause even intra-species variation, thus offering an inexhaustible source of novel bioactive compounds (Jiao et al., 2011) with unique physiological and chemical properties, including anti-inflammatory, antioxidant, antimicrobial, antiviral, anti-coagulant, and antitumor activities (Lee et al, 2017). Most recently, sulfated polysaccharides (fucoidans) extracted from the brown algae *Saccharina japonica* were able to effectively inhibit SARS-CoV-2 *in vitro* (Kwon et al, 2020). Nevertheless, marine-derived polysaccharides are still under-exploited, thus paving the way for a new trend in the discovery of compounds from a sustainable source. This study aimed to conduct comprehensive research and determine biological activities (antioxidant, antibacterial, and antifungal) of water-soluble polysaccharide extracts from 10 marine species from the Croatian coast of the Adriatic Sea: 2 green algae (*Ulva lactuca* and *Codium bursa*), 5 brown algae (*Fucus virsoides, Padina pavonica, Cystoseira barbata, Halopteris scoparia*, and *Cystoseira compressa*), 1 coral (*Eunicella cavolini*), 1 sea squirt (*Aplidium conicum*) and 1 sponge (*Chondrosia reniformis*), along with embryotoxicity and mutagenic potential to determine their safety for non-target organisms and humans.

Materials and methods

Ten marine organisms were collected from the coastal area of Croatia. Extraction of polysaccharides was conducted in acidic conditions (H_2SO_4) under high temperature (80°C). Dry residues of polysaccharide extracts were dissolved in a distilled water. Chemical characterization was performed using high-performance liquid chromatography (HPLC). Antioxidant activity was evaluated by implementing four *in vitro* assays - oxygen radical absorbance capacity (ORAC), reduction of radical cation ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay, and ferric reducing antioxidant power assay (FRAP). The mutagenic potential was evaluated by AMES test following ISO 16240 (2005) while anti-microbial activity was analyzed by the implementation of disk diffusion and microdilution methods on 4 bacterial strains and 1 yeast strain. The toxicity of samples was determined using the zebrafish embryotoxicity test (OECD 236, 2013).

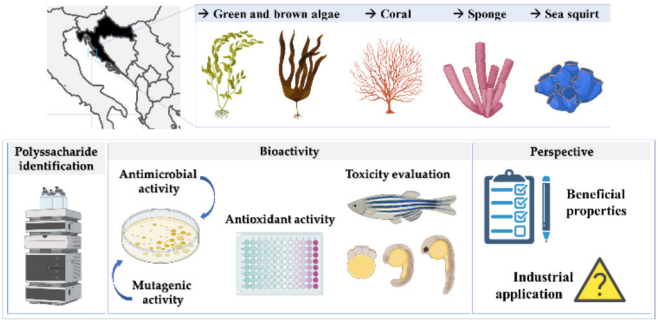


Fig. 1. Schematic overview of the experimental design used in this research.

(Continued on next page)

Results

The highest extraction yields were obtained for *A. conicum* (21.98%) and *U. lactuca* (18.89%). Chemical analysis revealed a high abundance of different polysaccharides within each sample. No clear halos were observed in the disk diffusion assay, but *C. reniformis* (42.0 ± 1.3 %), *C. barbata* (45.7 ± 12.4 %), and *F. virsoides* (37.7 ± 4.6 %) were able to inhibit the growth of *B. subtilis*, while *C. reniformis* (34.0 ± 1.2 %) and *C. barbata* (24.0 ± 3.3 %) showed antifungal activity against *C. albicans*. The highest antioxidant activity was obtained for *C. barbata* and *C. compressa* extracts using DPPH, ABTS, and FRAP assays, while ORAC assay revealed the highest activity for *C. reniformis* and *H. scoparia* extracts. Tested polysaccharide extracts showed no mutagenic or embryotoxic potential, with an exception for *C. reniformis* extract.

Discussion and conclusion

The optimization of the polysaccharide extraction is vital for obtaining high extraction yields with no interfering compounds. Pretreatment with organic solvents for the removal of polyphenols and pigments was conducted and extraction in acidic conditions followed by precipitation was performed. All samples showed a relatively high extraction yield, from 5.66 to 21.98 %. The chemical analysis supported these findings with specific chromatograms without interfering peaks. Different organisms have shown to have diverse polysaccharide compositions which contributed to various biological activities. Although antimicrobial activity was not present for most of the tested extract, some activity was obtained and was positively correlated with the increased antioxidant activity. All samples had medium to high antioxidant activity, which shows the potential usage of these compounds in different industrial applications. However, this application is limited if the extracted compounds are toxic or harmful. Combined with the AMES mutagenic test, the obtained results of embryotoxic testing have proven the safety of almost all isolated polysaccharide extracts, except for *C. reniformis* extract, thus indicating the importance of marine-derived compounds in different areas of research and applications, from food supplements to higher pharmaceutical development.

References

Shi Q., Wang A., Lu Z., Qin C., Hu J., and J. Yin. 2017. Overview on the antiviral activities and mechanisms of marine polysaccharides from seaweeds. Carbohydrate Research 453–454: 1-9.

Gopu M., and K. Selvam. 2020. Polysaccharides from marine red algae *Amphiroa rigida* and their biomedical potential: An in-vitro study, Biocatalysis and Agricultural Biotechnology 29: 101769.

Jiao G., Yu G., Zhang J., and H. S. Ewart. 2011. Chemical structures and bioactivities of sulfated polysaccharides from marine algae, Marine Drugs 9: 196–233.

Lee Y.-E., Kim H., Seo C., Park T., Bin Lee K., Yoo S.-Y., Hong S.-C., Tae Kim J., and J. Lee. 2017. Marine polysaccharides: therapeutic efficacy and biomedical applications, Archives of Pharmacal Research 40: 1006–1020.

Kwon P. S., Oh H., Kwon S. J., Jin W., Zhang F., Fraser K., Hong J. J., Linhardt R. J., and J. S. Dordick. 2020. Sulfated polysaccharides effectively inhibit SARS-CoV-2 *in vitro*. Cell Discovery 6: 50.

International Organization for Standardization (ISO). (2005). ISO 16240:2005 - Water quality — Determination of the genotoxicity of water and wastewater — Salmonella/microsome test (Ames test). In *International Standard International Standard* (Vol. 2005).

OECD. (2013). Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, July, 1–22.

LATE HIGH TEMPERATURE PROMOTES FAST GROWTH AND FEMALE SEX DIFFERENTIATION IN EUROPEAN SEA BASS *Dicentrarchus labrax*, WHILE EARLY PHOTOPERIOD HAS NO EFFECT ON EITHER TRAIT

F. Clota*[1,2], B. Geffroy [1], A.Vergnet [1], M.O. Blanc [1], S. Lallement [1], F. Ruelle [1], A. Goikoetxea [1], M. Leitwein [1], F. Allal [1] and M. Vandeputte[1,2]

[1] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots France
[2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy en Josas, France
E-mail: frederic.clota@inrae.fr

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 higher weight than males. However, most farmed fish batches show a highly male-skewed sex-ratio. The sea bass has a complex system of sex determination, combining both genetic (polygenic) and environmental (temperature) influences. Low initial temperatures (<17°C) favor female sex determination (Piferrer et al., 2005). In contrast, a cold treatment prolonged until the late post-larval phase has been recently shown to promote male differentiation (Vandeputte et al., 2020). In other species with environment sex determination (ESD) like *Leurestes tenuis*, both temperature and photoperiod can affect the sex-ratio (Brown et al., 2014). In this experiment, we tested the effect on the sex-ratio of *D. labrax*, of two photoperiods (12L:12D and 10L:14D), applied during the larval phase, from 9 to 90 days post hatching (dph), at a temperature of 16°C, crossed with the effect of four temperatures (19; 21; 23 and 25°C) applied from 90 dph until the fish were about 80 mm length, a time where sex can be considered definitively fixed.

Material and methods

All experiments were conducted in the Experimental Marine Aquaculture platform of Ifremer at Palavas-les-Flots, France. Fish were from a complete factorial mating of 10 males and 8 females from a West Mediterranean strain. Fertilized eggs (23,000 / tank) were directly dispatched into six 500 L tanks. Photoperiod treatments (12L: 12D and 10L:14D) were done in triplicate, from 9 to 90 dph, with water temperature kept at 16°C. Temperature was gradually increased starting at 85 dph. Fish were transferred to another room at 87, 88, 89 and 90 dph, when temperature reached 19, 21, 23 and 25°C, respectively. The other room had four independent recirculating systems with four tanks of 110L each, stabilized at 19, 21, 23 or 25°C. Two tanks in each circuit received 240 fish from the 12D:12D photoperiodic treatment and the other two 240 fish from the 10L:14D treatment. The photoperiod applied until the end of the experiment was 12L:12D for all groups. Thermal treatment was stopped when fish reached a length about 8 cm (1500 day.degrees, calculated on a 10°C basis – $DD_{10°C}$), a size at which sex is completely determined. At this time fish were individually weighed, measured and tagged with RFID glass tags, and transferred to 1.5 m^3 tanks at 22°C. All fish from the same temperature treatment were pooled in the same tank, and at 242 dph, were individually measured and transferred to a 5 m^3 tank. Fish from the 19°C and 21°C treatments were pooled in a same 5 m^3 tank, and a second tank received fish from the 23 and 25°C treatments. They were grown at 23°C until sexing at 409 dph, where 1926 fish were measured and sexed. Data were analyzed using R software. Temperature, photoperiod, interactions and tank effect on sex-ratio were tested with a logit generalized linear mixed model fitted with lme4 package in R. Sex, temperature, photoperiod, interactions and tank effect on final weight were tested with a linear mixed model. Post-hoc differences between treatments were tested by multiple comparisons of means with Tukey adjustment.

Results and discussion

Photoperiod had no significant effect on sex-ratio ($p>0.45$), while temperature effect on sex-ratio was highly significant ($p< 0.0001$). At 19°C, the proportion of females was 30.4%; it was 36.5% at 21°C, 44.0% at 23°C and 49.0% at 25°C (see fig. 1). Compared to 19°C, odds-ratios were 1.31 for 21°C, 1.78 for 23°C and 2.17 for 25°C, meaning that at high temperature (23 and 25°C), the probability to be (or become) a female was twice that observed at 19°C. No interaction between temperature and photoperiod was found ($p>0.088$). Up to now, high temperatures during the sex determination period were known to promote male development, in sea bass but also in most fish species with TSD (Ospina-Alvarez and Piferrer, 2008). Here we showed that high temperatures applied to larvae after a cold (16°C) first period, can significantly increase the percentage of females in European sea bass. Early photoperiod had no effect on the final weight of fish ($p>0.33$), while early temperature treatments showed a long-term effect on weight later on, at 409 dph. At this age, the mean weight was 101±1.92g for the 19°C treatment; 107±1.98g for 21°C; 124±1.92g for 23°C and 119±2.13g for 25°C. Sex also had an effect on weight ($p<0.001$ males: 102±1.18g, females: 123±1.39g). No effect of interaction between temperature, sex or photoperiod, on the weight at 409 dph was found ($p>005$). Since the differences between 23 and 25°C treatment are not significant neither for sex-ratio nor for body weight, we propose a treatment at 23°C during the post larval stage (90-160 dph), following an initial 90 days at 16°C, to significantly increase both female ratio and final weight, with a moderate energy cost, in a West Mediterranean strain of sea bass.

(Continued on next page)

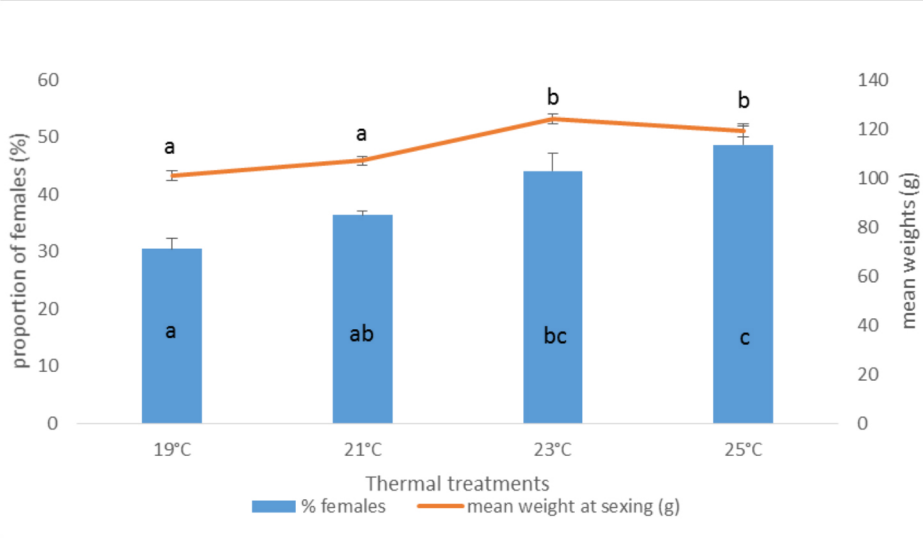


Fig. 1: Effect on the sex-ratio (proportion of females), and on the mean weight at sexing of *Dicentrarchus labrax* of late thermal treatments, from 87-90 dph to 1500 $DD_{10°C}$, after initial rearing at 16°C. Different letters indicate significant differences ($P<0.05$)

Acknowledgements

This study was supported by the French Ministry of Environment under grant CRECHE[2020]

References:

Brown E.E., Baumann H., Conover D.O. 2014. Temperature and photoperiod effect on sex determination in a fish. J. Exp.l Mar. Biol. Ecol. **461**: 39-43.

Ospina-Alvarez N. and Piferrer F. 2008. Temperature-dependent sex determination in fish revisited : prevalence, a single sex ratio response pattern, and possible effects of climate change. PLoS ONE **3**:e2837.

Piferrer, F., Blazquez M., Navarro L., Gonzalez A. 2005. Genetic, Endocrine and Environmental Components of Sex Determination and Differentiation in the European Sea Bass (*Dicentrarchus labrax* L.). Gen.Comp. Endocrinol. **142**:102-110.

Vandeputte et al. 2020. Low temperature has opposite effects on sex determination in a marine fish at the larval/post-larval and juvenile stages. Ecol. Evol.**10**:13825-13835.

MUSSEL MEAL AS A PROTEIN SOURCE IN DIETS OF GILTHEAD SEABREAM JUVENILES

R. Colen[1*], C. Aragão[1,2], L.T. Antelo[3], X.A. Álvarez-Salgado[3], S. Engrola[1]

[1] Centre of Marine Sciences (CCMAR), 8005-139 Faro, Portugal
[2] Universidade do Algarve, 8005-139 Faro, Portugal
[3] CSIC, Institute of Marine Research (IIM - CSIC), 36208 Vigo, Spain

[*]E-mail: rcolen@ualg.pt

Introduction

In 2020 world population was projected to increase from 7.8 billion to 9.9 billion by 2050 (http://worldpopulationreview.com). To overcome the stagnation of wild fisheries in supplying seafood for the growing population, Aquaculture will need to address several technical challenges to secure food demand (FAO, 2020). One of the challenges is to expand the number of sustainable raw materials to increase flexibility in the formulation of highly nutritious aquafeeds.

The Galicia Region (Spain) is the main producer of farmed mussels (Mytilus galloprovincialis) in Europe (FAO, 2020). Although the main production is channelled for human consumption, processing is generating side-streams. A new solution for mussel side-streams is the production of high-quality mussel meal. To combine a new resource originated from a low trophic species side-stream with feed production will contribute to the valorisation of aquaculture value-chains and to the circular economy approach in the sector.

The aim of the present study was to evaluate the effects of fishmeal replacement by mussel meal as the main source of marine-derived protein in dietary formulations for gilthead seabream (Sparus aurata) juveniles. The dietary effects were assessed through the analysis of key performance indicators, feed utilization, and biochemical parameters.

Material and Methods

Three practical diets were formulated to be isonitrogenous (crude protein, ~48.6%), and isoenergetic (gross energy, ~ 21MJ/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 proteins. Diets were manufactured at SPAROS Lda. (Olhão, Portugal).

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). Upon arrival, fish were adapted to new conditions for two weeks in a flow-through system with aeration, during which they were fed a commercial diet.

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 500L 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, for evaluation of several key performance indicators.

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 -20ºC 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.

(Continued on next page)

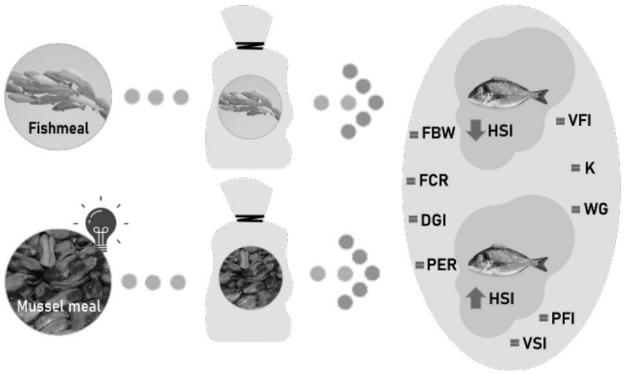


Figure 1. Growth performance and feed utilization indicators of seabream juveniles fed the experimental diets (Mussel 50 and 100) compared with fish fed the Commercial diet, at the end of the trial.

Results and Discussion

The partial or total replacement of fishmeal by mussel meal had no detrimental effect on key growth performance indicators (Figure 1). 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 ($P<0.05$).

More parameters, such as biochemical, immune, metabolic and antioxidant responses are under analysis. Based on growth performance and feed utilization data, the replacement of fishmeal by mussel meal might be a viable strategy to tackle the identified technical challenge.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 818173 and by the Portuguese Foundation for Science and Technology (Ministry of Science and Higher Education, Portugal) through project UIDB/04326/2020 to CCMAR and contract DL 57/2016/CP1361/CT0033 to CA. This abstract reflects the views only of the AquaVitae consortium, and the European Union cannot be held responsible for any use which may be made of the information it contains.

References

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

ECO-EFFICIENT FEEDS FOR EUROPEAN AQUACULTURE

L.E.C. Conceição[1], G. V. Pereira[1], A.M. Fernandes[1], J. Dias[1], C. Hoerterer[2], J. Petereit[2], B. Buck[2,3], G. Micallef[4], J.A. Calduch-Giner[5], F. Naya-Català[5], M.C. Piazzon[5], A. Sitjà-Bobadilla[5], J. Pérez-Sánchez[5], F. Faccenda4[6], M. Povinelli[6], R. Newton[7], B. Glencross[7], C. Kreiß[8], B. Costas[9], J.M.O. Fernandes[10], J. Johansen[11]

[1]SPAROS Lda. Olhão, Portugal
[2]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany
[3] University of Bremerhaven, Appl. Mar. Biol., Bremerhaven, Germany
[4]GIFAS, Inndyr, Norway
[5]Institute of Aquaculture Torre de la Sal (IATS-CSIC), Castellón, Spain
[6]Fondazione Edmund Mach, San Michele all'Adige, Italy
[7]University of Stirling, Scotland (UK)
[8]Thunen Institute, Bremerhaven, Germany
[9]CIIMAR, University of Porto, Porto, Portugal
[10]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
[11]Salten Havbrukspark AS, Nygårdsjøen, Norway

Introduction

The current drive for aquaculture sustainability has been pushing feed formulators for the design of novel aquafeeds, based on ingredients such as insect meals, by-products of fisheries and animal production, microbial biomasses, and algae-based products. All these are viable alternatives, often with inclusion levels restrictions, to those commonly used up to date, e.g. fish meal, fish oil, soy products. A challenge associated with this transition is ensuring that novel feeds provide all required nutrients, without affecting, and when possible improving farming performance. Effects on growth, health status, feed conversion, environmental impacts, and fish biochemical composition (e.g., n-3 HUFAs) from a consumer perspective, have all to be evaluated. However, these emerging ingredients have mostly been tested by researchers and on a one-by-one basis. Our approach was to test different formulation concepts using combinations of these ingredients, rather than one-by-one. Therefore, the aim was not to test individual emerging ingredients, but rather formulation scenarios that may be relevant in the near future. The GAIN project aims to promote eco-intensification of the European aquaculture, and therefore a more sustainable and efficient use of resources in aquafeeds, including circularity towards zero-waste is paramount. The GAIN aquafeed formulation concept starts in replacing cost-effectively fish meal and fish oil from feed-targeted fisheries, together with commodity ingredients from vegetables such as soy, wheat, and corn. Besides the obvious impact of fisheries, using plant protein and oil sources is an increasing concern, due to pressure on farmland resources, resulting deforestation, negative effects on global food security, EU reliance on imports of major crops, and carbon footprint of transportation of some of the raw materials across continents. The present work summarizes the GAIN project effort in testing several alternative formulation concepts in five farmed species: rainbow trout, Atlantic salmon, gilthead seabream, European seabass, and turbot.

Methods

A total of nine nutritional trials were performed using 4 main formulation concepts (see Fig. 1). These nutritional trials were performed in optimal rearing conditions for each species, at partner facilities (rainbow trout at FEM, Italy; Atlantic salmon at GIFAS, Norway; gilthead seabream at SPAROS, Portugal, and IATS-CSIC, Spain; European seabass and turbot at AWI, Germany). The feed formulations were developed for each of the target species, taking into their nutritional requirements and tolerance to different ingredients. Feeds were produced by extrusion with oils added by vacuum coating at the SPAROS pilot feed mill (Olhão, Portugal).

Results and Discussion

All feed formulations gave good growth performances and feed conversions for salmon, seabream, seabass, and turbot and very good ones for rainbow trout. GAIN formulations reached, in general, very similar values compared to the control feeds. However, some formulations performed slightly worse in terms of feed conversion: MIX and PAP in the case of seabream, and PAP in the case of salmon. Moreover, available results for the other key performance indicators assessed (e.g.: nutrient retention and digestibility; biomarker genes, enzymes, and metabolites; skin/gut histology; organoleptic evaluation) suggest that the novel formulations affect fish physiology, with fish likely driven towards a new allostatic balance, but no detrimental effects to fish health could be identified.

(Continued on next page)

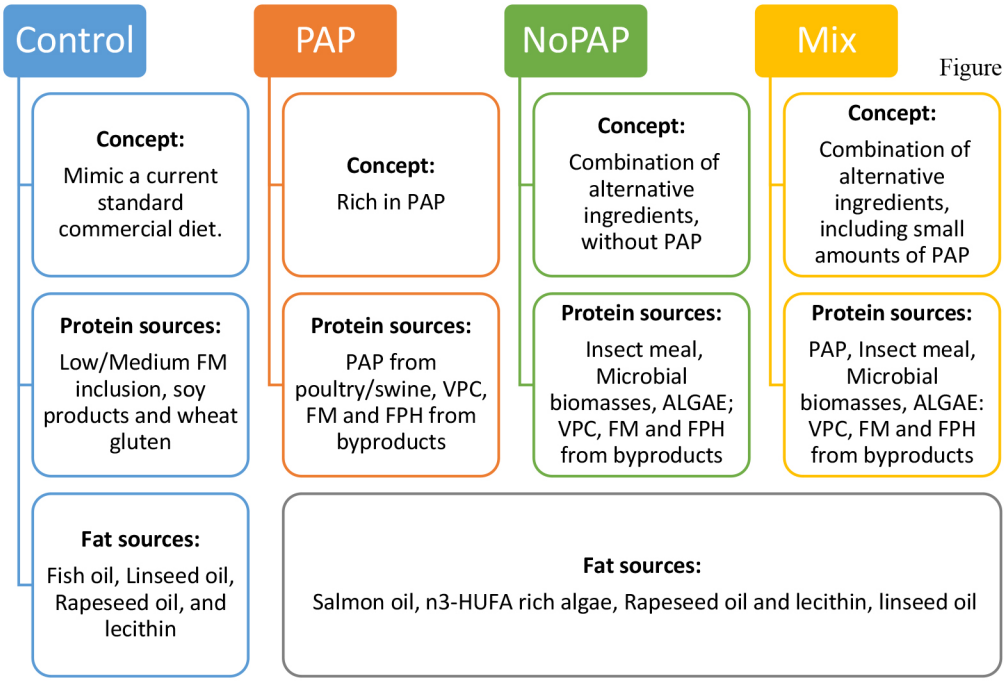


Figure 1: FM – fish meal; FO – fish oil; PAP – processed animal protein from farmed animals (e.g., poultry meal, feather meal, and blood meal); VCP – vegetable (e.g., pea, rapeseed) protein concentrates from European origin; FPH - fish protein hydrolysates from fisheries/ aquaculture byproducts (e.g., fish trimmings, heads, and frames); ALGAE: Microalgae and Macroalgae rich in Se and other minerals; Salmon oil - a by-product from the salmon farming industry.

Conclusions

Overall, these results suggest that the novel GAIN feed formulations tested are viable options shortly, if not immediately. In any case, the major farmed European species can be fed on more sustainable and resource-efficient aquafeeds, towards a zero-waste industry.

Acknowledgments:

This project was financed by the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773330 (GAIN).

EVALUATING COST-EFFECTIVENESS OF COMMERCIAL MICRODIETS FOR WHITELEG SHRIMP (*Penaeus vannamei*) POST-LARVAE

A. Barreto[1], W. Pinto[2], R.J.M. Rocha[1], L.E.C. Conceição[2*]

[1]RIASEARCH Unipessoal Lda, Murtosa (Portugal)
[2]SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão (Portugal)

*E-mail: luisconceicao@sparos.pt

Introduction

Whiteleg shrimp (*Penaeus vannamei*) is currently the second major species produced in aquaculture (FAO, 2020), representing in 2018 a share of 6 % of globally traded species. During the early developmental stages, shrimp larvae and post-larvae are intensively reared in hatcheries that originate around 5 million tonnes of adults per year. These are critical stages of shrimp development, with the transition from live-feeds to inert microdiets (weaning) being severely influenced by diet quality. When optimal nutritional standards are not met, sub-optimal growth, low survival, cannibalism, high size dispersion and reduced disease resistance are often verified. The latter assumes a high dependence of optimal zootechnical conditions and nutrition during weaning, as shrimp depend uniquely on their innate immune system to avoid pathogenic outbreaks and maintain a good health status. Hence, problems related to microdiet quality have a large impact on shrimp performance in the long-term, affecting the downstream production of high-quality juveniles. Although several commercial diets are currently available in the market, the room for optimizing a weaning diet for shrimp larvae/post-larvae is still large. For this purpose, this work aimed at evaluating the cost-benefit relationship of three microdiets (standard, premium and ultra-premium) for shrimp post-larval performance. Criteria such as growth, survival and economical conversion ratio were used to select the most adequate strategy to employ when selecting a microdiet for the early stages of shrimp development.

Materials and methods

Three commercial microdiets currently available in the European market for shrimp post-larvae were initially selected: A – a standard diet; B – a premium diet with a cost around 53% higher than diet A; and C – an ultra-premium diet with a cost around 144% higher than diet A. White leg shrimp post-larvae (PL13, mean wet weight 5 mg) were reared under standard procedures in triplicate tanks (0.28 m^2; density of 1076 post-larvae/m^2) at RIASEARCH Lda facilities (Portugal) and fed on these commercial microdiets *ad libitum* for 21 days. Water temperature throughout the experiment was kept at around 27 ºC. At the end of the trial, shrimp were counted and weighted to determine survival and growth performance. The amount of feed administrated to each tank was registered daily to allow the calculation of feed conversion ratio, whereas the commercial cost of each diet was taken into account to determine economical conversion ratio. These criteria were evaluated to determine the cost-benefit relationships of each diet.

Results

At the end of the experimental period, no significant differences were obtained for post-larval survival, with high results being achieved for all dietary treatments (A – 86.7 ± 6.4%; B - 74.9 ± 12.8%; C - 85.4 ± 4.5%). Shrimp post-larvae fed the ultra-premium diet achieved a significantly higher final body weight (Figure 1) and relative growth rate (C - 19.8 ± 0.1%/day) than those fed the remaining diets (A – 15.6 ± 0.5%/day; B – 17.1 ± 0.7%/day). Concomitantly, shrimp fed the standard diet achieved a significantly lower final body weight and relative growth rate than the premium diet. Nevertheless, no significant differences were found regarding ECR (Figure 1), even though diet B tended to have a higher value.

Discussion

The results obtained in this study show that the use of an ultra-premium quality diet resulted in a significant increase in post-larval weight after 21 days (+ 111 and + 62 % than the standard and premium, respectively). However, no significant differences were detected between treatments for survival and economical conversion ratio. Although the latter was not significantly different between dietary treatments, it can be inferred that higher quality diets can still be advantageous in this critical phase of production. The benefits may be related to an increase in the nutritional adequacy to shrimp dietary requirements and larval robustness/quality, which can assume a pivotal importance in shrimp production during disease outbreaks or stress periods (Racotta et al., 2003). Furthermore, it should be taken into account that higher growth may lead to savings in operational costs, as well as ultimately allowing to reduce the time shrimp take to reach commercial size and eventually allow to increase the number of yearly production cycles. Thereby, results from this study suggest that it would be advantageous to feed shrimp post-larvae with ultra-premium diets during the early developmental stages.

(Continued on next page)

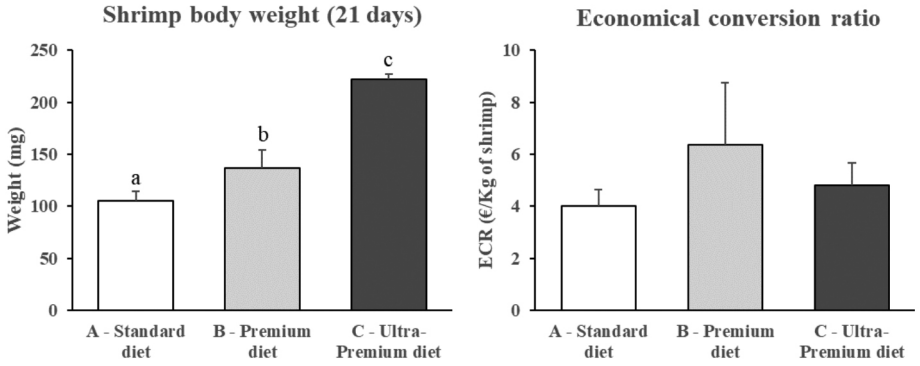


Figure 1. Shrimp final body weight at the end of the experiment and economical conversion ratio (€ spent in feed/Kg shrimp biomass gain) of the three different commercial microdiets. Results expressed as mean (n = 3 experimental units). Different superscript letters indicate statistical differences ($P<0.05$) between treatments in a One-way ANOVA.

References

FAO, 2020. The state of world fisheries and aquaculture - 2020. Sustainability in action, Rome, pp. 206.

Racotta, I.S., Palacios, E., Ibarra, A.M., 2003. Shrimp larval quality in relation to broodstock condition. Aquaculture 227, 107-130.

Acknowledgements

This work is part of the project FA_05_2017_005 SHELLWIN, financed by the Blue Fund program of the Ministry of the Sea, Portuguese Republic.

RECENT ADVANCES IN MARINE ANIMALS' TRANSPORT

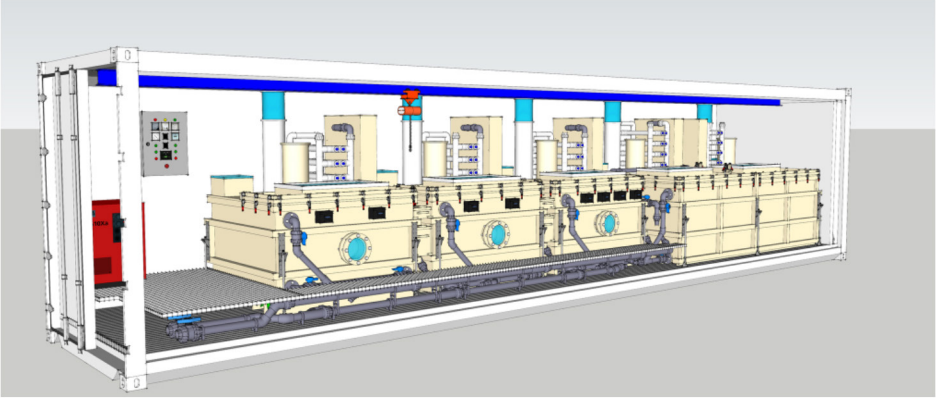
João Correia*, David Silva, Gonçalo Graça, Ivan Beltran, João Reis, Luís Silva, Pedro Marques, Rui Guedes, Telmo Morato

Flying Sharks Lda., Rua Farrobim do Sul 116, 9900-361 Horta, Portugal info@flyingsharks.eu

The opening of the Oceanário de Lisboa, where Flying Sharks' founding staff originated from, involved the collection and transport of animals from literally every corner of the planet to Lisbon, which was a monumental exercise in the development of long-term transport techniques. These techniques were then refined over two decades, allowing for the collection and transport of species once considered 'impossible', such as *Scomber* sp., *Sarda sarda*, *Mola mola*, *Naucrates ductor*, and an assortment of jellyfish, among multiple others. Such advancements include the replacement of 12 V systems for 220 V, while ammonia and pH are no longer a concern, thanks to recent developments in quenching and buffering agents. Additionally, a new paradigm in marine animal transport is presented, whereas buffering agents are used preventively and not correctively, while life support systems designed for long-term maintenance and not just transport conditions. This turned our 'transport unit' into a 'mobile holding station'.

This mobile station 's objective is to allow for long-term maintenance of live marine animals and consists of a 40 foot shipping container, which allows for movement by sea or road. Some of the more relevant technical aspects include:

- Operational volume of 17 m^3;
- Temperature controlled environment;
- Remote monitoring of water quality parameters, such as oxygen, pH, temperature and ammonia
- Remote monitoring through video of systems and animals as well;
- Multiple power inputs, such as 12V, 24V, 110V, 240V, and 380V, to allow for easy adaptation to different geographical;
- Amongst many other aspects which will be presented.



MICROALGAE AS NUTRITIONAL FEED INGREDIENTS

Joana Silva*, Inês Guerra, Nádia Correia, Pedro Quelhas, Helena Cardoso, Margarida Costa

ALLMICROALGAE Natural Products S.A., R&D department, Rua 25 de Abril s/n, 2445-413 Pataias, Portugal
Email: joana.g.silva@allmicroalgae.com

Introduction

The need to substitute classical aquafeed ingredients is becoming a continuous concern due to their sustainability issues. Higher plants face problems related to low digestibility and an unbalanced essential amino acid profile. On the other hand, the diverse group of microalgae has a biochemical profile that suits the nutritional needs of the aquatic animals. Microalgae can have several other industrial applications that include food, cosmetics, biofertilizers, among others. These organism's high digestibility, protein and lipid content, and other essential nutrients make them key ingredients for feed formulations. Besides, microalgae can also produce bioactive metabolites, such as immunostimulants, or antioxidants, which may have a role in fish health performance.

Material and methods

Different five different microalgae were cultivated during the present study, namely, *Chorella vulgaris*, *Nannochloropsis oceanica*, *Chlorococcum amblystomatis*, *Phaeodactylum tricornutum* and *Tetraselmis chui*. All microalgae were grown autotrophically in outdoor pilot photobioreactors with 2.6 m^3, at Allmicroalgae facilities. After cultivation, the cells were collected by centrifugation and further spray-dried for biochemical analysis.

CHN composition was determined using a Vario el III (Vario EL, Element Analyser System, GmbH, Hanau, Germany), according to the procedure provided by the manufacturer. The total protein was calculated by multiplying the percentage of nitrogen by the 6.25 factor (Richmond and Hu, 2004). The total ash was determined by gravimetric analysis. The total lipid content was determined following the Bligh and Dyer method (1959). The carbohydrate content was determined by subtracting the weight of proteins, lipids and ashes from the total DW of biomass.

Results

Among the studied microalgae, *C. vulgaris* was the one presenting higher productivity in 2.6m^3 tubular photobioreactors, reaching 0.261 g/L/day, followed by *T. chui*, with 0,222 g/L/day. *P. tricornutum* and *N. oceanica* grew at 0.124 and 0.104 g/L/day, respectively. *C. amblystomatis* presented the lowest productivity rate, growing at 0.090 g/L/day.

C. amblystomatis presented the higher protein content (55.7%), followed by *C. vulgaris* (54.0%). *N. oceanica* was the species presenting the lowest protein content, 29.6%. *P. tricornutum* appeared to be a protein-rich microalga (51.2%), but as well a lipid-rich microalga (29.5%). This alga was the one presenting the higher lipid content, followed by *N. oceanica*, *C. vulgaris* e *C. amblystomatis*, with 22.0%, 20.0%, and 18.3%, respectivelly. *T. chui* was the microalga with the lowest lipid content, 6.5%. Regarding carbohydrates, the concentrations ranged from 16.6%, in *C. vulgaris* to 37.8%, in *N. oceanica*.

Ash concentrations were low in all tested microalgae, being *P. tricornutum* and *T.chui* the species with the highest content, 14.0%.

Discussion and conclusions

Microalgae cultivated at Allmicroalgae facilities meet the nutritional needs for a good fish meal and fish oil substitute. Fish feed is characterized by its high protein content, which has a crucial role in fish muscle tissues (Delgado and Reyes-Jaquez, 2017). Among the studied microalgae, *C. amblystomatis*, *C. vulgaris, P. tricornutum* showed to contain protein levels above 50%, being within the range of the one found in the classical ingredient (Cho and Kim, 2010). Except for *N. oceanica*, the carbohydrate content of the studied microalgae was below 30%, representing an advantage, considering the fish's poor capacity to digest carbohydrates (Delgado and Reyes-Jaquez, 2017).

The high lipid content found in the studied microalgae, namely *P. tricornutum*, *N. oceanica* and *C. vulgaris*, reinforces the potential of these organisms to be used in the substitution of fish oils. Besides, low levels of ashes were found in all tested algae.

This study clearly shows the potential of using microalgae as fish oil and fish meal substitutes. As demonstrated, microalgae can grow at high productivities and do not need arable land, not competing with other feedstocks. Further complementary studies are needed to access the digestibility of the microalgae, their bioactivities, as well as *in vivo* feed experimentation.

(Continued on next page)

References

Bligh, E. and Dyer, W. 1959. A rapid method of total lipid extraction and purification. The Canadian Journal of Biochemistry and Physiology, 37(8): 911–917.

Cho, J and Kim, I. 2010. Fish meal – nutritive value. Journal of Animal Physiology and Animal Nutrition, 95(6): 685-692.

Delgado, E. and Reyes-Jaquez, D. 2017. Extruded Aquaculture Feed: A Review, Extrusion of Metals, Polymers and Food Products, Sayyad Zahid Qamar, IntechOpen.

Richmond, A. and Hu, Q. 2004. Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition. Blackwell Publishing.

OOGENESIS IN WILD AND REARED GREATER AMBERJACK *Seriola dumerili* (RISSO, 1810)

A. Corriero[1*], C. Pousis[1], R. Zupa[1], C. De Virgilio[2] and C. C. Mylonas[3]

[1]Department of Emergency and Organ Transplantation, Section of Veterinary Clinics and Animal Production, University of Bari Aldo Moro, 70010, Valenzano, Bari (Italy)
3Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, 70125, Bari (Italy)
[3]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, 71003, Heraklion, Crete (Greece)
E-mail: aldo.corriero@uniba.it

Introduction

The incorporation of new species in the aquaculture industry necessitates to control the reproductive function in captivity and to produce high numbers of high-quality eggs. Greater amberjack *Seriola dumerili* (Risso, 1810) caught from the wild and reared in captivity have been shown not to develop further than early vitellogenesis or if they did complete vitellogenesis, they failed to undergo oocyte maturation and required exogenous hormonal therapies to induce ovulation and spawning (Mylonas et al., 2004).The present work represents an overview of the results obtained in a study on the oogenesis of wild and captive-reared greater amberjack carried out within the EU FP7 project Diversify (Zupa et al, 2017; Pousis et al., 2018, 2019).

Material and Methods

Twenty-one wild and twelve captive-reared greater amberjack females were sampled during 2014, 2015 and 2016 at three different phases of the reproductive cycle: early gametogenesis (EARLY), late April-early May (wild fish = 5; captive-reared fish = 4); advanced gametogenesis (ADVANCED), late May-early June (wild fish = 4; captive-reared fish = 4); spawning (SPAWNING), late June-early July (wild fish = 12; captive-reared fish = 4). Wild fish were sampled on board a professional purse-seine fishing vessel operating around the Pelagie Islands (Sicily, Italy); captive-reared individuals belonged to a broodstock captured as juveniles and moved to a sea cage of Argosaronikos Fishfarming S.A. (Salamina Island, Greece). For each fish, biometric data (fork length, FL, in cm; body mass, BM, in kg; testis mass, TM, in g) were registered and gonadosomatic index (GSI = 100 × TM/BM) was calculated. Liver samples were store at -80°C and subsequently used for the analysis of vitellogenin (*vtga*, *vtgb* and *vtgc*) expression through RT-PCR. Ovary samples were used for histological analysis and for vitellogenin receptor (*vtgr* and *lrp13*) expression analysis through RT-PCR. Blood samples were centrifuged and plasma was stored at -20°C for the analysis of testosterone, 17β-estradiol and 17,20β-dihydroxypren-4-en-3-one by ELISA assays.

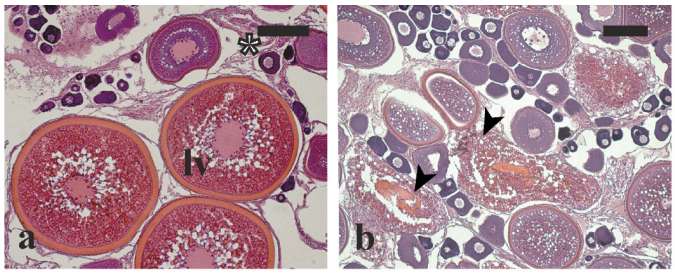


Fig. 1. Micrographs from greater amberjack ovaries sampled during the ADVANCED phase of the reproductive season. (a) Wild fish showing late vitellogenic oocytes and post-ovulatory follicles. (b) Captive-reared fish showing extensive atresia of late vitellogenenic follicles. Haematoxylin-eosin staining. Magnification bars = 150 μm. Arrowheads: atretic late vitellogenic follicles; asterisk: post-ovulatory follicle; lv: late vitellogenic follicle.

(Continued on next page)

Results and Discussion

The GSI and all the sex steroid plasma levels were lower in captive-reared fish. During the EARLY phase, wild and captive-reared fish displayed perinucleolar or early vitellogenesis as the most advanced oocyte stage. During the ADVANCED phase, when the wild greater amberjack breeders were already in spawning condition (Fig. 1a), ovaries of captive-reared breeders showed extensive atresia of late vitellogenic oocytes (Fig. 1b). During the SPAWNING period, all captive-reared fish had regressed ovaries, while wild breeders still displayed oocytes at late vitellogenesis and maturation stages as well as postovulatory follicles.

The expression levels of *vtga*, *vtgb* and *vtgc* did not differ significantly between captive-reared and wild females. Ovarian *vtgr* and *lrp13* transcription was more active during early gametogenesis, suggesting that vitellogenin receptor transcripts were synthesized by previtellogenic oocytes and remained in the cellular mRNA pool until oocytes resumed meiosis and entered vitellogenesis. A reduced *vtgr* and *lrp13* transcription was observed in captive-reared compared wild greater amberjack during the EARLY phase. The observed reproductive dysfunction, leading to oocyte atresia and reduced gonadosomatic index, arose during the early phase of oogenesis, when transcription of vitellogenin receptor genes appeared to be reduced, and did not appear to be associated to a lower liver capacity to synthesize the egg yolk precursors. Severe reproductive dysfunctions were observed also in males of the same broodstock and involved low sex steroid plasma concentrations and precocious cessation of spermatogenesis (Zupa et al., 2017). Preliminary data obtained within the H2020 project NewTechAqua indicate that hatchery-produced greater amberjack reared in sea cages in Salamina (Greece) have similar GSI compared with wild fish sampled in the same period of the reproductive cycle (early June 2021). Although further analyses are required, the available data indicate that hatchery-produced individuals might be less affected by captivity-induced stress than wild-caught breeders.

Financial grant provided by the European Union´s Programmes FP7 (GA 603121, DIVERSIFY) and H2020 (GA 862658, NewTechAqua).

References

Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P. 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture, 237: 141-154.

Pousis, C., Mylonas, C. C., De Virgilio, C., Gadaleta, G., Santamaria, N., Passantino, L., Zupa, R., Papadaki, M., Fakriadis, I., Ferreri, R., Corriero A. 2018. The observed oogenesis impairment in greater amberjack *Seriola dumerili* (Risso, 1810) reared in captivity is not related to an insufficient liver transcription or oocyte uptake of vitellogenin. Aquaculture Research, 49: 243-252.

Pousis, C., Rodríguez, C., De Ruvo, P., De Virgilio, C., Pérez, J.A., Mylonas, C. C., Zupa, R., Passantino, L., Santamaria, N., Valentini, L., Corriero A. 2019. Vitellogenin receptor and fatty acid profiles of individual lipid 1 classes of oocytes from wild and captive-reared greater amberjack (*Seriola dumerili*) during the reproductive cycle. Theriogenology, 140: 73-83.

Zupa, R., Rodríguez, C., Mylonas, C. C., Rosenfeld, H., Fakriadis, I., Papadaki, M., Pérez, J. A., Pousis, C., Basilone, G., Corriero, A. 2017a. Comparative study of reproductive development in wild and captive-reared greater amberjack *Seriola dumerili* (Risso,1810). PLoS ONE 12(1): e0169645.

EVIDENCE OF MULTIPLE GENOME DUPLICATION EVENTS IN *MYTILUS* EVOLUTION

A. Corrochano-Fraile, A. Davie, S. Carboni, M Bekaert

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
E-mail: ana.corrochano-fraile@stir.ac.uk

Introduction

The marine mussel *Mytilus* is among the foremost cosmopolitan marine genera and is present in estuarine and oceanic habitats, in both the subtidal and intertidal zones (Koehn, 1991). In Scotland, mussel aquaculture is a growing industry, dominated by the production of *Mytilus edulis* (Dondero et al., 2006). To improve knowledge of distribution and genetic structure, this report presents the first draft genome assembly for *M. edulis* (Linnaeus, 1758) and the comparison with two other genomes from the same genera: *Mytilus Coruscus* (Gould, 1861) and *Mytilus Galloprovincialis (Lamarck, 1819)*. The *Mytilidae* have the capability to become a model shellfish for climate change adaptation using genome-enabled systems biology and multi-disciplinary studies of interactions between abiotic stressors, pathogen attacks, and aquaculture practises.

Material and Methods

An adult specimen of *M. edulis* was collected from a wild costal population located in St. Andrews (Scotland). Gills were dissected and genomic DNA was extracted. High-weight DNA was used for library preparation and sequencing using both Oxford Nanopore and Illumina platforms. The genome comparisons were carried with *M. coruscus* (Assembly GCA_011752425.2; Li et al., 2020) and *M. galloprovincialis* (Assembly GCA_900618805.1; Gerdol et al., 2020). The mitochondrial genome was retrieved manually from the genome assembly.

Complete annotated nuclear genomes of Bivalvia (class) were collected. Blast+/BlastP v2.10.0 (Altschul et al., 1990)was used to search for duplicated sequences in protein-coding genes between each genome. Duplicate pairs were identified as sequences that demonstrated over 70 % sequence similarity, mutual protein coverage > 80%, protein length > 30 amino-acid from an all-against-all search.

A set of core-orthologs was constructed from the three complete annotated nuclear genomes of *Mytilinae* (subfamily), *M. edulis, M. galloprovincialis and M. coruscus* and were used to identity cluster of orthologous genes with a 1:1:1 ratio.

Results

This study presents the annotated genome sequence assembly of the blue mussel, *M. edulis*. The genome was assembled into 3,339 scaffolds with a total length of 1.83 Gb and a scaffold N50 of 1.10 Mb. Annotation of the *M. edulis* genome assembly identified a total of 69,265 genes. To assess the paleo-history of the Mytilinae (subfamily), we performed a comparative genomic investigation. A total of 2,293 gene duplications younger than Ks = 5 were inferred across the total data set of 16,291 assembled unigene clusters in *Mytilinae* (*M. coruscus, M. edulis* and *M. galloprovincialis*). The functional analysis of positively selected orthologs has also allowed for the identification of gene duplications assembled unigene clusters involved in key physiological processes.

Bibliography

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Dondero, F., Dagnino, A., Jonsson, H., Caprì, F., Gastaldi, L., & Viarengo, A. (2006). Assessing the occurrence of a stress syndrome in mussels (Mytilus edulis) using a combined biomarker/gene expression approach. *Aquatic Toxicology*, *78*(SUPPL.), 13–24. https://doi.org/10.1016/j.aquatox.2006.02.025

Gerdol, M., Moreira, R., Cruz, F., Gómez-Garrido, J., Vlasova, A., Rosani, U., Venier, P., Naranjo-Ortiz, M. A., Murgarella, M., Greco, S., Balseiro, P., Corvelo, A., Frias, L., Gut, M., Gabaldón, T., Pallavicini, A., Canchaya, C., Novoa, B., Alioto, T. S., … Figueras, A. (2020). Massive gene presence-absence variation shapes an open pan-genome in the Mediterranean mussel. *Genome Biology*, *21*(1), 275. https://doi.org/10.1186/s13059-020-02180-3

Koehn, R. K. (1991). The genetics and taxonomy of species in the genus Mytilus. *Aquaculture*, *94*(2–3), 125–145. https://doi.org/10.1016/0044-8486(91)90114-M

Li, R., Zhang, W., Lu, J., Zhang, Z., Mu, C., Song, W., Migaud, H., Wang, C., & Bekaert, M. (2020). The Whole-Genome Sequencing and Hybrid Assembly of Mytilus coruscus. *Frontiers in Genetics*, *11*(May), 1–6. https://doi.org/10.3389/fgene.2020.00440

THE GILL MICROBIOTA COMPOSITION IN SEA FARMED ATLANTIC SALMON (*Salmo salar*): LOCATION MATTERS

Costelloe, E.[1], Douglas, A.[1], Lorgan-Richie, M.[1], Valdenegro, V.[2], Bickerdike, R.[3], Noguera, P.[4], Król, E.[1] and Martin, S.A.M.[1]

[1]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK
[2] BioMar AS, Pb 1282 Sluppen, N-7462 Trondheim, Norway
[3] Scottish Sea Farms, Laurel House, Laurelhill Business Park, Stirling, FK7 9JQ, UK
[4] Health and Welfare, Marine Scotland Science, Aberdeen, UK
Email: e.costelloe.18@abdn.ac.uk

Introduction

There is a growing interest in the gill health of economically important species such as Atlantic salmon (*Salmo salar*) as this organ is central to many physiological functions and overall fish performance. As a mucosal surface, gills can be colonised by pathogens and act as a site of pathogen entry. Numerous pathogens and environmental factors contribute to the gill inflammation, but the underlying response mechanisms to the disease are poorly understood. The terms "complex gill disorder" (CGD) and "proliferative gill disease" (PGD) reflect the complexity and multifactorial aetiology of gill inflammation. Microbiota associated with the gill mucosal surface that remain in homeostasis are believed to be an essential part of the gill immunity and health. Over the last decade, the interaction between microbiota and mucosal immunity has become an active field of research, aiming to improve understanding of gill health. The main objective of this study is to further our understanding of gill health in Sea farmed Atlantic salmon.

Materials and methods

We sampled Atlantic salmon from three different marine production sites in Scotland (A on Isle of Mull and B and C in Shetland) and examined the gills at three different levels of organization: gross morphology with the use of PGD scores (macroscopic examination), histopathology (microscopic examination) and microbial community composition (samples and other parameters fully described in Król et al. 2020). Gill mucus swabs were taken from 75 fish in total and used to extract DNA. Gill microbiota composition was evaluated by sequencing the V3/V4 variable region of the bacterial 16S using an Illumina MiSeq platform, followed by analysis of amplicon sequence variants (ASVs) with a DADA2 pipeline (Callahan et al. 2016). Functionality was inferred using Piphillin (Iwai et al. 2016).

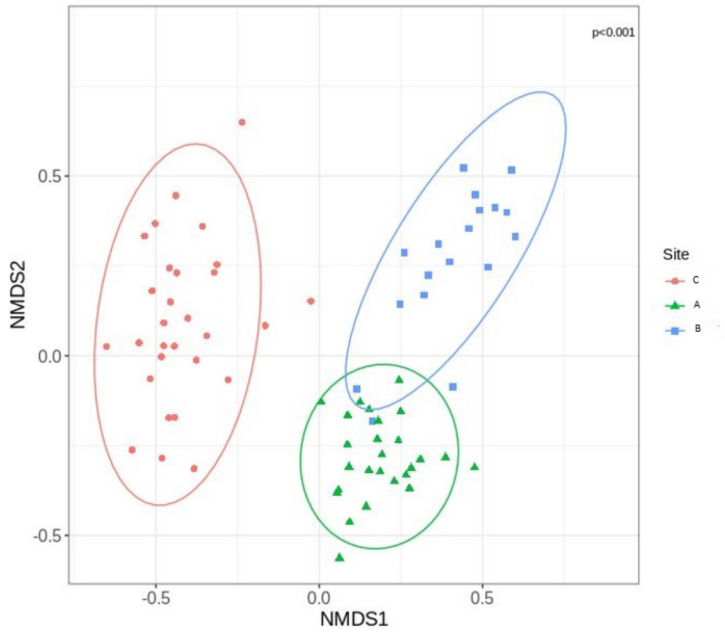


Figure 1: Beta-diversity based on Bray Curtis distances visualised in an NMDS plot. Different colours specify Atlantic Salmon separated by site (A, B and C) as described in Król et al. 2020. Data ellipses are based upon an assumed multivariate t-distribution at a level of 0.95.

(Continued on next page)

Results

Fish from the three different sites (A, B and C) had significantly different gill microbiota composition ($p < 0.001$, Fig. 1). The dominant gill microbial phyla were *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, which is consistent with other salmonid studies (Brown et al., 2019). Site C, which had lower gill histopathology scores than sites A and B (Król et al. 2020), was characterised by significantly higher alpha diversity indices. All three sites were clearly separated by beta diversity. Despite the differences, all fish had a core gill microbiota that was shared between the sites. The functional analysis of the gill microbiota composition revealed the differences associated with the metabolic pathways.

Discussion and conclusions

The diversity of the gill microbiota composition was predominantly associated with the origin of fish (sites A, B and C), suggesting the importance of spatial and temporal drivers in shaping gill microbiota and gill health. Gill microbiota diversity was the highest in the fish with the lowest histopathology scores (site C), although the causality of this observation remains unknown. Oppositely, fish with the decreased gill microbiota diversity had higher gill histopathology scores (sites A and B). The relationship between gill microbiota composition and gill histopathology as well as their impacts on gill health require further studies.

References:

BROWN, R.M., WIENS., G.D., and SALINAS., I. 2019. Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout. Fish and Shellfish Immunology 86(3), 497-506.

CALLAHAN, B.J., MCMURDIE, P.J., ROSEN, M.J., HAN, A.W., JOHNSON, A.J.A. and HOLMES, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods,* **13**(7), pp. 581-583.

IWAI, S., WEINMAIER, T., SCHMIDT, B.L., ALBERTSON, D.G., POLOSO, N.J., DABBAGH, K. and DESANTIS, T.Z., 2016. Piphillin: Improved Prediction of Metagenomic Content by Direct Inference from Human Microbiomes. *PLOS ONE,* **11**(11), pp. e0166104.

KRÓL, E., NOGUERA, P., SHAW, S., COSTELLOE, E., GAJARDO., K., VALDENEGRO, V., BICKERDIKE, R., DOUGLAS, A., and MARTIN, SAM., 2020. Integration of transcriptome, gross morphology and histopathology in the gill of sea farmed Atlantic Salmon (Salmo salar): Lessons from multi-site sampling. *Frontiers in Genetics*, 11, 610.

HEAT-TREATED SOYBEAN MEAL: EFFECTS ON DIETARY ISOFLAVONE GENISTEIN CONTENT AND *Sparus aurata* JUVENILES GROWTH INDEXES, IMMUNE, ANTIOXIDANT, LIPOGENIC & DIGESTIVE SYSTEMS

E. Cotou[1]*, M. Halabalaki[2], M. Henry[1], A. Vasilaki[1], N. Politakis[1], M. Kotsiri[1], E. Fountoulaki[1], I. Nengas[1]

[1] Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Centre for Marine Research, 46.7 km Athinon-Sounion Ave, 19013 Anavyssos, Greece
[2] Department of Pharmacy, Division of Pharmacognosy and Natural Products Chemistry, National and Kapodistrian University of Athens, Panepistimioupoli Zografou, 15771 Athens, Greece
*e-mail: ecotou@hcmr.gr

Introduction

Soybean meal (SBM) contains several anti-nutritional compounds (ANCs) (e.g. lectins, trypsin inhibitors, soy antigens, isoflavones) that are major hindrances towards the nutritive value of SBM in fish diet [1]. Several methods including thermal treatments are being used to inactivate or eliminate many of these ANCs before SBM can be used in fish diets. The degree to which SBM must be heated may vary among fishes [2]. Isoflavones are a class of phytoestrogens with structure & function similar to synthetic estrogens having a range of estrogenic-like activities as well as antifungal, anti-oxidative and anti-inflammatory properties. The isoflavone genistein is present in soybeans in high amounts [3]. Dietary estrogenic-like effects of genistein-enriched diets may have positive but also negative effects in fish metabolism & health status [4]. There is good evidence that genistein affects the lipid metabolism in different fishes and alters plasma triglyceride and cholesterol levels as well as overall growth rates [5]. Yet, heat treatment of soybean products can affect not only the content but also the profile of isoflavones and their antioxidant activity [6]. The objectives of this study were to compare the feasibility of replacing FM protein in formulated diets with either a conventional SBM or a thermal treated SBM and evaluate the impacts on growth indexes & overall health status of gilthead seabream (*Sparus aurata*) juveniles, an important carnivorous sparid for marine cage culture, using selected growth indexes & biomarkers responses.

Materials and Methods

Three diets (isoproteinic & isolipidic) were produced in our lab: a 30% fish meal based diet (FM) & two other diets in which the FM was being replaced with a 28 % of SBM (Untreated-SBM) or with a 28% of SBM heat treated (Treated-SBM) (Table 1). Fabrication of all diets involved weight of raw materials, addition of 3% fish oil and mixing (Hobart), extrusion in pellets (Clextral) and drying at 40°C for 5 hours. The remaining amount of fish oil was added with a coater (Dinessen) under vacuum. Heat treatment involved addition of 20-30% hot water (80-90°C) in raw SBM following continuous mixing and heating at 60-65°C for 15 minutes and then drying at 50°C for 2 hours. Genistein was qualitatively & quantitatively analyzed in the methanolic extracts by UPLC-HRMS (Table 2). Juveniles of *S. aurata* (31.4 ± 0.5 g) were reared in 300 l tanks (30 fish/tank) in an open flow through seawater system (salinity 35‰, 22°C) and fed for two months. Trials were run in triplicates. At the end all fish were anesthetized counted & weighted. Blood samples were taken for immune biomarkers analysis. Liver & whole intestines were removed, snap-frozen in liquid nitrogen & stored at -80°C until analysis of enzymatic antioxidant, lipogenic & digestive biomarkers responses. Data were subjected to proper statistics analysis with the SPSS 21.

Results & Discussion

Replacement of FM with untreated SBM significantly affected the selected growth indexes and biomarkers responses. Further, heat treatment of SBM increased the content of genistein (Table 2). Most of the growth indexes were affected but only FCR, fish total body length and digestibility of lipids & carbohydrates were statistical significantly influenced compared to untreated-SBM (Table 3 & 4). Changes of lipogenic & digestive biomarkers responses were not significant after heat treatment (Table 5), whereas the most immune and enzymatic antioxidant biomarkers were significantly altered after SBM heat treatment (Table 6 & 7). Results obtained pointed out to take into consideration the possible consequences of SBM treatment and dietary isoflavone genistein on nutritional value of fish diet and *S. aurata* juveniles growth and health status.

(Continued on next page)

Table 1. Composition of the diets

%	FM	Untreated-SBM	Treated-SBM
FM 70	30	12	12
Wheat meal	18	4.7	4.7
Wheat gluten	15	20	20
Corn gluten	20	13.5	13.5
SBM HP48	-	30	30
Fish oil	14	15.5	15.5
Premix	0.25	0.25	0.25
Vit C 35	0.057	0.057	0.057
$Ca(H_2PO_4)_2$	1.4	2.5	2.5
Lysine	0.8	0.9	0.9
Methionine	0.3	0.4	0.4
Choline	0.15	0.15	0.15
Y_2O_3	0.05	0.05	0.05

Table 2. Genistein content

	Genistein (µg/gr)
In Raw material	
SBM untreated	0.78
SBM heat treated	1.96
SBM heat treated + dried	1.69
In Diets	
FM	< detection limit
Untreated SBM	0.006
Treated SBM	0.02

Table 3. Growth indexes of *S. aurata*

	FM	Untreated-SBM	Treated-SBM
WG (g)	66.80 ± 1.40	68.20 ± 4.40	62.40 ± 3.90
SGR (%)	1.75 ± 0.02	1.78 ± 0.09	1.69 ± 0.06
FCR	0.92 ± 0.00 **a**	1.01 ± 0.02 **b**	1.05 ± 0.00 **c**
FI (%)	1.58 ± 0.02 **a**	1.75 ± 0.05 **b**	1.75 ± 0.04 **b**
Body Length (cm)	18.7 ± 0.8 **ab**	18.80 ± 0.70 **a**	18.5 ± 0.7 **b**
HSI	1.83 ± 0.31 **a**	1.44 ± 0.25 **b**	1.44 ± 0.17 **b**

Table 4. Digestibility of the experimental diets

%	FM	Untreated-SBM	Treated-SBM
Proteins	95.81 ± 0.18 **a**	94.12 ± 0.22 **b**	94.50 ± 0.12 **b**
Lipids	95.18 ± 0.70 **a**	91.79 ± 1.30 **b**	92.99 ± 0.92 **ab**
Carbohydrates	99.82 ± 0.00 **a**	98.42 ± 0.11 **b**	98.83 ± 0.12 **c**

Table 5. Lipogenic & Digestive biomarkers responses

Specific activity of	FM	Untreated-SBM	Treated-SBM
(mU/mg protein)			
Malic enzyme	8.12 ± 0.95 **a**	4.57 ± 0.44 **b**	4.50 ± 0.44 **b**
G6PD	62.0 ± 8.4 **a**	50.4 ± 5.8 **b**	49.8 ± 5.4 **b**
(nM/min / mg protein)			
ALP	25.3 ± 2.8	23.5 ± 3.2	25.3 ± 2.7
Trypsin	82.76 ± 9.87	79.93 ± 9.52	81.99 ± 6.13
Alanine peptidase	48.2 ± 6.3	44.4 ± 6.2	44.5 ± 7.8

Table 6. Immune biomarkers responses

	FM	Untreated-SBM	Treated-SBM
Nitric Oxide (µM)	1.23 ± 0.42	1.45 ± 0.36 ↑	3.31 ± 0.93 ↑
Lysozyme (U/ml)	212.7 ± 16.7	192.9 ± 15.9 ↓	226.3 ± 18.1 ↑
Complement (% bact. killing/min)	62.26 ± 1.57	61.77 ± 1.82	60.92 ± 1.75 ↓
Ceruloplasmin (U/ml)	30.53 ± 7.12	31.53 ± 6.06	17.81 ± 5.63 ↓
Trypsin inhibition (%)	85.14 ± 2.69 **a**	52.43 ± 5.29 **b**	45.82 ± 2.96 **b**
Myeloperoxidase (U/ml)	0.013 ± 0.005 **a**	0.014 ± 0.005 **a**	0.066 ± 0.015 **b**

Table 7. Enzymatic Antioxidant biomarkers responses

Specific activity of	FM	Untreated-SBM	Treated-SBM
Total GPx (nM/min/mg prot.)	67.70 ± 7.84 **a**	42.80 ± 6.00 **b**	47.60 ± 5.69 **b**
Se-GPx (nM/min/mg prot.)	65.10 ± 7.84 **a**	46.80 ± 9.00 **b**	70.60 ± 5.98 **ab**
GR (nM/min/mg prot.)	5.76 ± 0.70 **a**	6.82 ± 0.75 **b**	7.98 ± 0.80 **c**
GST (nM/min/mg prot.)	294.6 ± 41.8 **a**	396.3 ± 52.2 **b**	457.4 ± 62.2 **c**
GSH *(nM/mg protein)*	0.14 ± 0.01 **a**	0.15 ± 0.02 **b**	0.15 ± 0.02 **ab**

References

1. NRC, 2011. Nutrient Requirements of Fish & Shrimp. NRC, Academy Press. Washington, DC.
2. Lim C., Akiyama M., 1992. Full fat SBM meal utilization by fish. Asian Fish. Sci., 5:181-197.
3. Franke, A.A., 1994. Quantitation of phytoestrogens in legumes by HPLC. Journal of Agricultural and Food Chemistry, 42, 1905-1013.
4. Ahmadifar E., Yousefi M., et al., 2020. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Reviews in Fisheries Science & Aquaculture, https://doi.org/10.1080/23308249.2020.1818689.
5. Torno C., Staats S., Fickler A., et al., 2019. Combined effects of nutritional, biochemical and environmental stimuli on growth performance and fatty acid composition of gilthead sea bream (*Sparus aurata*). PLOS ONE, https://doi.org/10.1371/journal.pone.0216611.
6. Chen L., Teng H., et al., 2019. A value-added cooking process to improve the quality of soybean: Protecting its isoflavones & antioxidant activity. Food Science & Human Wellness, 8:195-201.

EMBRYONIC AND EARLY LARVAL STAGES DEVELOPMENT OF THE SEA CUCUMBER HOLOTHURIA SANCTORI: A NEW POTENTIAL SPECIES FOR AQUACULTURE

G. Courtois de Viçose, M. Magdy

Instituto ECOAQUA/GIA Universidad De Las Palmas De Gran Canaria, Juan de Quesada 30, 35001 Las Palmas de Gran Canaria Spain
Email: gtricor@hotmail.com

Introduction

The continuous and increasing demand for sea cucumbers and new target species leads to certain overfishing scenario in some regions of the world requiring responsible management of catches and sustainment of market needs by other sources such as aquaculture. Therefore, several studies have investigated sea cucumber aquaculture, including hatchery production, as preliminary steps to the success of aquaculture of such species. Various species have been studied in Europe (Domínguez-Godino and González-Wangüemert, 2018; Laguerre et al., 2020 and Rakaj et al., 2019). However, no hatchery production studies have been performed on *H. sanctori* that exists naturally in the Mediterranean Sea and the eastern Atlantic Ocean. Hence, the study of its life cycle is of interest for production and environmental needs. Thus, the present study aims to test spawning induction methods, report embryonic and larval development and investigate larval rearing techniques of the sea cucumber *Holothuria sanctori*.

Materials and methods

Broodstock collection and maintenance

Adult specimens of *H. sanctori* were collected by scuba diving and were maintained in a biofilter reservoir, naturally filled with sediments, with flowing seawater at ambient temperature and under natural photoperiod until their transfer to experimental units for spawning induction and larval production experiments.

Spawning induction

Prior to the induction procedure, broodstock were washed with fresh seawater and placed in 300 L tanks at a density of 7 specimen/m^2 and then subjected to three different spawning induction treatments, that combines mechanical shock, thermal shock and algal stimulation. Each treatment was tested in triplicate.

Embryonic and larval culture

The fertilized eggs were stocked at two different densities in 200 L conical bottom larval tanks, filled with 1 **µm** filtered fresh seawater and UV sterilized, and fed at two different algal densities when the auricularia stage was reached. Three treatment were tested in triplicate: Treatment (A): 0.3 egg/ml and algal density from $5x10^3$ cells/ml to $2x10^4$ cells/ml. Treatment (B): 0.3 egg/ml and algal density from 10^4 cells/ml to $4x10^4$ cells/ml. Treatment (C): 1 egg/ml and algal density from 10^4 cells/ml to $4x10^4$ cells/ml. Tanks were maintained under natural photoperiod, constant temperature and provided with aeration and constant water flow. The algae tested as a source of feed were *Nannochloropsis* sp. and *Amphora* sp., provided in equal proportions (3 times/day) with concentrations gradually increasing until reaching a fixed algal concentration at the end of the experiment.

Embryonic and larval development

Embryonic and larval development was monitored microscopically from fertilization of the eggs up to the late auricularia stage. Embryos were observed every hour to observe cell divisions and take measurements. Daily water samples were taken from each treatment, for 26 days, to monitor larval development, and register larval size (length and width) of 10 larvae per tank.

Results

Spawning induction

Spawning was induced by mechanical shock and algal stimulation and spawning behaviour exhibited consisted in specimens clinging to the walls of the tanks close to the water surface. Male and female gametes were expulsed from a single gonopore at the top dorsal anterior surface of the body.

(Continued on next page)

Embryonic and larval development

Once fertilized the eggs were observed to go through different cleavage and gastrulation stages until reaching a fully elongated blastopore 57h30 min after fertilization. Early, mid and late doliolaria stages were observed until reaching until length and width of 1013.16 ± 151.46 **µm**, and 681.32 ± 112.6 **µm**, respectively.

Larval growth

Larve presented significantly higher lengths and widths ($P < 0.05$) al lower larval density while their length was not significantly affected ($P > 0.05$) by algal feeding density throughout auricularia larval development.

Discussion

The observed embryonic and larval development of *H. sanctori* were in line with the previous studies performed on several sea cucumber species. *H. sanctori* fertilized eggs had a medium size of 143 **µm**, lower than higher values reported for *H. polii, H. forskali, H. tubulosa*, and *H. mammata* (Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018; Rakaj et al., 2019; Laguerre et al., 2020) and higher than lower values reported for *A. mauritiana* (Ramofafia et al., 2003), while the early auricularia size of 563 **µm** exceeded the ones reported for these species. The timing of the emergence of auricularia larvae in *H. sanctori* (3 days 6 hours) was close to that observed in *H. polii* and *H. tubulosa* (3 days) (Rakaj et al., 2018; Rakaj et al., 2019), bred at a similar temperature. The larval growth results corroborate the ones of previous study on *H. scabra* suggesting that the best larval density is between 0.3 – 0.7 larvae/ml (James, 1996), but are also contrary to work protocols used in hatchery production for other species where the larval stocking density is reported to be 1 larva/ml (Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al.,2018). However, similarly to *H. tubulosa* (Rakaj et al., 2018) the low concentration of microalgae administered to the larvae was found to provide better growth and development than in higher concentration regimes.

References

Domínguez-Godino, J.A., González-Wangüemert, M., 2018. Breeding and larval development of *Holothuria mammata* , a new target species for aquaculture. Aquac. Res. 49, 1430-1440.

James, D.B., 1996. Culture of sea-cucumber. Bull. Cent. Fish. Res. Inst. 48, 120-126.

Laguerre, H., Raymond, G., Plan, P., Améziane, N., Bailly, X., Le Chevalier, P., 2020. First description of embryonic and larval development, juvenile growth of the black sea-cucumber *Holothuria forskali* (Echinodermata: Holothurodea), a new species for aquaculture in the north-eastern Atlantic. Aquac. 521, 734961.

Ramofafia, C., Byrne, M., Battaglene, S., 2003. Development of three commercial sea
cucumbers *Holothuria scabra*, *Holothuria fuscogilva* and *Actinopyga mauritiana* : larval structure and growth. Mar. Freshw. Res. 54, 657-667.

Rakaj, A., Fianchini, A., Boncagni, P., Lovatelli, A., Scardi, M., Cataudella, S., 2018. Spawning and rearing of *Holothuria tubulosa*: A new candidate for aquaculture in the Mediterranean region. Aquaculture Research 49, 557-568.

Rakaj, A., Fianchini, A., Boncagni, P., Scardi, M., Cataudella, S., 2019. Artificial reproduction of *Holothuria Polii*: a new candidate for aquaculture. Aquaculture 498, 444-453.

Acknowledgements

This research was supported by the Aquainvert project (MAC2/1.1a/282; https://aquainvert.eu) funded by Programa de Cooperación INTERREG MAC 2014-2020.

INTEGRATION OF THE SEA CUCUMBER, *Holothuria sanctori,* TO ABALONE, *Haliotis tuberculate coccinea,* GROW-OUT PROCESSES

G. Courtois de Viçose, M. Magdy

Instituto ECOAQUA/GIA Universidad De Las Palmas De Gran Canaria, Juan de Quesada 30, 35001 Las Palmas de Gran Canaria Spain.
Email: gtricor@hotmail.com

Introduction

Various studies have demonstrated the interest of including deposit feeders, as an integrated element of IMTA systems devoted to the consumption of organic materials (Cubillo et al., 2016). Sea cucumbers are of interest as deposit feeder, for their environmental value, their interesting growth potential and low mortality rates (Zamora and Jeffs, 2012). Various sea cucumber species within IMTA systems have been tested for co-culture with a variety of fish (Hannah et al., 2013), molluscs (Kang et al., 2003; Zhou, 2006) and seaweed (Beltran-Gutierrez et al., 2016). The interest of Holothurians as deposit feeders was demonstrated in these studies and their mitigation ability within different IMTA systems was further tested, indicating that sea cucumbers can optimise the net use of wastes (Reid et al., 2013).

Given the existing research, the current study investigates the potential for the integrated culture of the sea cucumber *H. sanctori* with the abalone *Haliotis tuberculata coccinea* in order to develop the integrated Land Based IMTA production of these species.

Materials and methods

Experimental specimens

Young individuals of *H. sanctori* (mean weight: 31.07 ± 14.51 g) 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 coccinea*) baskets (in which abalone were fed macroalgae). After their indoor acclimation, they were then respectively allocated to experimental IMTA units.

Adult abalone of *H. tuberculata coccinea* (mean weight: 37.32 ± 9.01g and mean size: 60.83 ± 5.30mm) were obtained from the abalone production unit of the ECOAQUA institute, University of Las Palmas de Gran Canaria.

IMTA experimental set up

Experimental IMTA systems were designed to provide two levels compartments; one for the fed abalone (*H. tuberculata coccinea*) of 50L capacity and the other for sea cucumber (*H. sanctori*) located below the abalone compartment. Together located in 300L tank capacity. The abalone compartment was perforated on the bottom and on the sides to allow water exchange and release of abalone wastes to reach the sea cucumber located below.

Stocking density

Tests were performed in triplicates in the IMTA experimental units at two sea cucumber densities (2.5 Specimen.m^{-2} and 3.75 Specimen.m^{-2}) that were placed under abalone baskets stocked at 50 Specimen/m^{-2} and weekly fed 0.8kg of IMTA produced macroalgae.

Growth performance and ingestion rate

Weight Gain (WG), and Specific Growth Rate (SGR) were estimated on a monthly basis while ingestion rate was estimated in mg/g/h

Biochemical analysis

Triplicate samples of sea cucumber body wall were collected from experimental and wild specimens and were analyzed for total lipids, protein, carbohydrate, and ash.

Results

Growth performance

Overall, the mean SGR and WG values differed significantly ($p < 0.05$) among high density and low-density treatments. All sea cucumber specimens within the density 3.75 sp.m^{-2}, were found to lose weights and exhibit negative SGR and WG values. On the contrary, for specimens stocked at a density of 2.5 sp.m^{-2}, their growth performance improved as all specimens could gain weight and exhibit positive SGR and WG values.

(Continued on next page)

Biochemical analysis

No significant difference ($p > 0.05$) was observed between the proximate biochemical composition of sea cucumbers produced in the tested IMTA system and the wild-caught specimens, supporting the suitability of such system for the production of this sea cucumber species.

Discussion

The loss of condition amongst sea cucumber fed abalone faeces at high density in the current experiment indicates that stocking density has a significant impact on *H. sanctori* growth. Similarly, several authors have reported an inverse proportionality between sea cucumber stocking density and their growth rates (Dong *et al*., 2010; Pei *et al*., 2012). Based on these results, a stocking density of 2.5 $sp.m^{-2}$ is suggested to sustain growth of *H. sanctori*. In terms of biochemical composition, the similarities observed between the experimental specimens and their wild counterparts suggest the ability of sea cucumbers to adjust their feed intake to satisfy their nutritional requirements and to maintain a steady nutrient composition within their body walls, this being of interest for future successful production of the species.

References

Beltran-Gutierrez, M., Ferse, S.C., Kunzmann, A., Stead, S.M., Msuya, F.E., Hoffmeister, T.S. and Slater, M.J. 2014. Co-culture of sea cucumber *Holothuria scabra* and red seaweed *Kappaphycus striatum*. *Aquaculture Research* 47, 1549-1559.

Cubillo, A.M., Ferreira, J.G., Robinson, S.M.C., Pearce, C.M., Corner, R.A. and Johansen, J. 2016. Role of deposit feeders in integrated multi-trophic aquaculture A model analysis. *Aquaculture* 453, 54-66.

Dong, S.L., Liang, M., Gao, Q., Wang, F., Dong, Y.W. and Tian, X.L. 2010. Intraspecific effects of sea cucumber (*Apostichopus japonicus*) with reference to stocking density and body size. *Aquaculture Research* 41, 1170-1178.

Hannah, L., Pearce, C.M. and Cross, S.F. 2013. Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multi-trophic aquaculture site. *Aquaculture* 406-407, 34-42.

Kang, K.H., Kwon, J.Y. and Kim, Y.M. 2003. A beneficial coculture: charm abalone *Haliotis discus hannai* and sea cucumber *Stichopus japonicus*. *Aquaculture* 216, 87-93.

Reid, G.K., Robinson, S.M.C., Chopin, T. and MacDonald, B.A. 2013. Dietary proportion of fish culture solids required by shellfish to reduce the net organic load in open-water integrated multi-trophic aquaculture: a scoping exercise with Co-cultured Atlantic salmon (*Salmo salar*) and blue mussel (*Mytilus edulis*). *J. Shellfish Res*. 32, 509-517.

Xia, B., Ren, Y., Wang, J., Sun, Y. and Zhang, Z. 2017. Effects of feeding frequency and density on growth, energy budget and physiological performance of sea cucumber *Apostichopus japonicus*, (selenka). *Aquaculture* 466, 26-32.

Zamora, L.N. and Jeffs, A.G. 2012a. Feeding, metabolism and growth in response to temperature in juveniles of the Australasian sea cucumber, *Australostichopus mollis*. *Aquaculture* 358-359, 92-97.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X. and Zhang, F. 2006. Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. *Aquaculture* 256, 510-520.

Acknowledgements

This research is supported by the EU H2020 research and innovation program within the collaborative project "AQUAVITAE– New species, processes and products contributing to increased production and improved sustainability in emerging low trophic, and existing low and high trophic aquaculture value chains in the Atlantic" (https://aquavitaeproject.eu/) under Grant Agreement No. 818173.

PROTECTIVE EFFECT OF POLYSACCHARIDES FROM THE ADRIATIC SEA MACROALGAE AGAINST H_2O_2-INDUCED OXIDATIVE STRESS IN ZEBRAFISH EMBRYO

R. Čož-Rakovac*[a,b], K. Begić[c], S. Babić[a,b], L. Čižmek[a,b], A. Dobrinčić[c], A. Martić[a,b], I. Strunjak-Perović[a,b], V. Dragović Uzelac[c]

[a]Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb (Croatia)
[b]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute
[c]Faculty of Food Technology and Biotechnology, University of Zagreb
E-mail: rrakovac@irb.hr

Introduction

Marine macroalgae represent a source of bioactive compounds capable of producing a myriad of secondary metabolites. Due to the wide range of biological activities, polysaccharides extracted from macroalgae have a wide variety of potential industrial applications including functional foods, nutraceuticals, etc (Garcia-Vaquero et al. 2016). The Adriatic Sea is a challenging habitat characterized by high salinity (35.0-38.5 ‰), large temperature oscillations (7-27°C), relatively shallow depth (~200 m depth), and increased impact of UVA and UVB radiation (Grbec et al. 2018). The ability of marine macroalgae to survive such harsh environmental conditions suggests the presence of unique protective compounds and mechanisms. Their structure and biological activity are highly influenced by growth location and harvesting season that cause even intra-species variation, thus offering an inexhaustible source of novel bioactive compounds (Jiao et al., 2011; Garcia-Vaquero et al. 2016). The present study was undertaken to evaluate the toxic potential and novel *in-vivo* antioxidant activity determination of polysaccharide fractions and to complete this research by developing a robust platform for the high-throughput screening of novel marine compounds with antioxidant properties.

Materials and methods

The 7 marine macroalgae [two green algae: *Ulva lactuca* (ULLA) and *Codium bursa* (COBU) and five brown algae: *Padina pavonica* (PAPA), *Halopteris scoparia* (HASC), *Cystoseira compressa* (CYCO), *Cystoseira barbata* (CYBA) and *Fucus virsoides* (FUVI)] were collected from the coastal area of Croatia. Extraction of polysaccharides from freeze-dried samples included the addition of acetone under constant stirring for 18h, followed by filtration and drying of residues which were afterward stirred in hot sulphuric acid and filtered. Polysaccharides were precipitated by the addition of ethanol, refrigerated, collected and dried after 24h. The extraction yield of polysaccharides, as well as the content of fucose, sulfuric groups and uronic acid was determined.

Zebrafish embryotoxicity test (ZET) was performed on 0.25, 0.50 and 1.00 mg/mL of each fraction using zebrafish *Danio rerio* embryos (WIK strain) in accordance with OECD 236 Guidlines (2013). Lethal and sub-lethal effects, as well as cardiotoxic and neurotoxic potential, were estimated at 96 h of exposure. The zebrafish embryo was further employed to confirm the protective effect of polysaccharide fractions on H_2O_2-induced ROS generation *in vivo*, following the developed protocol described in the study of Jerković et al. (2021).

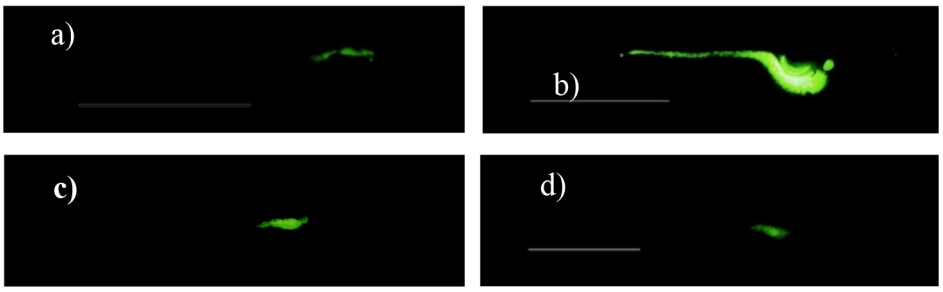


Figure 1. *In vivo* evaluation of antioxidant potential of polysaccharide fractions in DCF-DA stained larvae. a) Non-treated control, b) Hydrogen peroxide-treated specimens. Specimens pretreated with: c) 0,5 mg/mL HASC, and d) 1,00 mg/mL HASC. Scale bar=100 μm.

(Continued on next page)

Results

Fractions of brown macroalgae contained a higher amount of sulphated polysaccharides (up to 95.17%), fucosis (up to 20.51%) and uronic acid (up to 8.45%) than fractions prepared from green algae. Tested polysaccharide fractions showed no embryotoxic, cardiotoxic and/or neurotoxic potential. The only exception was COBU that increased mortality and heartbeat rate at 1 mg/mL, which was excluded for further testing of antioxidant activity. The H_2O_2 treated embryos exhibited significantly high fluorescence intensity (386.7%, $p<0.05$) compared to the non-treated group (100%). Embryos pretreated with CYCO, CYBA and HASC polysaccharide fractions exhibited a statistically significant decrease of fluorescence intensities (Figure 1), subduing the effect generated via H_2O_2. The highest antioxidant activity was noticed on HASC (fluorescence intensity decreased for 60.27% comparing to the group on H_2O_2).

Discussion

Among seven tested polysaccharide fractions, a protective effect against H_2O_2-induced oxidative stress was proven for CYBA, CYCO and HASC fractions. Concentration of CYCO and HASC fractions in 1 mg/mL showed the highest antioxidant activity, which corresponds well with the high amount of polysaccharide groups (95.17 and 70.47%, respectively). Amongst tested fractions, CYCO contained the highest amount of uronic acid, well known for its antioxidant properties (Ai et al. 2020). Moreover, synergistic action in such a complex mixture should be taken into account. Collectively, the results obtained in the study suggest that polysaccharides isolated from the Adriatic Sea macroalgae might be a potent source of natural antioxidants that could be sustainably utilized in industrial applications. Due to the high degree of genetic similarity among zebrafish and humans, our findings suggest that zebrafish is a valuable model not only in determination of biomolecule's safety for human usage but also in the prediction of their mechanism of action in the human organism.

References

Ai Z, You Y, Li W, Fan J, Wang Y, Huang J, Wang J, Wang J, Liu J 2020 Enhanced uronic acid content, antioxidant, and anti-inflammatory activities of polysaccharides from ginseng fermented by *Saccharomyces cerevisiae* GIW□1. Journal of Food Processing and Preservation, 44(11), e14885.

García-Vaquero M, Rajauria G, O'Doherty JV, Sweeney T (2017). Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. Food research international, 99, 1011-1020.

Grbec B, Matić F, Beg Paklar G, Morović M, Popović R, Vilibić I 2018 Long-term trends, variability and extremes of in situ sea surface temperature measured along the eastern Adriatic coast and its relationship to hemispheric processes, Pure Appl. Geophys. 175, 4031–4046.

Jerković I, Cikoš AM, Babić S, Čižmek L, Bojanić K, Aladić K, Ul'yanovskii NV, Kosyakov DS, Lebedev AT, Čož-Rakovac R, Jokić S 2021 Bioprospecting of less-polar constituents from endemic brown macroalga *Fucus virsoides* J. Agardh from the Adriatic Sea and targeted antioxidant effects *in vitro* and *in vivo* (Zebrafish Model). Marine drugs, 19(5), 235.

Jiao G, Yu G, Zhang J, Ewart HS 2011 Chemical structures and bioactivities of sulfated polysaccharides from marine algae, Marine Drugs 9(2), 196–233.

OECD Guidelines for the testing of chemicals. Fish Embryo Acute Toxicity (FET) Test. 2013. Organization for Economic Cooperation and Development Publishing.

EFFECTS OF *BMP15* KNOCKOUT ON ATLANTIC SALMON GAMETOGENESIS

D. Crespo[(1)*], E. Kjærner-Semb[(1)], K. O. Skaftnesmo[(1)], E. Andersson[(1)], P. G. Fjelldal[(2)], T. J. Hansen[(2)], R. B. Edvardsen[(1)], R. W. Schulz[(1,3)], A. Wargelius[(1)], L. Kleppe[(1)]

[(1)] Institute of Marine Research, Bergen, Norway
[(2)] Institute of Marine Research, Matre Research Station, Matredal, Norway
[(3)] Utrecht University, The Netherlands
E-mail: diego.crespo@hi.no

Inducing sterility represents an alternative strategy for sustainable Atlantic salmon (*Salmo salar*) aquaculture since that can avoid genetic introgression of farmed escapees into wild populations and unwanted precoucius male sexual maturation. One possibility is to target proteins essential for germ cell formation or survival, such as previously identified gonad-specific factors. Bone morphogenetic protein (BMP15) is a critical regulatory factor in mammalian female reproduction that plays a crucial role in ovary development and oocyte maturation, while inactivating mutations in *BMP15* result in sterility. Despite its importance in female fertility, little is known regarding the biological function(s) of BMP15/Bmp15 in male gonads in vertebrates. Previous work of our group revealed, as reported in mammalian models, a gonad- and germ cell-specific expression of *bmp15* in salmon. To further investigate the role of Bmp15 in gonadal development, we generated in this study *bmp15*-edited Atlantic salmon using CRISPR/Cas9. Gamete quality analysis showed that fertilized eggs from highly mutated F0 generation *bmp15* CRISPR-ed fish had low or no survival, suggesting an important role of Bmp15 in the production of functional gametes, and/or in embryonic development in salmon. However, surviving larvae were kept in order to stablish an F1 generation and to investigate potential phenotypes during gametogenesis. For that purpose, F1 *bmp15* knockout (KO) mutants and control fish were reared in a common garden, gonad samples were collected, body weight and length measured and gonado-somatic (GSI) indices scored at 3 different time points (18, 22 and 26 months). At 18 months of age, ~21% of the *bmp15* KO females investigated had 1 ovarian bulb only and the majority (83%) of the *bmp15* KO males showed germ cell-poor testes, which was accompanied by reduced *vasa* expression levels compared to the control group in both sexes. In addition, transcripts levels of genes involved in estrogen biosynthesis and signaling (*cyp19a1a* and *esr1*) were stimulated in *bmp15* KO females. In the following samplings (22 and 26 months of age), no significant difference was recorded for the females in none of the parameters analyzed. In contrast, the stage of spermatogenesis seemed advanced in *bmp15* KO males compared to the control group, showing significantly elevated GSI values and higher cumulative frequency of spermiating testes (~50 *vs*. 22%) but lower expression levels of steroidogenic enzymes (*cyp17a1* and *star*). However, the effect of *bmp15* loss-of-function on sperm quality and viability requires further investigation. Taken together, our results suggest that Bmp15 plays a role in the regulation of germ cell development during early stages of both oogenesis and spermatogenesis in salmon, and that genetic ablation of *bmp15* in males advances the stage of spermatogenesis but sperm production is normal. Unfortunately, due to the long generation time in female salmon, data on later stages of maturation and ovulation are not available yet and further analysis will have to clarify whether *bmp15* loss-of-function leads to obvious ovarian phenotypes as described in mammals.

A NOVEL ENVIRONMENTAL METHOD FOR CIRCULAR INNOVATIONS: EMERGY ASSESSMENT OF RAS BY-PRODUCT VALORISATION OPTIONS

S. Cristiano[1]*, H. Baarset[2], L. Svenningsson, C. Bruckner[3], and R. Pastres[1,4]

[1] Università Ca' Foscari Venezia, Campus Scientifico, via Torino 155, 30172 Mestre, Venice (Italy)
[2] Waister AS, Åslyveien 15, 3170 Sem (Norway)
[3] Salten Havbrukspark, Sjøfossen Næringsutvikling, 8140 Inndyr (Norway)
[4] Bluefarm s.r.l., Centro Vega ed. Pegaso, via delle Industrie 15, 30175 Marghera, Venice (Italy)
E-mail: silvio.cristiano@unive.it

Introduction

Driven by environmental concerns, resource optimisation goals, costs abatement, and market competitivity, the aquaculture sector is not exempt from seeking circular and less impacting solutions. By definition, RAS systems allow for direct recirculation of water. Here, a step forward is analysed, addressing by-products that are currently not recirculated in the plant nor in outer human economies, in turn implying safety and environmental threats in addition to disposal expenditures: namely, wastewater and fish mortality. The former yields sludge that is converted into biogas, while the recirculated water still averagely contains relevant quantities of nitrogen (N, 0.028 mg/L), ammonium (NH_4, 0.036 mg/L), Chemical Oxygen Demand (COD, 219 mg/L), and suspended solids (SS, 100 mg/L) when leaving a regular RAS. The latter is mostly treated by the technology of ensilage, with biomass treated with formic acid, thus causing a hazardous by-product to be transported and safely disposed of, while exposing humans to a liquid that is able to cause acid etching to the skin, eyes, lungs, and more; the waste liquid may also produce gases that are harmful to human health and that can be explosive. Targeting such two by-products, some eco-innovations were developed within the Horizon 2020 project GAIN – Green Aquaculture Intensification in Europe (2018–2021), aimed at abating costs and impacts for the farm, and valorising current by-product as new resources for fertiliser mixes, pet food, cement production and/or energy generation. Carried out by Norwegian industrial partners Salten Havbrukspark (SHP) and Waister AS (WAS), such proposals were independently evaluated by Italian public research partner Università Ca' Foscari Venezia through an emerging comprehensive environmental accounting method – i.e. EMergy assessment – allowing to enlarge the evaluation boundary compared to well-oiled Life-Cycle Assessment (LCA), which is anyway also used here for comparisons.

Materials and methods

This study presents the environmental assessment of several options per innovation. In both, scenarios (A) represent reference conditions depicting business-as-usual approaches. Regarding wastewater, innovative scenario SHP(B) shows the consequences of a filtered and dried sludge, i.e. more purified water to be recirculated and a resulting powder, rich in nutrients, to be recirculated and valorised in outer economies: (B1) as a fertiliser; (B2) as bio-energy in a cement factory; and (B3) as biogas substrate. Regarding fish mortalities, drying innovations differ for the cooling media of the new machinery, i.e. water in WAS(B) and a mix of water and glycol in WAS(C), and for the consequent fact that in (B) hot water is recirculated in the RAS, allowing to save electricity in the fish farm. For such innovation, end-of-life valorisation options are represented by the reuse of the dried product as a pet food ingredient, as bio-energy in a cement factory, and as biogas substrate. Both eco-innovations are referred to demonstration plants in Norway; all inputs, including transportation ones to the final valorisation sites, are computed for that geo-economic context, yet allowing for exportation and adaptation. The economic implications of savings from the avoided disposal or even by the sale of by-products are also evaluated in the present assessment. Indeed, this is possible through the thermonidamics- and systems ecology-based EMergy accounting (EMA) approach (Odum, 1996; Brown & Ulgiati, 2016): changing the perspective from the final user (receiver) to the environment (donor), material and immaterial inputs and outputs are associated with their direct and indirect requirements to the geobiosphere. LCA results from the standardised Life-Cycle Assessment (LCA) (Arvanitoyannis, 2008) are also offered and discussed. Through EMA, the reliance upon natural resources is further explored compared to LCA, focusing more on impacts. Such approaches may be seen as complementary; for this reason, they are increasingly used together for more comprehensive views on a process' sustainability.

(Continued on next page)

Results

The eco-innovations' potentials, limits, and margins for improvement are presented and discussed. The eco-innovations for fish mortality treatment seem to perform better than business-as-usual ensilage. Through LCA, environmental gains larger than –80% are obtained in most indicators, even when the product is simply disposed of, and larger environmental gains arise from the reuse of the dried product in the processing of pet food, implying avoided disposal and savings in alternate ingredients. Positive LCA results, although smaller than in the other innovation, also come from innovations addressing wastewater, with no marked preference among all end-of-life options. In both innovations, smaller, neutral, and in some cases contrasting results are reached on water consumption. Compatible trends seem to be confirmed by EMA, anyway still in progress while submitting the present proposal, allowing for further insights about the mineral and fossil fuel scarcity, and adding up novel information about flows that are instead not accounted for in LCA: among these, the most interesting ones for the assessment at hand seem to derive from free-of-charge renewable inputs, from human labour, and from the environmental significance of the monetary costs associated with the purchase and selling of inputs, waste, and valorised by-products.

Acknowledgements

The research leading to these results has received funding from the European Union's HORIZON 2020 Framework Programme under Grant Agreement no. 773330.

References

Arvanitoyannis, I. S. (2008). ISO 14040: life cycle assessment (LCA)–principles and guidelines. *Waste management for the food industries*, 97-132.

Brown, M. T., & Ulgiati, S. (2016). Emergy assessment of global renewable sources. *Ecological Modelling*, *339*, 148-156.

Odum, H. T. (1996). *Environmental accounting: emergy and environmental decision making*. Wiley.

EFFECT OF *Perkinsus olseni IN VITRO* INFECTION ON *Ruditapes decussatus* CONDITIONING

Andreia Cruz[1], Catarina Duarte[1], Eric Guévélou[1], Joana Sousa[1], Sandra Joaquim[2], Domitília Matias[2], Sergio Fernández-Boo[3]

[1] Oceano Fresco S.A, Porto de Abrigo, 2450-075 Nazaré, Portugal.
[2] IPMA - Instituto Português do Mar e da Atmosfera, Av. 5 outubro s/n, Olhão, Portugal
[3] Animal Health and Aquaculture (A_2S), CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Porto, Portugal.

Presenting author email: andreia.cruz@oceano-fresco.pt

Introduction

Ruditapes decussatus (grooved carpet shell) is an European native clam species with high economic and gastronomic value. 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). Of the *Perkinsus* species, *P. olseni* can have a severe impact on bivalve molluscs, namely in clam species (*R. decussatus*, *R. philippinarum* and *Venerupis corrugata*) and has been associated with high mortality rates in different molluscan species worldwide, resulting in severe economic losses (Ruano et al., 2015).

It is known that heavy *P. olseni* infection provokes the accumulation of large granulocytomas invading clam's gonad, and thus reducing the reproductive area (Casas & Villalba, 2012). In addition to the physical effects of *P. olseni* on clam's organ structure, heavy infections also cause a reduction in host condition index (Casas, 2002). The energy consumed by the parasite in heavily infected clams may suppose a considerable subtraction of the energy available for growth and reproduction (Choi et al., 1989). Studies available in the literature showed contradictory results in clams. Park et al. (2006) found that spawning frequency and oocyte production were negatively affected in *R. philippinarum* females heavily-infected by *P. olseni*. Contrary, Casas & Villalba (2012) did not find a significant effect of infection on gonadal index, fecundity or spawning efficiency. However, to our knowledge no studies have investigated the effect of *P. olseni* on *R. decussatus* maturity and reserve storage during hatchery conditioning. In this work, the effect of *P. olseni in vitro* infection of *R. decussatus* on gametogenesis.

Materials and Methods

Perkinsus free adults of *R. decussatus* were divided randomly in three groups: a control group (non-infected, injected with saline solution), and two *in vitro* infected groups by injection - low infection (LI) (5000 *Perkinsus*/clam) and high infection (HI) (500.000 *Perkinsus*/clam). Clams were held in tanks in a flow-through circuit containing filtered seawater, at 19ºC and were fed daily with a mixed diet.

Sampling were done at the beginning of the experiment (T0), after 34 days (T1) and at the end of the conditioning period (58 days) (T2) for the determination of condition index, gametogenic stage, gross biochemical composition (total proteins, glycogen and total lipids) and *Perkinsus* infection level by RFTM standard method. The software SigmaPlot (version 12.5 Systat Software, Germany) was used to perform all statistical analyses.

Results and discussion

It was observed an increase of *Perkinsus* infection directly correlated with the dose injected: control (no infection); low dose -> medium infection; high dose -> high infection.

During the conditioning period, it was observed the decrease of glycogen reserves *vs* the increase of total lipids in all treatments, indicating *de novo* synthesis of total lipids to gamete formation.

The decreased of glycogen contents observed during the conditioning period, was more accentuated in the first 34 days and in the treatments with clams infected. Behind the consumption of glycogen to gametogenic process, the clams infected with *Perkinsus* seem to spend more energy for their metabolism.

The effect of *Perkinsus* infection in clam's conditioning was evident, since in HI treatment was observed a delay in the gonadal development comparatively with the two other treatments. This fact was also supported by the higher values of total lipids observed in the HI, at the end of conditioning period.

(Continued on next page)

Acknowledgements
This research was supported by the project 'EUROCLAM – Unlocking the potential of Euro-native bivalves' (CENTRO-01-0247-FEDER-113950) co-financed by CENTRO 2020, PT2020 and Fundo Europeu de Desenvolvimento Regional and by the project 'Tools4Breed – Challenge test and genetic markers for *Perkinsus* as a tool for *Ruditapes decussatus´* selective breeding' (FA_05_2017_025) co-financed by Fundo Azul and República Portuguesa. Catarina Duarte was supported by a grant funded by FA_05_2017_025 project.

References

Casas, S.M., La Peyre, J.F., Reece, K.S., Azevedo, C., Villalba, A. (2002). Continuous *in vitro* culture of the carpet shell clam *Tapes decussatus* protozoan parasite *Perkinsus atlanticus*. Diseases of Aquatic Organisms. 52(3): 217-231.

Casas, S.M. & Villalba, A. (2012). Study of perkinsosis in the grooved carpet shell clam *Ruditapes decussatus* in Galicia (NW Spain). III. The effects of *Perkinsus olseni* infection on clam reproduction. Aquaculture, 356, 40-47.

Choi, K.S., Wilson, E.A., Lewis, D.H., Powell, E.N., Ray, S.M. (1989). The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. Journal of Shellfish Research 8, 125-131.

Matias, D., Joaquim, S., Margarete, A., Moura, P., Teixeira, J., Sousa, D., Sobral, P., Leitão, A., 2013. The reproductive cycle of the European clam *Ruditapes decussatus* (L, 1758) in two Portuguese populations : Implications for management and aquaculture programs. Aquaculture 406–407, 52–61.

Park, K.I., Figueras, A., Choi, K. S. (2006). Application of enzyme-linked immunosorbent assay (ELISA) for the study of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca: Bivalvia): II. Impacts of *Perkinsus olseni* on clam reproduction. Aquaculture, 251(2-4), 182-191.

Ruano, F., Batista F., Arcangeli G. (2015). Perkinsosis in the clams *Ruditapes decussatus* and *R. philippinarum* in the Northeastern Atlantic and Mediterranean Sea: A review. Journal of Invertebrate Pathology. 131: 58-67.

EUROCLAM - UNLOCKING THE POTENTIAL OF EURO-NATIVE BIVALVE SPECIES THROUGH SCIENCE-DRIVEN AQUACULTURE

Andreia Cruz, Eric Guévélou, Joana Sousa

Oceano Fresco S.A, Porto de Abrigo, 2450-075 Nazaré, Portugal.

Presenting author email: andreia.cruz@oceano-fresco.pt

Introduction

Oceano Fresco is a private company that develops and produces bivalves varieties with superior performance, respecting environmental sustainability and ensuring consumer safety.

There are a few breeding programmes for bivalve species in the United States, Australia, New Zealand, and France. These programmes have mainly focused on oysters and their diseases. In clams, there are some studies dealing with the heritability of growth in *R. philippinarum* (Manila clam), mostly at a research level (Huo et al. (2016) and Zhao, et al. (2012)). However, presently and to our knowledge, there is not one single commercial breeding program for European native clams. Despite the clam market being larger and having a higher price per kilogram than the oyster market, the clam aquaculture sector is still very rudimentary – i.e. made up of small players with little capacity to invest in R&D or verticalize their business. Thus, a clear business opportunity exists.

The market potential of high-value clams was confirmed directly by Oceano Fresco's team during a field study conducted when the company was launched in key aquaculture geographies (Portugal, Spain, Italy, China, South Korea, US and Norway) as mentioned above. We have since then in-depth validated that there is material commercial value to have both our target species (*Rudipates decussatus* and *Venerupis corrugata*) available at the same time to create scale and productivity. This is because of technical fit reasons (ie. some producing areas or plots are more suitable to produce either submerged or partially submerged clams) and also commercial reasons driven by geo consumption patterns impact on sales and distribution coverage (ie. some areas consume both while others only one of the species).

On its State of the World's Aquatic Genetic Resource for Food and Agriculture report of 2019, FAO concluded that there is a tremendous opportunity to sustainably improve aquaculture productivity through the widespread adoption of genetic improvement "with a focus on well-managed and long-term selective breeding for continuous genetic improvement programs, into which other genetic technologies can be integrated".

Oceano Fresco and its R&D partners will conduct a selective breeding program that will allow a sharp increase in the production of high-quality and high market-value clam species – *R. decussatus* and *V. corrugata*, resulting in the sustainable improvement of aquaculture production. This will be done at EUROCLAM project, where various cutting-edge tools and methodologies will be applied.

This project started in January 2021 and has a duration of 2 years.

Materials and Methods

At EUROCLAM project the following work packages are planned: i) production optimization of elite stocks of *Ruditapes decussatus* and *Venerupis corrugata*; ii) Large-scale test model for *Perkinsus olseni* resistance; iii) Development of genomic tools for *R. decussatus*; iv) GWAS experiments and genotyping and update of breeding plan for *R. decussatus*; v) Commercialization, communication, and management.

Results and discussion

At the end of the EUROCLAM project, it is expect to have (1) applied a science-based approach, at the industrial level, for breeding and cultivation of European clams; (2) developed a large-scale test model for the clam pathogen *Perkinsus olseni*; (3) sequenced the genome of a European clam and develop several genomic tools for selection; and (4) implemented an industrial breeding plan for *Ruditapes decussatus*.

This blue growth project will increase the production of a high nutritional protein, restoring and preserving biodiversity, and contributing for non-damaged ecosystems.

(Continued on next page)

Acknowledgements

This research is supported by the project 'EUROCLAM – Unlocking the potential of Euro-native bivalves' (CENTRO-01-0247-FEDER-113950) co-financed by CENTRO 2020, PT2020 and Fundo Europeu de Desenvolvimento Regional.

References

Huo et al. (2016). Growth performance of larval and juvenile manila clam (*Ruditapes philippinarum*) from divergently selected individuals of a full-sib family. Journal of Ocean University of China, 15(6), 1046-1050.

Zhao, et al. (2012). Divergent selection for shell length in the Manila clam, *R. philippinarum*. Journal of the World Aquaculture Society.

SHELLFISH REGULATORY ECOSYSTEM SERVICES—POTENTIAL FOR NUTRIENT CREDIT TRADING

A.M. Cubillo[1*], S.B. Bricker[2], J.G. Ferreira[1,3], A.S. Lopes[1], H. Moore[4], M. Service[4]

[1] Longline Environment Ltd., 88 Wood St, London, EC2V 7RS, United Kingdom
[2] National Oceanic and Atmospheric Administration (NOAA), National Ocean Service, National Centers for Coastal Ocean Science, 1305 East West Highway, Silver Spring, MD, United States
[3]DCEA-FCT, NOVA, Monte de Caparica 2825-516 Portugal
[4] Fisheries and Aquatic Ecosystems Branch, Agri-Food and Biosciences Institute (AFBI), 18A Newforge Lane, Belfast BT9 5PX, United Kingdom

* Corresponding author, alhambra.cubillo@longline.co.uk

Introduction

Nutrient-related water quality degradation is a global challenge to coastal waterbody health. Bivalve aquaculture can help reduce impacts of eutrophication through filtration, which removes particulate organic material (POM). Some filtered POM is assimilated into tissue and shell, and rest is expelled as faeces, pseudofaeces and ammonia. The potential contribution of bivalve aquaculture to reduction of nutrient impacts in European (EU) coastal waters was evaluated to develop a framework for nutrient credit trading in the EU that will include bivalve shellfish producers. An economic valuation of the removed nutrients was also made to show the value within the larger context of the shellfish industry and nutrient management, and to show the potential compensation that might be paid to shellfish producers for the nutrient removal service provided, if they were included in a nutrient credit trading program.

Methods

Nutrient Loads: Data for nutrient loads to Regional EU Seas were compiled, updating a previous evaluation (Ferreira and Bricker, 2016). Sources included: (i) Skarbøvik et al. (2013) for Norwegian and Barents Seas; (ii) HELCOM (2015, 2018) for the Baltic Sea; (iii) OSPAR (2017) for the Greater North Sea, Celtic Seas and Bay of Biscay; and (iv) Bouraoui et al. (2011) for Greater North, Black, and Mediterranean (Med) Seas.

Shellfish production and associated nutrient removal: Bivalve production data for blue mussel, Med mussel, Pacific oyster, European oyster, and Manila clam were sourced from META (Longline Environment Ltd, 2020) and Eurostat (Eurostat, 2020) websites. Net nutrient removal was estimated in two ways: (i) through elemental analysis of shellfish flesh and shell for each species which was then scaled to production, and (ii) by application of the Farm Aquaculture Resource Management (FARM) model. The model calculates nutrient removal and potential economic value, providing a substitution or 'avoided' cost of land-based nutrient removal that would serve as additional revenue to the farmer in a nutrient credit trading program (Cornwell et al. 2016; Ferreira and Bricker 2016; Bricker et al. 2018).

Scaling nitrogen removal to country and European scale: Nitrogen (N) removal estimates were upscaled to estimate bioextraction potential and associated economic value for the regulatory ecosystem service provided by each shellfish species at country and EU level. Total N removal by shellfish aquaculture was compared to total N load to EU Seas.

Table 1. Nitrogen loading to European seas in 2018 and N removed by shellfish harvest at each area as percentage of total loads. Percentages were calculated based on the country of production.

	Nitrogen loading ($t\ y^{-1}$)	Nitrogen removal (%)	
		Elemental analysis	FARM
Norwegian Sea	64 600	-	-
Barents Sea	11 010	-	-
Baltic Sea	601 900	-	-
Greater North Sea	1000 000	0.05%	0.06%
Celtic Seas	275 000	0.06%	0.09%
Bay of Biscay and Iberian Coast	450 000	0.54%	0.89%
Black Sea	700 000	-	-
Mediterranean Sea–Europe only	950 000	0.16%	0.37%
Total	4052 610	0.12%	0.22%

(Continued on next page)

Results and Discussion

Nutrient Loads: The total N load to EU seas was 4777 × **10**^{3} tonnes (t) y^{-1} (Table 1). The Greater North Sea (1500 × 10^3 t y^{-1}), Med Sea (950 × 10^3 t y^{-1}) and Baltic Sea (826 × **10**^{3} t y^{-1}) received the highest loads, while the Arctic region received the lowest: Norwegian Sea (65 × 10^3 t y^{-1}) and Barents Sea (11 × 10^3 t y^{-1}).

Shellfish production and associated nutrient removal: The five species selected for this study accounted for >95% of total EU farmed shellfish production and 40-45% of all farmed aquatic organisms (Eurostat, 2020). The largest EU producers were Spain and France. Spanish bivalve production was 42% of EU output, dominated by Med mussel, while France accounted for 24% of EU farmed bivalves with Pacific oysters as the main species (65% in 2018), followed by blue mussels (31%).

Scaling nitrogen removal to country and European scale: The total estimated N removed by farmed bivalves in EU Seas was 8.9 × **10**^{3} t y^{-1} and 4.9 × **10**^{3} t y^{-1} by the FARM model and elemental analysis, respectively. Comparison of removal estimates by the two approaches shows that FARM model results were higher for all species except blue mussel culture for which estimates showed good agreement. Both approaches showed the bulk of N removed was attributed to Med mussel aquaculture (between 61 and 68%). Pacific oysters were the second largest contributor to N removal, 8.0-15% of total, depending on approach. Spain was the primary contributor to N removal due to high production of Med mussels, accounting for 45-49% of total removal depending on the approach. Italy and France each account for 15-20% of total N removal. The elemental analysis approach showed Med and blue mussels had the highest capacity for N removal, reaching ~9-10 t of N removed per 1000 t harvested. Oysters and Manila clams ranged from 2.9-3.7 t of N removed per 1000 t harvested. The modelling approach showed that Manila clams have greatest removal capacity followed by flat oyster and Med mussel, which had similar removal.

Shellfish production in the EU removed between 0.12% (elemental) and 0.22% (FARM) of total 2018 N inputs to European Seas (Table). This varies by waterbody; N removal in the Greater North Sea is ~0.05% of inputs, while in the Bay of Biscay and Iberian coast it was as high as 0.89% due mostly to Med mussel production. While this seems small, the estimated avoided cost value of removed N, using the Virginia Chesapeake Bay Nutrient Credit Exchange Association value of US$8.33 (6.89€) per credit, is 34-61 million € or ~3-6% of the total production value (974 million €). This could be compensation to growers if they were included in a nutrient credit trading program. If shellfish growers are not included, this valuation could still enhance public awareness of water quality issues, and could help shift attitudes to allow increased opportunities for shellfish aquaculture that would improve water quality, especially helping reduce difficult to manage non-point sources, and stimulate local economies.

References

Bouraoui F, et al. (2011) Long term nutrient loads entering European seas, EUR 24726 EN, Publications Office of the European Union, Luxembourg, 2011, ISBN 978-92-79-19319-4, doi:10.2788/54513, JRC 62873. 82 pp.

Bricker SB, et al. (2018) The role of shellfish aquaculture in reduction of eutrophication in an urban estuary. Environmental Science & Technology 52 (1): 173– 183.

Cornwell J, et al. (2016) Panel recommendations on the oyster BMP nutrient and suspended sediment reduction effectiveness determination decision framework and nitrogen and phosphorus assimilation in oyster tissue reduction effectiveness for oyster aquaculture practices. Report to the Chesapeake Bay Program. http://www.chesapeakebay.net/documents/Oyster_BMP_1st_Report_Final_Approved_2016-12-19.pdf. Accessed 4 Jan 2021.

Eurostat. (2020). Eurostat. Retrieved 12 October 2020, from https://ec.europa.eu/eurostat/data/database. Accessed January 2021.

Ferreira, JG & SB Bricker. 2016. Goods and services of extensive aquaculture: shellfish culture and nutrient trading. Aquacult. Int. 24:803–825.

HELCOM (2015) Updated Fifth Baltic Sea pollution load compilation (PLC-5.5). Baltic Sea Env. Proceedings No. 145.

HELCOM (2018) Sources and pathways of nutrients to the Baltic Sea. Baltic Sea Env. Proceedings No. 153.

Longline Environment Ltd. (2020). META - Maritime and Environmental Thresholds for Aquaculture. Retrieved 12 March 2021, from www.longline.co.uk/meta.

OSPAR (2017) Eutrophication Status of the OSPAR Maritime Area. OSPAR Commission, London, OSPAR publication no. 694/2017, ISBN: 978-1-911458-34-0, 166 pp.

Skarbøvik E, et al. (2013) Riverine inputs and direct discharges to Norwegian coastal waters – 2012, Norwegian Institute for Water Research Report No. M-80/2013, ISBN 978-82-577-6319-0.

COMPARATIVE LIFE CYCLE ASSESSMENT OF SEMI-INTENSIVE AND EXTENSIVE POND IMTA AND SEMI-INTENSIVE CONVENTIONAL FISHPOND PRODUCTION

M.E. Cunha[1]*, E. Malta[2], B. Partida[2], M.M. Agraso, L. Ribeiro[1]

[1] - IPMA - Instituto Português do Mar e da Atmosfera, Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
[2] - CTAQUA- Andalusian Aquaculture Technology Center, Muelle Comercial s/n, 11500, El Puerto de Santa María (Cádiz), Spain
E-mail: micunha@ipma.pt

Introduction

In southwest Iberia extensive and semi-intensive fish culture in earthen ponds are traditional activities in saltmarsh. The most used commercial fish species are gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Since most of this activity takes place in protected areas, intensifying the production is out of the question, however increasing product diversification with IMTA, may provide an answer by creating a balanced system for environmental sustainability of earthen ponds while increasing their profitability and social acceptability. Life cycle assessment (LCA) was used to evaluate the environmental footprint of semi-intensive and semi-extensive pond IMTA in comparison to semi-intensive conventional fishpond production (POLY).

Methods

The studied systems:

Species	Semi-intensive IMTA [# pond^{-1}/MW]	Semi-extensive IMTA [# pond^{-1}/MW]	Semi-intensive POLY [# pond^{-1}/MW]
Argyrosomus regius	2,700/175	-	8,000/9.5
Diplodus sargus	500/236	-	-
Sparus aurata	-	1,500/20	500/29.4
Mugil cephalus	320/268	-	-
Crassostrea gigas	24,000/1.46	44,875/0.32	
Ulva spp.	+	+	-
Final fish density (kg m^{-2})	2.5	0.1	2.5
Final oyster density(kg m^{-2})	1	0.5	-

- number; MW – Individual mean weight in gram

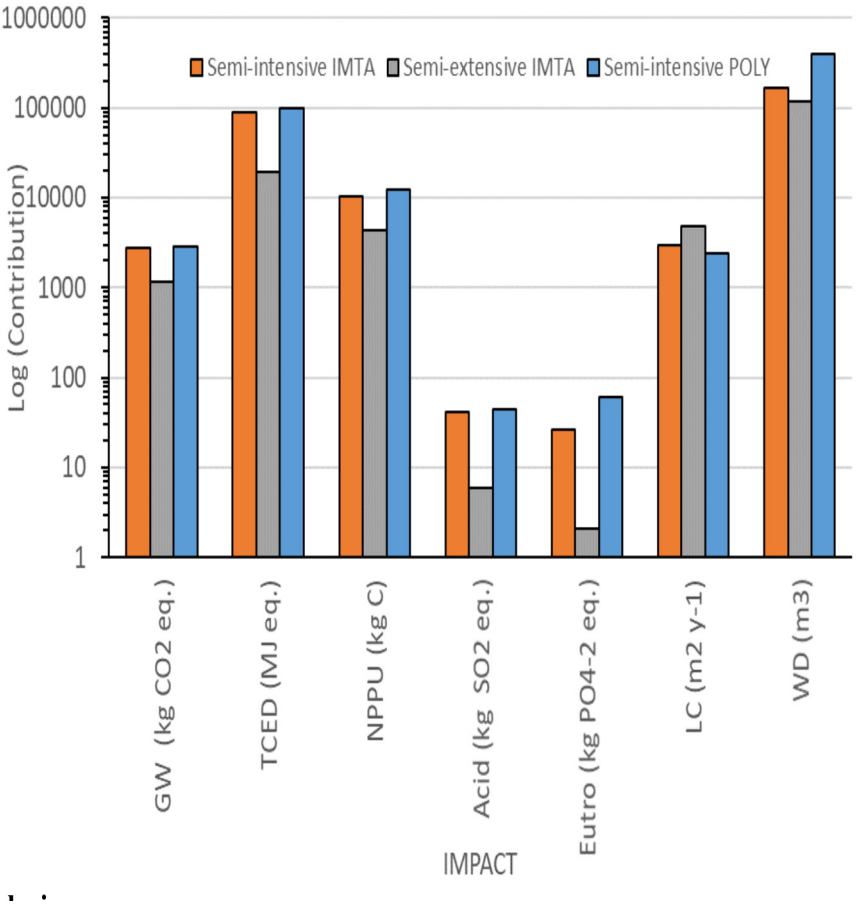
Scenario data for the systems considered in the study:

	Semi-intensive IMTA	Semi-extensive IMTA	Semi-intensive POLY
CYCLE LENGTH (months)	5.3	8.5	26
INPUTS			
Feed (kg)	1,888	89	10,975
Used sea water (m^3)	101,110	7,185	1,201,764
Fish biomass weight (kg)	677	45	91
Oyster flesh biomass weight (kg)	5.5	14.4	-
Main infrastructure components			
Total farm area (m^2)	90,000	285,000	90,000
Total farm pond area (m^2)	23,600	200,000	23,600
Area of production ponds (m^2)	700	665	1,700
OUTPUTS			
Fish biomass gain (kg)	1,099	65	6,137
Oyster flesh biomass gain (kg)	130	94	-
Associated downstream production			
Macroalgae biomass gain (kg)	1,224	35,400	-
Zootechnical performance			
Fish feed conversion ratio	1.72	0.56	1.79

The impact categories:

Global Warming (GW) in kg CO_2-equivalents, Net Primary Production Use (NPPU) in kg C, Total Cumulative Energy Demand (TCED) in MJ-equivalents, Acidification (Acid) in kg SO_2-equivalnets, Eutrophication (Eutro) in kg PO_4^- equivalents, Land Competition (LC) in m^2, and Water Dependence (WD) in m^3.

(Continued on next page)



Results

Comparative contributions of different impact categories of each rearing system calculated for 1 tonne of aquatic products as functional unit (Fig. bellow) show that eutrophication (Eutro), acidification potential (Acid) and total cumulative energy demand (TCED) showed the largest difference between the semi-intensive and semi-extensive production systems. Except for land competition (LC), IMTA systems had lower contribution to the impacts when compared to the polyculture system. This was most noticeable for the semi-extensive IMTA system that had the lowest impacts. The impact of the semi-intensive IMTA system on global warming was only slighter lower than that of the polyculture semi-intensive system. However, eutrophication and water dependence were also much lower.

Conclusions

Conclusions from the environmental impact study of pond integrated multi-trophic aquaculture (IMTA) in semi-extensive and semi-intensive systems in comparison to semi-intensive fishpond polyculture show that:

With exception of land competition, semi-extensive IMTA has an overall lower environmental impact compared to semi-intensive IMTA and semi-intensive fish polyculture,

To produce the same amount of fish biomass the semi-intensive IMTA is much less eutrophic and water dependent than semi-intensive fish polyculture,

The semi-intensive IMTA used less feed than the semi-intensive fishpond polyculture to produce the same amount of seafood biomass.

Acknowledgements

The authors want to acknowledge INTERREG V A Espanha Portugal (POCTEP) program, project 0750_AQUA_AMBI_2_5_P – Apoio à gestão das zonas húmidas do litoral do Sudoeste Ibérico: interação entre a Aquacultura e o meio Ambiente na região transfronteiriça Alentejo-Algarve-Andaluzia – FASE 2

INTERACTIVE EFFECTS OF YGEIA+ ON THE EUROPEAN SEABASS (*Dicentrarchus labrax*) HEALTH CONDITION AND INFLAMMATORY RESPONSE

A. Cunha[1,2*], P. Santos[1,2,3], I. Ferreira[2,4], C. Teixeira[1,2,6], A. Afonso[1,2], S. Magalhães[5], T. Aires[5], E. Matos[5,7], B. Costas[1,2]

[1] Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[2] Centro de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal
[3] Instituto Politécnico de Leiria, Peniche, Portugal
[4] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.
[5] Sorgal S.A, São João de Ovar, Portugal.
[6] SPAROS Lda., Olhão, Portugal
[7] B2E Associação para a Bioeconomia Azul – Laboratório Colaborativo, Leça da Palmeira, Portugal
* E-mail: andre.cunha.96@gmail.com

Introduction

Nutrition can have significant health implications for animals and, particularly in fish, best practices on diet formulation are of major importance, as feeds probably represent the leading expenditure to the aquaculture industry (Kiron, 2012). The term functional or fortified feeds is used to describe fish feeds that have added benefits beyond the fish essential nutritional requirements, with both health status and growth expected to improve (Li et al., 2009). Therefore, a shift away from chemotherapeutic and antibiotic treatments would be possible. Among functional commercial feeds like Ygeia+ (Sorgal), which are characterized as capable of providing the required nutrients for normal development, but also giving additional benefits, there is still a need to better understand their modulatory effects on immune response and disease resistance.

The aim of the present study was two-fold: i) to study the effects of Ygeia+ short-term feeding on immune-condition and oxidative stress status of European seabass (*Dicentrarchus labrax*); and ii) to assess the interactive effects of short-term feeding with Ygeia+ and vaccination in the immune response and disease resistance of European seabass.

Material and Methods

Two independent trials were performed with European seabass weighting 12-36 g in recirculating seawater systems (temperature 18 ºC; salinity 35; photoperiod 12L:12D). In both trials fish were fed for 5 and 10 days a control (commercial) diet or the Ygeia+ diet, both produced and provided by Sorgal. In the first trial, fish were inoculated with heat-inactivated *Photobacterium damselae piscicida* (1×10^6 cfu/mL) at the end of each feeding time, and the inflammatory response was assessed for 4, 24 and 48h. In the second trial, fish were vaccinated with AVAC VR/PD/TM (HIPRA) or sham injected after each feeding period. Following vaccination procedures, all fish were fed the control diet for 3 weeks and sampled for the assessment of immune parameters and oxidative stress biomarkers. Fish were also bath challenged with *Tenacibaculum maritimum* to evaluate if Ygeia+ can induce protection and the synergistic dietary effects with the vaccine.

Results

The first trial pointed to a positive effect of Ygeia+ with a tendency to increase circulating monocyte and neutrophil numbers following inflammation in fish fed Ygeia+ for 5 days. Neutrophilia and monocytosis tended to correlate well with the increase of plasma lysozyme at 4 h following an inflammatory insult. Humoral lysozyme levels also tended to be higher in Ygeia+ fed animals during all sampling points. Lipid peroxidation was significantly lower in Ygeia+ fed animals, when compared with control at 24 hours after inflammation, in animals fed for 5 days. Superoxide dismutase activity was also significantly lower in Ygeia+, in the same sampling time.

During the second trial, specific IgM augmented in all vaccinated groups, which also showed improved survival compared to non-vaccinated individuals. Moreover, vaccinated seabass fed Ygeia+ for 10 days increased total glutathione levels in liver, compared to their counterparts fed the control diet.

Discussion

In summary, functional diets such as Ygeia+ are important sustainable prophylactic strategies that deserve further attention, particularly regarding conditions where oxidative stress may occur. Indeed, the observed lower levels of lipid peroxidation and superoxide dismutase can be linked to a positive effect of Ygeia+ in the reduction of free reactive oxygen species in the liver. Considering all data from both studies, other feeding times should be considered to fine-tune Ygeia+ as a feeding strategy to improve European seabass robustness.

(Continued on next page)

Acknowledgments

This study was supported through projects PP-IJUP2019-SOJADEPORTUGAL-24 and UID/Multi/04423/2020, partially supported by SORGAL, the European Regional Development Fund (ERDF) through the COMPETE2020 - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology. BC, IF and CT were also supported by FCT (IF/00197/2015, SFRH/BD/147750/2019 and PD/BDE/135541/2018, respectively).

References

Kiron V. Fish immune system and its nutritional modulation for preventive health care. Animal Feed Science and Technology (2012) 173:111–133.

Li P, Mai K, Trushenski J, Wu G. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino acids (2009) 37:43–53.

THE EFFECT OF SETTLEMENT SUBSTRATES AND FEED ON EARLY STAGE GROWTH OF THE SEA URCHIN *Tripneustes gratilla*: IMPLICATIONS FOR HATCHERY PROTOCOLS AND ECHINOCULTURE DEVELOPMENT

M.D. Cyrus[1,2]*, M. Bennett[2], B.M. Macey[1,2], V.E. Coyne[2] and J.J. Bolton[2]

[1]Department of Forestry, Fisheries and the Environment, Cape Town, 8001, South Africa
[2]University of Cape Town, 7701, South Africa
Email: MCyrus@environment.gov.za

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.

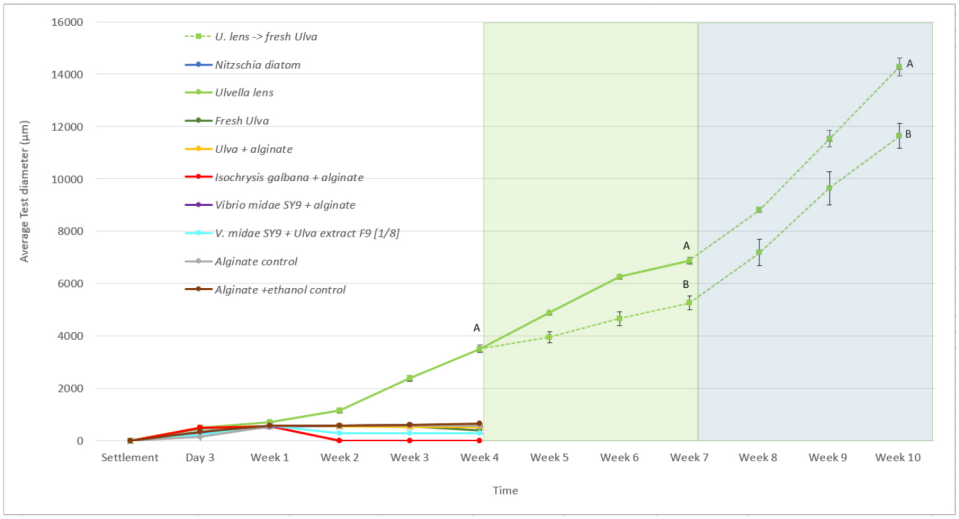


Figure 1: Average test diameter of juvenile *T. gratilla* over a 10 week period post settlement fed a variety of natural and artificial diets. Shaded areas represent dietary changes during weaning, from Ulvella to Ulva as indicated in the figure legend.

(Continued on next page)

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*; 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).

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.

References

Mos, B., Cowden, K., Nielsen, S. & Dworjanyn, S., 2011. Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. PLoS One, p. 6.

Affan, M.A., Khomayis, H.S., Lee, J.B., Al-Harbi, S.M., Touliabah, H.E.S. and Abdulwassi, N.I., 2015. Settlement and growth of larval and juvenile abalone on single and mixed strains of benthic diatoms. Thalassas: An international journal of marine sciences, 31(2), pp.59-65.Akakabe, Y. & Kajiwara, T., 2008. Bioactive volatile compounds from marine algae: feedging attractants. Journal of Applied Phycology, 20(5), pp. 211-214.

GENOME-WIDE ASSOCIATION STUDY OF INFECTIOUS PANCREATIC NECROSIS IN TWO SUCCESSIVE GENERATIONS OF RAINBOW TROUT

J. D'Ambrosio[1*], P. Patrice[1], Y. François[1], T. Morin[3], J. Cabon[3], J. Ruche[4], A. Desgranges[4], P. Haffray[1], F. Phocas[2]

[1] SYSAAF, French Poultry and Aquaculture Breeders Association, 35042 Rennes, France
[2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[3] ANSES, Ploufragan-Plouzané-Niort Laboratory, Unit Virology, immunology and ecotoxicology of fish (VIMEP), 29280 Plouzané, France
[4] SARL Milin Nevez, 29610 Plouigneau, France

Email: jonathan.d'ambrosio@inrae.fr

Introduction

In aquaculture, disease resistance is one of the main selected traits with growth performance, processing traits and flesh quality (Chavanne et al. 2016). Infectious Pancreatic Necrosis (IPN) is a highly contagious disease with vertical and horizontal transmissions (Dopazo 2020). IPN is encountered worldwide with initial episodes described in North America and Europe, but also in Asian countries since the 1980s (Dopazo 2020).

Different studies in rainbow trout have shown moderate genetic variability for IPN resistance (Rodríguez et al. 2019). However, there is little work to precisely characterize the architecture of IPN resistance in rainbow trout and analyses only included a single cohort of phenotyped fish (Ozaki et al. 2001, 2007; Rodríguez et al. 2019). Therefore, the two objectives of this study were to accurately estimate heritability of disease resistance and to detect quantitative trait loci (QTLs) associated with IPN resistance in two successive generations of a commercial French rainbow trout line.

Materials and Methods

The fish studied were produced in the 8th and 9th generations of selection (G8 and G9) of a commercial line of Milin Nevez breeding company (Bretagne Truite Group, France). In total 4,000 fish (2,000 from each generation) were challenged by immersion with IPN virus at the ANSES-SYSAAF Fortior Genetics platform. For the infection challenge in G8, a dose of approximately 1.10^5 $TCID_{50}$/mL of the strain NN193 was used in a total volume of 20L/tank. For the challenge in G9, a dose of approximately 1.10^5 $TCID_{50}$/mL of the strain NPI11125 was used in a total volume of 7L/tank. Fin samples were stored in alcohol filled jars and kept at +5°C (+/- 3°C). All the 372 parents of the phenotyped individuals as well as 1,885 and 2,000 phenotyped fish of G8 and G9, respectively, were genotyped for 57,501 SNPs with the Axiom™ Trout Genotyping array at the INRAE genotyping Platform Gentyane. After quality control, we retained for the GWAS 1,799 G8 and 1,978 G9 individuals genotyped for 25,512 SNPs. IPN resistance was calculated as a binary survival trait (S37, death/life at 37 days) and time to death (TD) with value of 40 attributed to all individuals still alive at 40 days. The average survival rate at 37 days was 61% and 78% for G8 and G9, respectively. The package BLUPf90 was used for the study with AIREMLf90 program for variance component estimation (Misztal et al. 2002) and POSTGSf90 program for GWAS (Aguilar et al. 2014).

Results

The heritability was estimated at intermediate values for the two traits: 0.20 and 0.25 for S37 and TD, respectively. Considering the joint analysis of data from the two cohorts, we identified the same two main QTLs for S37 and TD on the chromosomes 1 and 16 (Tableau 1).

Chr	Peak Position* (Mb)	Peak SNP	-log(p-value)	QTL Start (Mb)	QTL End (Mb)	% variance explained for QTL
1	11.96	Affx-88908615	8.94	10.17	17.06	3.1
16	0.30	Affx-88936423	7.96	0.30	2.75	1.2

Table 1: Summary statistics for QTL of IPN resistance (S37) detected based on GBLUP model with a threshold -log(p-value)=6.4, corresponding to a genome-wide significance threshold of 1% after Bonferroni correction.
* on the Arlee genome reference assembly (USDA_OmykA_1.1.).

(Continued on next page)

These QTLs were also observed in each of the analyses of single fish cohorts. The QTLs explained 3% and 1% of the genetic variance for chromosome 1 and 16, respectively.

In G8 cohort, the survival of fish with genotype Q_1Q_1 for Affx-88908615 is 65%, the survival of heterozygous fish Q_1q_1 is 56% and it is only 37% for q_1q_1 fish. In G9 cohort, survival rates were higher: respectively 81%, 71% and 64% for Q_1Q_1, Q_1q_1 and q_1q_1 genotypes at the peak SNP on chromosome 1. Furthermore, the combination of favorable alleles for the QTLs on chromosomes 1 and 16 allows an additional survival gain, with 87% of survival for Q_1Q_1-Q_2Q_2 fish and 73% of survival for Q_1Q_1-q_2q_2 fish in G9 cohort (respectively 72% and 60% in G8).

Discussion and Conclusion

The results show a moderate heritability for IPN resistance, similar to previous estimates in the literature. We detected two significant QTLs that are located on chromosomes 1 and 16 which were already identified in the literature as playing a role on IPN resistance (Santi et al. 2019; Ozaki et al. 2001, 2007). The QTL on chromosome 1 is located in the same region as previously described by Santi et al. (2019). The two QTLs are observed in each of the two studied cohorts and are therefore consistent QTLs in the population. The association of favourable alleles across QTLs allows an increase in survival up to 87% for G9 cohort. These QTLs offer the possibility of marker-assisted selection for rapid dissemination of genetic improvement for IPN resistance.

References

Aguilar I, et al. PREGSF90-POSTGSF90: Computational Tools for the Implementation of Single-step Genomic Selection and Genome-wide Association with Ungenotyped Individuals in BLUPF90 Programs. 10th WCGALP. 2014;Vancouver(Canada).

Chavanne H, et al. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquac Int. 2016;24(5):1287–307.

Dopazo CP. The infectious pancreatic necrosis virus (IPNV) and its virulence determinants: What is known and what should be known. Pathogens. 2020;9(2).

Misztal I, et al. BLUPF90 and related programs (BGF90). Proc 7th World Congr Genet Appl to Livest Prod. 2002;2.

Ozaki A, et al. Identification of additional quantitative trait loci (QTL) responsible for susceptibility to infectious pancreatic necrosis virus in rainbow trout. Fish Pathol. 2007;42(3):131–40.

Ozaki A, et al. Coimbra MRM, Akutsu T, et al. Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (Oncorhynchus mykiss). Mol Gen Genet. 2001;265(1):23–31.

Rodríguez FH, et al. Genome-wide Association Analysis for resistance to infectious pancreatic necrosis virus identifies candidate genes involved in viral replication and immune response in rainbow trout (Oncorhynchus mykiss). G3 Genes, Genomes, Genet. 2019;9(9):2897–904.

Santi N, Moen T, Ødegard J. US20190241980A1. 2019. p. 107.

QUANTIFICATION OF GRAZING EFFICACY, GROWTH AND HEALTH SCORE OF DIFFERENT LUMPFISH (*Cyclopterus lumpus* L.) FAMILIES: POSSIBLE SIZE AND GENDER EFFECTS

A.K.D. Imsland[1,2]*, P. Reynolds[3], T.A. Hangstad[4], L. Kapari[4], S.N. Maduna[5], S.B. Hagen[5], Ó.D.B. Jónsdóttir[1], F. Spetland[6] and K.S. Lindberg[7]

[1]Akvaplan-niva, Iceland Office, Akralind 4, 201, Kópavogur. Iceland
E-mail: aki@akvaplan.niva.no
[2]Dept. of Biosciences, Univ. of Bergen, High Technology Centre, 5020, Bergen, Norway
[3]GIFAS AS, Gildeskål, 8140 Inndyr, Norway
[4]Akvaplan-niva, Framsenteret, 9296 Tromsø, Norway
[5]Norwegian Institute of Bioeconomy Research, Svanhovd, 9925 Svanvik, Norway
[6]Lumarine AS, Stadionveien 21, 4632 Kristiansand, Norway
[7]Senja Akvakultursenter AS, Rubbestadveien 401, 9304 Vangsvik, Norway

Introduction

The biological control of sea lice using cleaner fish has become a feasible option due to the increased occurrence of resistance towards medical treatments in salmon lice, *Lepeophtheirus salmonis*. Previous studies have shown up to 93–97% less sea lice infestation (adult female lice) in sea cages with lumpfish compared to salmon in sea cages without lumpfish present (Imsland et al., 2014a). Significant individual differences in feed intake and preference for sea lice has been seen (Imsland et al., 2014a-b, 2018), and genetic influence has been suggested to be a possible factor (Imsland et al., 2016a). The present study aims to investigate the observed variation in sea lice (*L. salmonis* and *Caligus elongatus*) foraging behaviour of lumpfish to reveal potential correlations between inclination to graze sea lice and genetic composition. Previous research has indicated a size-related (Imsland et al., 2016b) and sex-related (P. Reynolds, Gifas, unpublished data) sea lice grazing of lumpfish but whether this can differ in different families of lumpfish has not been studied before.

Material and methods

To investigate the possible family influence on sea lice grazing of lumpfish on Atlantic salmon, ten families of lumpfish (N = 480) with a mean (± SD) weight of 54.8 ± 9.2 g were distributed among ten sea cages (5 × 5 × 5 m) each stocked with 400 Atlantic salmon with a mean (± SD) weight of 621.4 ± 9.2 g. All the ten cages were stocked with 48 lumpfish (12% stocking density). The stocking of cages was such that each cage consisted of two random families where full- and paternal half-sib families were randomly allocated to the different cages. During the trial period gastric lavage was performed every two weeks to assess the feeding preferences of individual lumpfish. At the end of the study, all fish from each family were arranged into 9 size classes from 40 to 230 g. The fish humanely dispatched (metacaine 600 mg l^{-1}) and dissected to determine the sex.

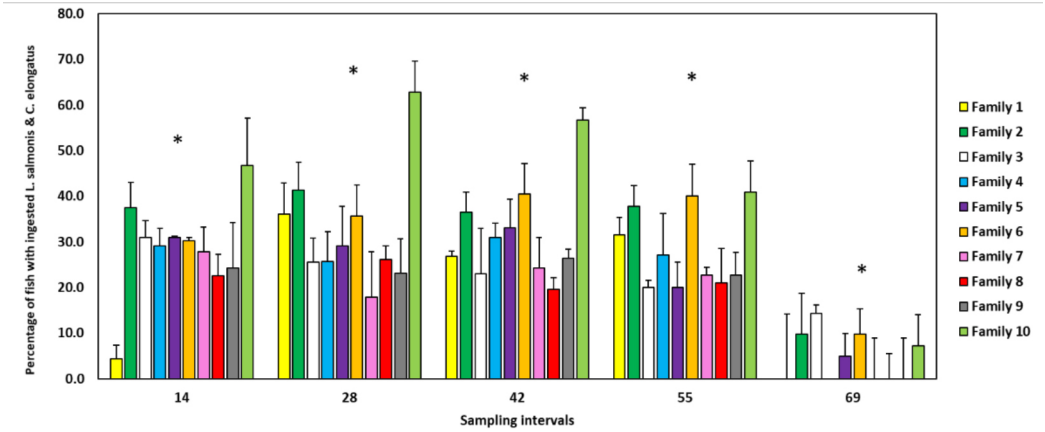


Fig. 1. Percentage values of eaten sea lice of the ten lumpfish families sampled at each sampling time point. Values are presented as means ± S.E. * indicates significant differences between the families.

(Continued on next page)

Results

Lumpfish from families 2, 6 and 10 had a significantly higher incidence of lice grazing of both *L. salmonis* and *C. elongatus* compared to the other families (Fig. 1). There was a trend for a reduction in sea lice grazing with increased size within each family. The results indicated that it was the smallest size classes of lumpfish (40-140 g) which exhibited higher sea lice grazing potential compared to the larger size classes within families. There were no clear differences in the lice grazing potential between male and female lumpfish within and between families

Discussion and conclusion

The lice grazing activity recorded during this study suggests a likely genetic effect, but influence may be from both male and female broodstock rather than an individual gender. Given that, the likely genetic effect can be used for future selection programmes for lumpfish grazing behaviour. Generally, it was found that it was the smallest size classes which exhibited higher sea lice grazing potential compared to the larger size classes.

The frequency of individuals to graze *L. salmonis* and *C. elongatus* on repeated occasions was significantly different between families. The highest percentage of repeat grazers were from families 2, 6 and 10 with the same individuals found to have consumed both species of sea lice in their stomachs in three or more of the five gastric lavage sampling points during the study period. As sea lice grazing efficacy is one trait that is strongly desirable in future breeding programmes then also the frequency of lice grazing by individuals within families should also be used as a selection criterion for such programmes.

References

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., Elvegård, T.A., 2014a. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo sala*r L.). Aquaculture 425-426, 18-23.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014b. Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433, 137-142.

Imsland, A.K., Reynolds, P., Eliassen, G., Mortensen, A., Hansen, Ø.J., Puvanendran, V., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Nytrø, A.V., Jonassen, T.M., 2016a. Is cleaning behavior in lumpfish (*Cyclopterus lumpus)* parentally controlled? Aquaculture 459, 156-165.

Imsland, A.K., Reynolds, P., Nytrø, A., Eliassen, G., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Jonassen, T.M., 2016b. Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. Aquaculture 465, 19-27.

Imsland, A.K., Hanssen, A., Reynolds, P., Nytrø, A.V., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018. It works! Lumpfish can significantly lower sea lice infections in large scale salmon farming. Biology Open 7, 7, bio036301. doi:10.1242/bio.036301

.

MORTALITY OF GOLDFISH, *Carassius auratus*, AFTER OUTBREAK OF *Gyrodactylus kobayashii* INFECTION IN SOME FARMS OF GUILAN PROVINCE, NORTHERN IRAN

J, Daghigh Roohi*, Shamsi, S, A. Dalir Ghaffari

Inland water Aquaculture research center, Iranian fisheries Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Anzali, Iran
Email: javad_daghigh@yahoo.com

Introduction: Goldfish, *Carassius auratus*, widely distributed across Eurasia, is one of the earliest fish domesticated for ornamental purposes (Tu et al., 2015). This species has been brought to Iran from eastern Asia for ornamental purposes about 85 years ago. Iranian people buy a few goldfish in a crystal bowl for the celebrating the new year known as Nowruz. For this reason in Iran goldfish is regularly cultured in some of the warm-water fish ponds (Daghigh Roohi et al., 2016). The purpose of this study was to determine the cause of gradual daily losses in three goldfish farms located in Rasht the capital of Guilan Province, northern Iran.

Material and methods: During November 2018 a total of 58 fish with an average weight of 7.7 gr and a total length of 7.28 cm were sampled from farms with losses and transferred to the laboratory with water from the same pools. The fish were kept in several aquariums equipped with aeration systems until the survey. After preparing wet mounts of skin and gills Gyrodactylid parasaites identified with significant prevalence and severity. *Gyrodactylus* isolates were fixed by Ammonium picrate glycerin (Malmberg, 1970). The infection rate and intensity of parasites were determined according to Bush et al. (1998). In order to morphometric assessments on captured images, Image J software were used for 16 point to point distances. Drawing of parasites were done by drawing tube and then compared by identification keys and parasites identified. For molecular investigation the genomic DNA was extracted from one parasite specimen and 5.8SrDNA and ITS2 region of *Gyrodactylus* specimens were amplified by related primers in PCR (Plaisance *et al*., 2005). Purified fragments of PCR products were sequenced from both forward and reverse sites of each PCR product. The acquired sequences were subjected to a nucleotide Basic Local Alignment Search Tool (BLAST) in NCBI GenBank to confirm the *Gyrodactylus* species.

Results: In this study, the infection of fish with *Dactylogyrus* spp. and *Trichodina* sp. were 25.8% and 39.6% respectively. The intensity of infection with these parasites were limited and not dangerous. On the other hand 70.71% of the fish were infected with *Gyrodactylus* on their skin. Morphometric evaluation of these parasite showed that its species was *Gyrodactylus kobayashii* and molecular research also confirmed this. The obtained sequence was deposited in GenBank with accession number MZ148586. The number of these parasites was counted in each fish. The range of *G. kobayashii* in studied fish was 1-379 worms per fish.

Discussion and Conclusions: In this survey although *Dactylogyrus* and *Trichodina* parasites were also isolated from the studied fish, but the prevalence and severity to *Gyrodactylus* parasites showed that it is the main cause of mortality in gold fishes. Investigation showed 70.71% of fishes were infected to *Gyrodactylus kobayashii* , the mean and maximum intensity of infection was 26 and 379 parasites per fish respectively. Although Gyrodactylids are potensially highly pathogenic (Bakke et al., 2002; Jalali et al., 2005) the pathogenicity of different *Gyrodactylus* species is variable and parasite induced host death is clearly dependent on host species and size (Bakke et al., 2007). In 2015 a report was published on massive goldfish mortality (79.4% of initial stocked biomass) due to the outbreak of *Gyrodactylus kobayashii* from central China. The mean intensity of *G. kobayashii* in goldfish in China was 264.7 by range 100-450 in each fish (Tu et al., 2015). In this study the number of goldfish losses was lower than China, which seems to be due to the lower intentensity of parasitic infection.

(Continued on next page)

References:

Bakke, T. A., Soleng, A., Harris, P., (2002). Host specificity dynamics: Observations on Gyrodactylid monogeneans. International Journal of Parasitology, 32: 281-308.

Bakke, T. A., Cable, J., Harris, P. D., (2007). The biology of Gyrodactylid monogeneans: the "Russian doll killers". Advance Parasitology, 64: 161-376.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A.W., (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology, 83(4), 575-583.

Daghigh Roohi, J., Ghasemzadeh, K., Amini, M., (2016). Occurrence and intensity of parasites in goldfish (*Carassius auratus* L.) from Guilan province fish ponds, north Iran. Croatian Journal of Fisheries 74, 20-24. DOI: 10.1515/cjf-2016-0004

Jalali, B., Shamsi, B., Barzegar, M., (2005). Occurrence of *Gyrodactylus* spp. (Monogenea: Gyrodactylidae) from Iranian freshwater fish. Iranian Journal of Fisheries Sciences, 4:19-30.

Malmberg, G (1970). The excretory systems and the marginal hooks as a basis for the systematic of *Gyrodactylus* (Trematoda, Monogenea). Arkiv for Zoology, 23(1/2): 1-235.

Plaisance, L., Timothy, D., Littlewood, J., Olson, P. D. and Morand, S (2005). Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of Indo-WestPacific butterflyfish hosts (Perciformes, Chaetodontidae). The Norwegian Academy of Science and Letters 2005 • Zoologica Scripta,34, 4, July 2005, pp425–436. doi:10.1111/j.1463-6409.2005.00191.x

Tu, X., Ling, F., Huang, A., Wang, G., (2014). An infection of Gyrodactylus kobayashii Hukuda, 1940 (Monogenea) associated with the mortality of goldfish (*Carassius auratus*) from central China. Parasitology Research 114: 737-745. DOI: 10.1007/s00436-014-4241-x

AN ATTEMPT AT WHOLE GENOME SEQUENCE IMPUTATION IN PACIFIC OYSTER (*Crassostrea gigas*)

B.S. Dagnachew[*], Lamy, J.B[#], Degremont, L.[#] Maurouard, E.[#], Heurtebise, S.[#] and Aslam, M.L.[*]

*Nofima, Osloveien 1, 1430 Ås, Norway;
[#] Laboratoire de Génétique et Pathologie des Mollusques Marins, Ifremer, France
Email: binyam.dagnachew@nofima.no

Introduction

Use of whole genome sequence data (WGS) is expected to improve identification of quantitative trait loci (QTL) and prediction of genomic breeding values (Van den Berg et al., 2019). However, sequencing of large number of individuals is not cost efficient. An alternative is to use genotype imputation. It involves whole genome sequencing of few key individuals, while most individuals are genotyped with a small subset of genome-wide distributed markers (low-density genotyping panel). These sequence data are then used to impute the non-genotyped markers for the individuals genotyped at low-density. The objective of this study was to determine accuracy of WGS imputation in Pacific oyster (*Crassostrea gigas*) and their application for genome-wide association studies (GWAS) and genomic predictions.

Materials and Methods

Whole genome sequence data were available from 67 individuals (10 grandparents, 22 F1 and 35 F2) and 1,530 F2 individuals were genotyped with a lower density SNP array. After genotype quality control, the WGS data contained ~365K markers and the genotype array had ~11K markers. The 11K genotypes were then imputed to WGS using the 67 sequenced individuals as a reference. Beagle V5.0 (Browning and Browning, 2016) software was used for the imputation. Survival phenotypes (dead or alive) from a natural filed outbreak of Oyster Herpes Virus (OsHV-1) were available from 1,530 F2 individuals which belong to 8 families.

Two sets of genomic breeding values (GEBV) were estimated using the 11K SNP array and imputed SNPs. The following mixed model was fitted into the data using ASReml 4.0 (Gilmour et al., 2015):

$$y = X\beta + Zu + e$$

Where is a vector of phenotypes coded as 0 and 1 for dead and survived respectively, is a vector of fixed effect of the mean, is a vector of breeding values, is a vector of random residual effects and are incidence matrices. It is assumed that and , where is the genomic relationship matrices, is additive genetic variance and is random residual variance. Accuracy of prediction was computed using a cross-validation scheme by masking the phenotypes of ~ 20% of the offspring. The cross-validation process was repeated 20 times and the accuracy was computed as the correlation of the estimated breeding value with the phenotype weighted by the square root of heritability. GWAS was performed using the two SNP data sets by applying the same model above in GCTA program (Yang et al., 2011) with first 4 PCAs included as covariate and the SNP effects were also computed.

Results and Discussion

Imputation of 356K markers from 11K SNPs resulted an average Beagle R^2 of 0.52 (Figure 1) and only ~82K SNPs had a Beagle R^2 value higher than 0.6. Given the structure of the dataset, the observed imputation accuracy is lower than expected. This could be as a result of errors in linkage map due to small size of families used to construct linkage map, species with high level of individuals variation for ploidy, repeat components and structural variation, and sub-optimal selection of markers for the SNP array. For example, comparing the genetic maps of sequence data and array data reveled that only the middle section of most of the chromosomes is covered by the array data and that might have contributed for the observed lower imputation accuracy. In addition, it was also observed low pairwise linkage disequilibrium (LD) and faster decaying LD, which indicates that there are low number of shared haplotypes in this population and possible mapping errors. However, compared to the 11K, use of imputed WGS data amplified a putative QTL region and led to identification of more genomic regions associated with OsHV-1 resistance. On the other hand, use of imputed WGS data did not improve genomic prediction accuracy compared to 11K. The results pointed out that even if the use of imputed WG sequence improved the power of QTL detection, improving the genomic resources of this species, such as better genome resources, would help to improve imputation accuracies and hence use of genomic data in this species.

(Continued on next page)

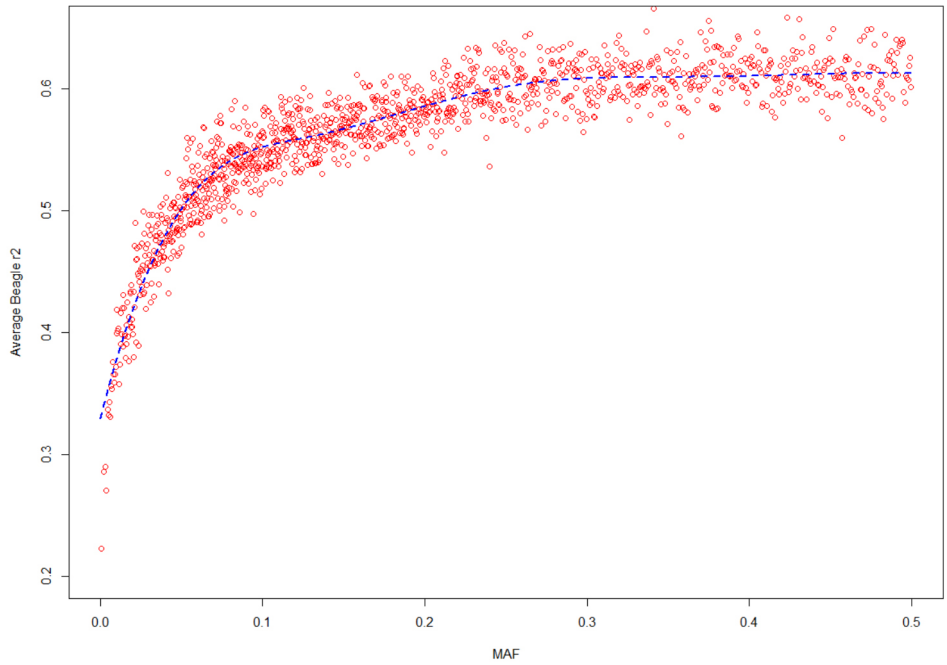


Figure 1: Imputation accuracy measured as average Beagle $R^{2.}$

References

Browning, S. R., and B L Browning (2016). Genotype imputation with millions of reference samples. Am J Hum Genet 98:116-126.

Gilmour AR, Gogel BJ, Cullis BR, Welham SJ, Thompson R (2015): ASReml User Guide Release 4.1 Structural Specification. In. Hemel Hempstead, UK: VSN International Ltd;

van den Berg, S., Vandenplas, J., van Eeuwijk, F.A. *et al. (2019)* Imputation to whole- genome sequence using multiple pig populations and its use in genome-wide association studies. *Genet Sel Evol* **51,** 2

Yang J, S.H. Lee, M.E. Goddard, P.M. Visscher (2011): GCTA: A tool for genome-wide complex trait analysis. *Am J Hum Genet*, **88**:76-82.

SELECTIVE GENOTYPING STRATEGIES FOR GENOMIC SELECTION FOR A DISEASE RESISTANCE TRAIT

B.S. Dagnachew*, A. Norris# and A. Sonesson*

*Nofima, Osloveien 1, 1430 Ås, Norway
#MOWI Genetics AS, Sandviksbodene 77A, NO-5035 Bergen, Norway
Email: binyam.dagnachew@nofima.no

Introduction

Genomic selection can increase genetic gain in aquaculture breeding for many traits, especially for traits which are not measured on the candidates themselves such as disease resistance traits. However, its implementation in this industry has been challenged by staggering genotyping cost due to many individuals needed to genotype. The aim of this study was to examine the potential of selective genotyping of reference population for genomic selection for a sib-evaluated binary trait using in silico approach.

Materials and Methods

Survival data from 7,298 belonging to 348 full-sib families from MOWI population challenged with salmonoid alphavirus (SAV3) were available. study Families with family size of greater than 20 were selected (there were 225 families). All individuals were genotyped with the customized NOFSAL3 ~55k SNP. Even though, genotype data for all individuals were available, for this study the genotypes of a varying fraction of reference sibs were masked according to the selective genotyping strategy.

There were five selective genotyping strategies tested: 1) *Full Genotyping (**FG**)*: Genotyping all training sibs. 2) *Top-Bottom Genotyping (**TBG**)*: genotyping of 10 training sibs per family, aiming at 5 dead and 5 live sibs. For families with less than 5 sibs of one category, additional sibs of the other category were genotyped. 3) *Minor Category Genotyping (**MCG**):* selective genotyping of sibs with the least common (i.e., minor) binary category phenotype. 4) *Random Across family genotyping (**RAG**):* genotyping of randomly selected sibs across families. 5) *Random Within family Genotyping (**RWG**):* genotyping of 10 random sibs per family regardless of their phenotype. The average number of training sibs to genotype by the different genotyping strategies were FG = 4,055, TBG = 2,250, MCG = 745, RAG = 2,250 and RWG = 2250. The number of genotyped individuals in MCG strategy was low due to there are many families with mortality rate higher than 90%.

The performance of three were tested, that is, Best Linear Unbiased Predictions of breeding values using pedigree-based relationships (PBLUP), genomic relationships for genotyped individuals only (GBLUP) and a single-step approach (ssGBLUP) using both pedigree and genomic. were estimated using BLUPF90 (Misztal et al., 2018) by fitting the following mixed linear model:

where is a vector of phenotypes coded as 0 and 1 for dead and survived fish respectively, is a vector of fixed effect of the sex (2 levels), 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, combination of pedigree and genomic, and pedigree-based relationship matrices respectively, is additive genetic variance and is random residual variance.

Prediction accuracy and the bias of the prediction models were assessed based on 100 replication of a cross-validation scheme. In the cross-validation, the phenotypes of 20% of individuals from each family were masked and their breeding values were estimated using the genotypes and phenotypes of the reference population. 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 trait (0.32). The bias was computed as the regression coefficient of phenotypes on either PEBV or GEBV. A regression coefficient that deviates from unity indicates either inflation or deflation of the breeding values. The means and standard errors for the prediction accuracy and biases were calculated from the 100 replications.

(Continued on next page)

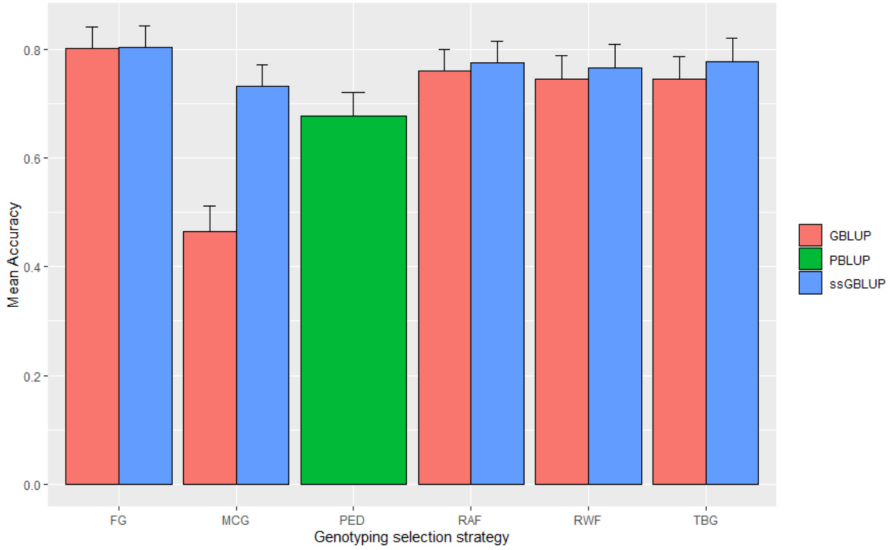


Figure 1: Mean prediction accuracy for the different selective genotyping strategies

Results and Discussion

Prediction accuracies for the different selective genotyping strategies are shown in Figure 1. As expected from the large family sizes, the average prediction accuracy for the classical family-based evaluation (i.e., PBLUP) was moderate, 0.67. The prediction accuracies for the different genotyping selection strategies were between 0.46 - 0.80 when standard GBLUP is used, and between 0.73 - 0.81 when ssGBLUP is implemented (Figure 1). Genomic models, except MCG with GBLUP, were found to increase prediction accuracies compared to PBLUP. For all the selection strategies, the prediction accuracy of ssGBLUP models were higher than the respective GBLUP models (Figure 1). This is because the ssGBLUP models use all the phenotype information by combing data from genotyped and ungenotyped individuals (Legarra et al., 2009). When applying ssGBLUP, the loss of prediction accuracy by genotyping a fraction of test sibs was limited for RAF, RWF and TBG approaches.

The prediction bias was lowest with the FG, RAG and RWG for both GBLUP and ssGBLUP models, while MCG and TGB strategies resulted in deflated GEBV. The results showed that selective genotyping strategies in combination with ssGBLUP approach could be used to minimize genotyping cost without compromising genomic prediction accuracy significantly.

References

Legarra, A., Aguilar, I., and Misztal, I. (2009). A relationship matrix including full pedigree and genomic information. *J. Dairy Sci*. 92, 4656–4663.

Misztal, I., S. Tsuruta, D. A. L. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica (2018). Manual for BLUPF90 family programs. University of Georgia

INCREASED SALINITY SIGNIFICANTLY AFFECTS PHYSIOLOGICAL AND IMMUNE RESPONSES IN STRIPED CATFISH (*Pangasianodon hypophthalmus*) JUVENILES

Dang Quang Hieu[1,2*], Bui Thi Bich Hang[2], Do Thi Thanh Huong[2], Garigliany Mutien[3], Nguyen Thanh Phuong[2], Frédéric Farnir , Patrick Kestemont[1]

[1] Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment (ILEE), University of Namur (UNamur), Rue de Bruxelles 61, B-5000, Namur, Belgium
[2] College of Aquaculture and Fisheries, Cantho University, Campus II, Cantho City, Viet Nam
[3] Department of Animal Production, Faculty of Veterinary Medicine, University of Liege, Liege 4000, Belgium
Email: quanghieudang.87@gmail.com

Introduction

Striped catfish *Pangasianodon hypophthalmus* is the most important aquaculture species in Vietnam, with export turnover reaching US$2.3 billion in 2019 (FAO, 2020). In recent years the sector has been negatively affected by climate changes and dam constructions along the Mekong River, especially salinity increase (Nguyen et al., 2014). In response to these factors, the striped catfish industry is compelled to adapt its culture methods to a brackish water environment to maintain its sustainability. However, the capacity of striped catfish to cope with salinity increase is not totally understood, especially with regards to its physiological and immunological responses, and this has been investigated in the present study.

Materials and Methods

Farmed striped catfish juveniles were submitted to a progressive increase of salinity at the rate of 0.25, 0.5, 0.75 and 1 ppt per day in order to reach 5, 10, 15 and 20 ppt, respectively after 20 days (D20). Then, the fish were continuously cultured in the corresponding salinity during two weeks (D34). Samplings were done at D20 and D34 to assay plasma osmolality and ion concentrations, lysozyme and peroxidase activities, hematology, gill histology, digestive enzyme activities and gene expressions related to physiological and immunological responses in head kidney.

Results

After 20 days, plasma osmolality gradually increased according to salinity with the lowest value of 271 ± 4.5 mosm and the highest value of 426 ± 12.8 mosm at 0 and 20 ppt respectively (Fig. 1a). Histological observations of gills clearly showed interlamellar cell mass (ILCM) in fish kept in freshwater, which completely disappeared in fish held at 10 ppt and 20 ppt (Fig. 1b). Besides, salinity led to a remarkable increase of digestive enzymes, including aminopeptidase, leucine alanine and pepsin at D20. The expression of osmoregulation genes as *naka1* and *cfrt* significant increased with the osmotic environment. Similarly, salinity upregulated the gene expressions of *hsp60* at D20 while *hsp70* remained unchanged, except in fish at 20 ppt (Fig. 2).

Discussion

As reported in other catfish species (Eckert et al., 2001), plasma osmolality gradually increased with salinity. Salinity also affected the transcript levels of *naka1* as previously reported by (Zhu et al., 2018). In fish reared at 0 ppt, a thick ILCM was visible, while this ILCM was substantially reduced upon exposure to hypoxic conditions, probably for creating a greater surface area for oxygen uptake (Phuong et al., 2018). The decrease of RBC in the present study can reduce the capacity of oxygen transportation. Therefore, the fish may increase gill surface for oxygen uptake through decrease of ILCM area. However, further research is required to understand the exact mechanism. As reported by Schmitz et al. (2016), the expression of *hsp70* did not change significantly with the increase of salinity up to 20ppt. Generally, salinity significantly affected the physiological and immunological responses, however most of the modifications occurred at short-term, with a partial recovering of the striped catfish after acclimation.

(Continued on next page)

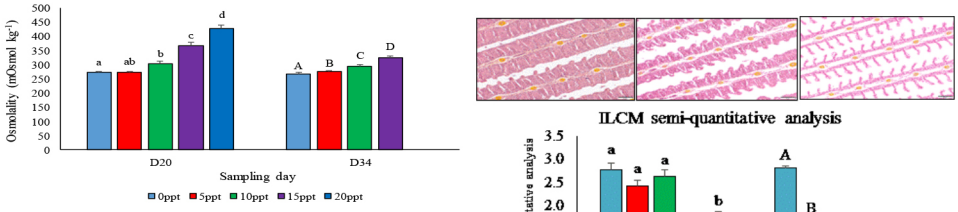


Fig. 1. Plasma osmolality (a) and gill histology (b) in striped catfish exposed to different salinities. Results are expressed as the mean ± SEM (n=4).

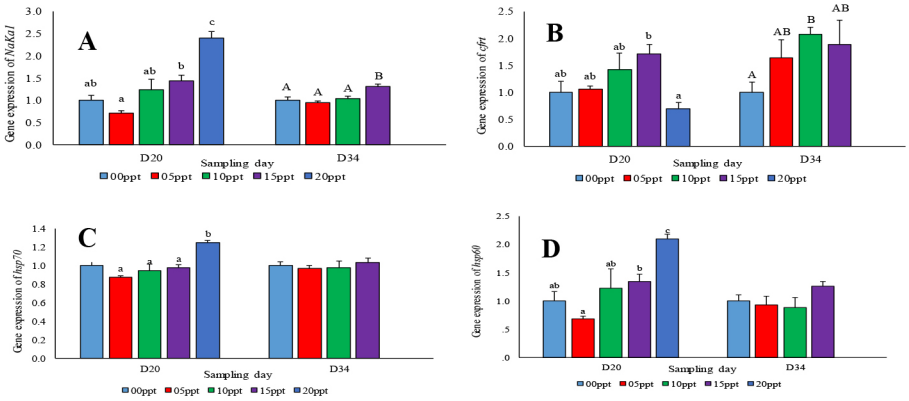


Fig. 2 Gene expressions in head kidney of striped catfish juveniles. (A) *naka1*, (B) *cfrt*, (C) *hsp70*, (D) *hsp60*. Results are expressed as the mean ± SEM (n=4).

References

Eckert, S.M., Yada, T., Shepherd, B.S., Stetson, M.H., Hirano, T., Grau, E.G., 2001. Hormonal Control of Osmoregulation in the Channel Catfish *Ictalurus punctatus*. Gen. Comp. Endocrinol. 122, 270–286.

FAO, 2020. GLOBEFISH Highlights January 2020 ISSUE, with Jan. – Sep. 2019 Statistics – A quarterly update on world seafood markets. Globefish Highlights no. 1–2020. Rome.

Nguyen, P.T.H., Do, H.T.T., Mather, P.B., Hurwood, D.A., 2014. Experimental assessment of the effects of sublethal salinities on growth performance and stress in cultured tra catfish (*Pangasianodon hypophthalmus*). Fish Physiol. Biochem. 40, 1839–1848.

Phuong, L.M., Huong, D.T.T., Malte, H., Nyengaard, J.R., Bayley, M., 2018. Ontogeny and morphometrics of the gills and swim bladder of air-breathing striped catfish *Pangasianodon hypophthalmus*. J. Exp. Biol. 221.

Schmitz, M., Douxfils, J., Mandiki, S.N.M., Morana, C., Baekelandt, S., Kestemont, P., 2016. Chronic hyperosmotic stress interferes with immune homeostasis in striped catfish (*Pangasianodon hypophthalmus*, S.) and leads to excessive inflammatory response during bacterial infection. Fish Shellfish Immunol. 55, 550–558.

Zhu, H., Liu, Z., Gao, F., Lu, M., Liu, Y., Su, H., Ma, D., Ke, X., Wang, M., Cao, J., Yi, M., 2018. Characterization and expression of Na+/K+-ATPase in gills and kidneys of the Teleost fish *Oreochromis mossambicus*, *Oreochromis urolepis hornorum* and their hybrids in response to salinity challenge. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 224, 1–10.

INCREASED SALINITY SIGNIFICANTLY AFFECTS INTESTINAL MICROBIOTA AND TRANSCRIPTOMICS IN STRIPED CATFISH (*Pangasianodon hypophthalmus*) JUVENILES

Dang Quang Hieu[1*], Jep Lokesh[2], Bui Thi Bich Hang[3], Do Thi Thanh Huong[3], Mutien-Marie Garigliany[4], Frédéric Farnir[4], Nguyen Thanh Phuong[3], Patrick Kestemont[1]

[1] Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment (ILEE), University of Namur (UNamur), Rue de Bruxelles 61, B-5000, Namur, Belgium.
[2] Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, NuMéA, St-Pée-sur-Nivelle, France.
[3] College of Aquaculture and Fisheries, Cantho University, Campus II, Cantho City, Viet Nam.
[4] Faculty of Veterinary Medicine, University of Liege, Liege 4000, Belgium.
Email: quanghieudang.87@gmail.com

Introduction

While most studies paid much attention to the physiological and immunological responses of striped catfish to salinity increase, how the intestinal-associated microbiota changes under hyperosmotic conditions in this species has not been investigated yet. The intestinal microbiota in striped catfish has not been studied using deep sequencing, except one study with limited phylum-level information (Sutriana et al., 2018). Moreover, the link between microbiota and host transcriptomics could shed light on how bacteria modulations interact with physiological and immunological aspects or vice versa.

Materials and Methods

Farmed striped catfish juveniles were submitted to a progressive increase of salinity at the rate of 0.25, 0.5, 0.75 and 1 psu per day in order to reach 5, 10, 15 and 20 psu respectively after 20 days (D20). Then, the fish were kept in the corresponding salinities during two weeks (D34). Intestinal samples were collected at D20 and D34 to assay microbiota using 16s rRNA sequencing and target gene expressions. For microbiota analysis, we used 16s rRNA and sequencing by Illumina Miseq. Data were processed using the UPARSE pipeline (Edgar, 2013). Alpha and beta diversity analyses (based on Bray-Curtis distance and ANOSIM test) were performed using QIIME2 (qiime2-2021.4). Relative abundances were plotted using phyloseq (1.34.0) in the R package (4.0.5). The significant abundant OTUs were calculated using Linear discriminant analysis effect size on Galaxy with p-value set at 0.05 and LDA log score threshold of 3.5. Regarding gene expression, extracted RNA was treated with a RTS DNAse™ kit (MO BIO Labs, USA) to avoid DNA contamination and then reverse transcribed to cDNA using a RevertAid RT Reverse Transcription Kit. The amplification of diluted cDNA was conducted using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Lab, USA) on QuantStudio™ 5 Real-Time PCR System.

Results

Hypersalinity generally decreased the OTU richness, evenness and phylogenetic diversity through the study, except the latter one with increasing trend at D34. Bacteroides were the most predominant genus across treatments at D20 however their compositions were different (Figure 1). Additionally, *Vibrio* was significantly risen with salinity increase and long exposure time. Regarding transcriptomic aspects, expression of osmoregulatory genes including *atp1a1, atp1b2a, slc12a1, slc12a2* were up-regulated while *cftr* and *aqp1* expressions were down-regulated with salinity gradient. Hypersalinity also increased expression of *hsp* genes (Figure 2).

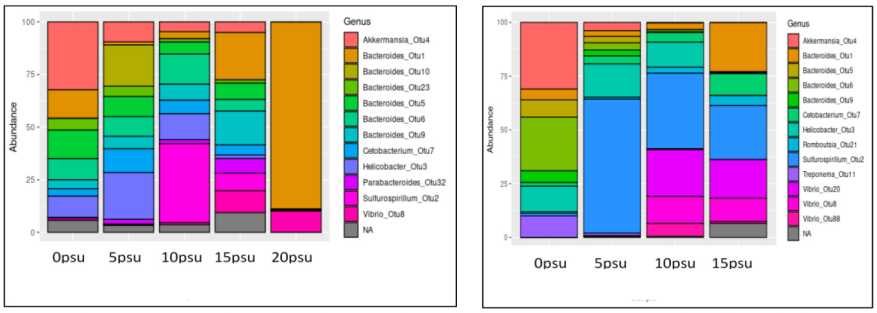


Figure 1. The mean relative abundance of predominant genera in striped catfish intestinal microbiota at D20 (A) and D34 (B).

(Continued on next page)

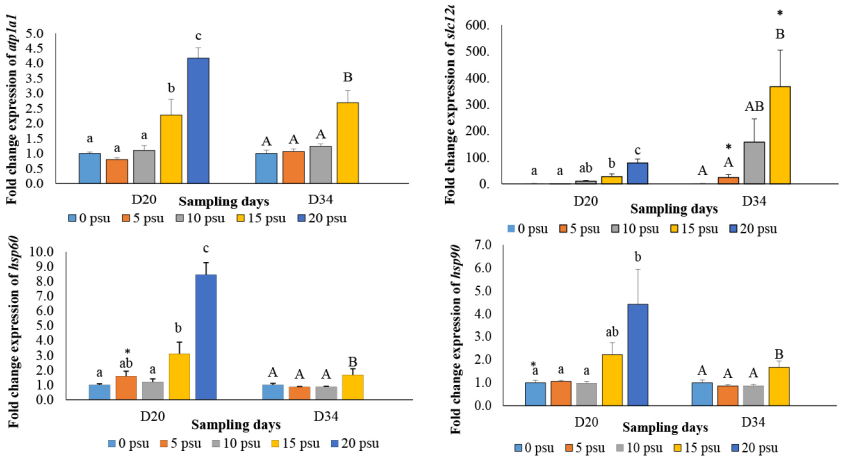


Figure 2. Fold change expressions of genes in intestinal striped catfish subject to different salinities. (A*) atp1a1*, (B) *slc12a1*, (C) *hsp60*, (D) *hsp90*

Discussion

Salinity was found to be a critical factor influencing microbiota communities, which reduced the diversity of intestinal microbiota (Dehler et al., 2017). The present study found that intestinal microbiota in freshwater striped catfish was predominant abundance of Bacteroidetes, Firmicutes, Verrucomicrobia and Proterobacteria. This was supported by previous research that these species were accounted for 90% in fish intestinal microbiota (Talwar et al., 2018). Vibrio was considered as a biomarker for salinity envrionment in fish intestinal microbiota (Zhao et al., 2020). The intestine contributed to the regulation of the osmoregulation process by altering the expression of genes related to ions and water transport. In general, the changes in the host transcriptome could lead to a decrease in the diversity of the microbiota under hyperosmotic conditions, i.e. 15 and 20 psu. This correlation might indicate that the anatomy and physiology including pH, osmolality, redox potential, compartment size and structure, passage rate and residence time, which are uneven in freshwater and seawater, significant affected the microbiota establishment (Ray and Ringø, 2014).

References

Dehler, C.E., Secombes, C.J., Martin, S.A.M., 2017. Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (*Salmo salar* L.). Sci. Rep. 7, 13877.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604

Ray, A.K., Ringø, E., 2014. The Gastrointestinal Tract of Fish. Aquac. Nutr., Wiley Online Books.

Talwar, C., Nagar, S., Lal, R., Negi, R.K., 2018. Fish Gut Microbiome: Current Approaches and Future Perspectives. Indian J. Microbiol. 58, 397–414.

Zhao, R., Symonds, J.E., Walker, S.P., Steiner, K., Carter, C.G., Bowman, J.P., Nowak, B.F., 2020. Salinity and fish age affect the gut microbiota of farmed Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 528, 735539.

SELECTION FOR ADAPTATION TO SALINE STRESS OF STRIPED CATFISH (*Pangasianodon hypophthalmus):* TOWARDS SUSTAINABLE CATFISH FARMING IN MEKONG DELTA, VIET NAM UNDER EFFECT OF CLIMATE CHANGE

Dao Minh Hai[1,2], Duong Thuy Yen[2], Dang Quang Hieu[2,3], Kestemont Patrick[3], Nguyen Thanh Phuong[2], Farnir Frédéric [1]

[1.] FARAH/Sustainable Animal Production, Faculty of Veterinary Medicine, University of Liege (B43), 4000-Liege, Belgium
[2.] College of Aquaculture and Fisheries, Can Tho University, Viet Nam
[3.] URBE, University of Namur, 5000-Namur, Belgium
Email: f.farnir@uliege.be

Introduction

Striped catfish (*Pangasianodon hypophthalmus*), a commercially important species cultured in Mekong Delta region in Southern Vietnam, is facing a significant challenge due to salinity intrusion as a result of climatic changes. Selection of new strains with high salinity tolerance allows economically feasible production of striped catfish in brackish environment. In this study, we demonstrated that survival rate and growth performance can be improved in striped catfish after a single generation of selection for ability to adapt to saline stress.

Materials and Methods

Production of base population: The fieldwork of the study has been conducted in College of Aquaculture and Fisheries, Can Tho University (CTU), Viet Nam from July/2017 to April/2021. The analysis of the samples and of the genetic data is performed in Liege University. Catfish broodstock have been selected from 3 different sources (An Giang, Vinh Long and Can Tho province, with 10 males and 10 females in each province), with average weights ranging from 5 to 7 kg. Principally, the selected broodstock had to be healthy, without visible injury or abnormal signs. A piece of fin (~1 cm^2) from each fish (i.e. 60 broodstock) has been collected and preserved in 95% ethanol for genetic analyses.

Spawning has been induced by injection with human chorionic gonadotrophin. A dry fertilization process has been used, where eggs and milt have been mixed gently. We have crossed twenty-nine females (~ 3000 eggs of each female) with thirty males to create 870 families (one female from Can Tho province could not be used due to the low quality of her eggs). Fertilization solution (3 g urea and 4 g salt in 1 L of water) has been added to the mixture of eggs and milt to trigger fertilization after 4 min. The fertilized eggs have then been transferred into boxes (one box per family) for incubation. The fertilized eggs have started to hatch 24 hours after fertilization. Fertilization rates varied from 83-90 % and the hatching rate was 61-73 %. The larvae have been transferred to rearing earthen ponds within 15 hours after hatching and fed live feed. From each family, 2000 good quality larvae (with no abnormal signs, uniform size, swimming actively and responding to external stimuli quickly) have been selected for nursing in two earthen ponds (leading to 1.740.000 larvae in total). After 47 days post-hatching (dph), 22.000 fries have been transferred from nursing ponds to CTU Recirculating Aquaculture System (RAS)

Selection principles: In RAS system, the fish were put progressively in the targeted saline conditions (10 ppt - parts per thousand). Subsequent sequential massal upward selection of 50% of the survival and healthy fast-growing fish at 3 stages of weight (100 g, 300 g and 1000 g) were implemented (selected group). In parallel, another set of fish was raised under similar conditions (pond, salinity, density, feed) and underwent a random selection process to serve as negative control (control group). At each time, approximately half of the fish were discarded. After 3 selection stages (~ one year), the average weight of selected group and random group were 1.380 ± 175 g and 793 ±230 g, respectively. In the third selection, all remaining fish were identified using Passive Integrated Transponders (PIT) tags. These PIT–tags were injected into muscle of fish and corresponding DNA samples were collected. The level of salinity was then decreased to 5 ppt and remained at this level until fish being mature.

(Continued on next page)

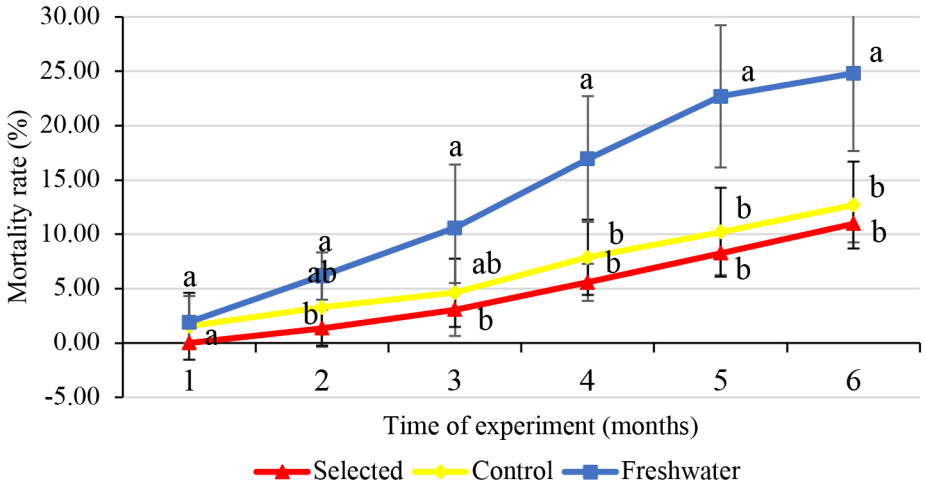


Figure 1: Accumulative mortality from three groups during culture experiment at 10 ppt

Evaluation of response to selection: To assess the efficiency of selection, the offspring of the selected, control and freshwater (fish matured in freshwater condition, representing normal broodstock in Mekong Delta) groups were challenged under different levels of salinity. To identify genetic relationships, a new algorithm using shallow sequencing information from the offspring was implemented, allowing parentage assignment in the selected and control groups based on genomic information. The rearing experiment was divided into four distinct periods according to each developmental stage of striped catfish: embryonic stage, larva to fry stage, fry to fingerling stage, fingerling to adult stage. For data collection, information on two major production traits (survival and growth) associated with the ability to adapt to salinity stress was recorded during the experiments.

Results

Preliminary results show that survival rate and growth performances of the offspring of the individuals from the selected group is better than for the two other groups, especially with freshwater group under saline stress. At 10 ppt level of salinity, in embryonic stage, hatching rate (%) of fertilized eggs is significantly higher in selected group (13.5%) compared to 10.4% in control group and 5% in freshwater group. For larva to fry stage, survival rate is also significantly higher in selected group (30%) compared to only 19% and 18% for control group and freshwater group, respectively. At 15ppt, only selected group and control group survived with 3.6% and 1.1% survival rates, respectively. We observed a similar trend for fingerling to adult stage, with selected group tending to live better in saline condition (Figure 1). For growth performance, in all experiments, the growth rate of selected group was higher than for control and freshwater group. These results suggest that selection to improve ability of striped catfish to adapt to saline stress can be achieved, in terms of increased survival rate and growth rate of the selected group following saline exposure.

PARENTAGE ASSIGNMENT OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) WITH SHALLOW WHOLE GENOME SEQUENCING DATA

Dao M. Hai [1,2], Duong T. Yen[2], Pham T. Liem[2], Bui M. Tam[2], Do T.T. Huong[2], Bui T.B. Hang[2], Vo N. Son[2], Dang Q. Hieu[2], W. Coppieters[5], M.M. Garigliany[3], P. Kestemont[4], N. A. Moussiaux[1], Nguyen T. Phuong[2], F. Farnir[1]

[1.] FARAH/Sustainable Animal Production, Faculty of Veterinary Medicine, University of Liege (B43), 4000-Liege, Belgium
[2.] College of Aquaculture and Fisheries, Can Tho University, Viet Nam.
[3.] FARAH/Veterinary Public Health, Faculty of Veterinary Medicine, University of Liege (B43), 4000-Liege, Belgium
[4.] URBE, University of Namur, 5000-Namur, Belgium
[5.] Genomics Platform, GIGA, University of Liege, 4000-Liege, Belgium
Corresponding author: f.farnir@uliege.be

Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is a species commonly cultured in the Mekong Delta region, Southern Vietnam. However, the genetic management of the broodstock of this species has not been studied extensively. This leads to potential risks associated to inbreeding, with potential negative effects on the growth and reproduction performances of the fish. Therefore, the pedigree information of broodstock in hatcheries, which is essential for a proper genetic management of the fish, is urgently required in order to sustain the high quality of seed to supply for catfish aquaculture in the Mekong Delta. Several previous studies on parentage assignment in other species have focused on using genetic data generated from micro-satellite markers and more recently from SNP arrays. In this study, we used the shallow whole genome sequencing (SWGS) data as an economic alternative to analyze parentage assignment of striped catfish instead of traditional array data.

Materials and Methods

Experimental fish and DNA sampling: The fieldwork of the study has been conducted in College of Aquaculture and Fisheries, Can Tho University, Viet Nam from July/2017 to April/2019. The analysis of the samples and of the genetic data has been performed in Liege University. Catfish broodstock have been selected from 3 different sources in three provinces (10 males and 10 females in each province). Spawning has been induced by injection with human chorionic gonadotrophin. Thirty females have been crossed with 30 males to create 900 full-sib families. From each family, 2,000 good quality larvae have been selected for nursing. After one-year culture, 500 individuals selected from the remaining offspring were used for parentage assignment. A piece of fin (~1 cm^2) from each fish (i.e. 60 broodstock and 500 offspring) has been collected and preserved in 95% ethanol for genetic analyses.

Preparation for genomic data: To prepare genomic data, we performed whole genome deep sequencing of one catfish with high fold coverage (~ 144 X), and used this information to establish a *de novo* draft reference genome. For 60 parents and 500 offspring, we used SWGS with fold coverage of ~ 1 to 2 X (parents) and ~ 0.5 X (offspring) per individual. We mapped SWGS data of 60 parents on the draft reference genome to identify genomic variants (5,900,000), including SNPs. Then, we extracted ~26,000 high quality SNPs from these variants and used the available sequencing data at these SNPs loci to infer the parents of the offspring.

Parentage assignment method: The use of SWGS data raises two challenges: First, for low-coverage (e.g., < 2 X), confirmed genotypes for offspring and parents are in most case not available. Second, read errors are common in next generation sequencing. To address these issues, we have developed a new parentage assignment algorithm based on a likelihood approach to identify the most suitable (i.e. likely) set of parents for each offspring. For each offspring, we have computed the likelihood for each possible parental couple, kept the most likely one and tested its significance as the putative couple of parents for this offspring

(Continued on next page)

Table 1: number of offspring per hatchery and sex of the breeder. The numbers between parentheses indicate the corresponding of parents involved in at least one couple.

Hatchery		An Giang	Can Tho	Vinh Long
Number of offspring	♀	430 (9)	34 (7)	6 (3)
	♂	114 (10)	204 (10)	152 (10)

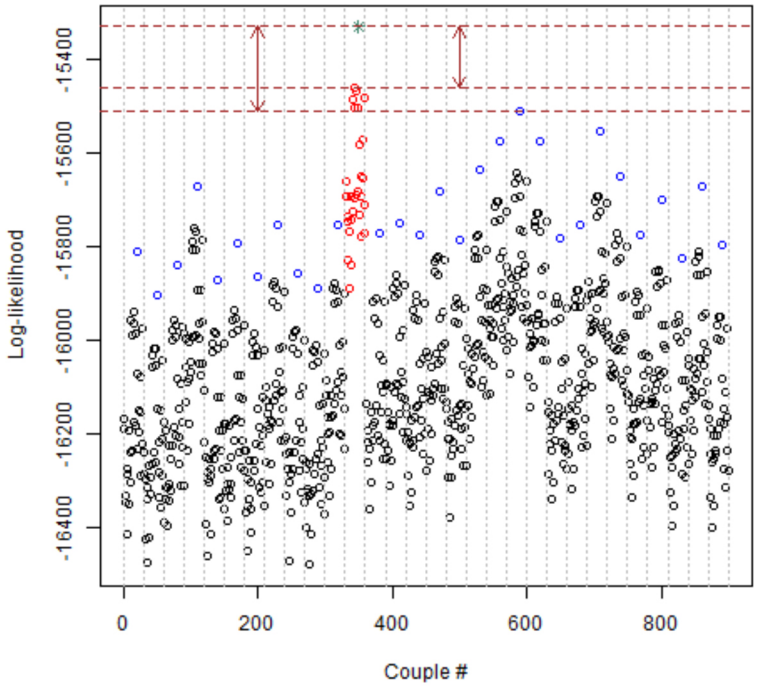


Figure 1 The results of parentage assignment for one offspring. Each dot represents the likelihood of one couple. The dotted vertical lines partition the graph into families sharing the same mother. The start represent the highest likelihood, the red dots correspond to likelihoods involving the "best" mother, and the blue dots to likelihoods involving the "best" father. The arrows represent the differences between the best and the next best likelihoods used to test the significance of the parental allocations.

Results

After running this analysis, all 470 striped catfish were significantly ($p < 0.05$) allocated to one couple of parents. Only one parent was unambiguously allocated for 26 of the remaining individuals (21 fathers and 5 mothers), and 4 could not be assigned to a unique couple. All fathers (30 fish) had offspring in the current population, while only 19 of the 30 mothers did. Most of the significantly allocated offspring (> 90%) were produced from mothers originating from An Giang hatchery, meanwhile, only 1.34% of offspring (6 individuals) originated from females from Vinh Long hatchery. The equilibrium was better for the males (Table 1). Significance in this study has been obtained by comparing the real differences between the best likelihood and the next best likelihood (Figure 1) involving the "best" father (mother) but not the "best" mother (father), to the corresponding distributions obtained by shuffling the sequencing data across the fathers and the mothers. These results suggest that SWGS data can be used for accurate parentage assignment using the appropriate algorithm and we believe that it will be a useful tool in parentage assignment not only for aquaculture but also for a wide range of animals.

ROTIFERS ENRICHED WITH MICROALGAE AS A NEW STRATEGY TO IMPROVE ZEBRAFISH REPRODUCTIVE PERFORMANCE

Daniela T. de Castro[1], Gil Martins[1,2], Patrícia Diogo[3], Tamára Santos[1], Inês B. Maia[1,3], Alexandre Rodrigues[3], Victória del Pino[3], João Navalho[3], Hugo Pereira[4] and Paulo J. Gavaia[1,4,5*]

[1] Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2] Faculty of Sciences and Technology, University of Algarve, Faro, Portugal
[3] Necton S.A., Olhão, Portugal
[4] GreenCoLab, University of Algarve, Faro, Portugal
[5] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
* Correspondence: pgavaia@ualg.pt

Introduction

Zebrafish (*Danio rerio*) has emerged as a successful animal model for many different areas such as aquaculture, genetics, development and human diseases[1]followed by Artemia nauplii until the fish reach subadult stage, the developmental time point at which they can be most easily transitioned onto a processed diet. However, the inclusion of Artemia is less than ideal, given its fluctuating availability and high costs. We tested whether or not we could replace Artemia with rotifers during our normal rearing sequence and still meet published performance standards for (i. Nowadays, it is considered the second most used model species worldwide, due to its advantageous characteristics such as the fully sequenced genome, 70% of homology with human genome, developed robust target mutagenesis technologies, embryo transparency, rapid growth and high fecundity[2]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. Live feeds like rotifers (*Brachionus* sp.) are commonly used in zebrafish feeding on the different life stages. The use of live feeds stimulate natural predatory behaviour and reduce captivity stress, working as environmental enrichment, thus improving fish welfare, which benefits fish reproduction[3]. However, their nutritional profile is poor in HUFAs that play important roles in reproduction, especially in females, such as DHA, ARA and EPA, which can be manipulated by enrichment using different microalgae species[4]**.** Microalgae are natural fish preys composed of a variety of nutrients generally with a balanced composition. However, little is known about the effects of rotifers enriched with microalgae on fish reproductive performance. Therefore, this work aimed at improving zebrafish reproductive performance through dietary supplementation with rotifers enriched with different microalgae, (*Nannochloropsis* sp., *Skeletonema costatum*, *Tisochrysis lutea* and *Dunaliella salina*) and their combinations.

Materials and methods

The experimental design included eight dietary treatments composed of two controls: ZF (Zebrafeed®, Sparos Lda, Portugal) and N (rotifers enriched with *Nannochloropsis* sp., Phytobloom® Green Formula, Necton S.A., Portugal) given to zebrafish females. The dietary experimental groups were co-fed for 12 weeks with ZF and microalgae enriched rotifers: NS (*Nannochloropsis* sp. and *Skeletonema costatum*), NT (*Nannochloropsis* sp. and *Tisochrysis lutea*), ND (*Nannochloropsis* sp. and *Dunaliella salina*), NST (*Nannochloropsis* sp., *S. costatum* and *T. lutea*), NSD (*Nannochloropsis* sp., *S. costatum* and *D. salina*) and NTD (*Nannochloropsis* sp., *T. lutea* and *D. salina*). The reproductive performance was evaluated by counting the number of layed eggs, egg and yolk length determination for 8 breeding events. At 5 dpf, total length was measured in 20 larvae per group and the operculum mineralization assay was performed at 6 dpf, as described in Tarasco et al.[5].

Results

Females fed with rotifers enriched with ND produced a significantly higher number of eggs, while females fed with ZF and NST produced a significantly lower number of eggs (Fig. 1A). Egg morphometry parameters showed that females fed with NT layed eggs with significantly higher diameters (Fig. 1B) and yolk area (Fig. 1C).

Females fed ZF and NSD produced larvae with a significantly larger length when compared to NST and NTD (Fig. 2A). Larvae from females fed with ZF displayed a significantly higher mineralization area on the operculum when compared to all the treatments, except for N and ND (Fig. 2B).

(Continued on next page)

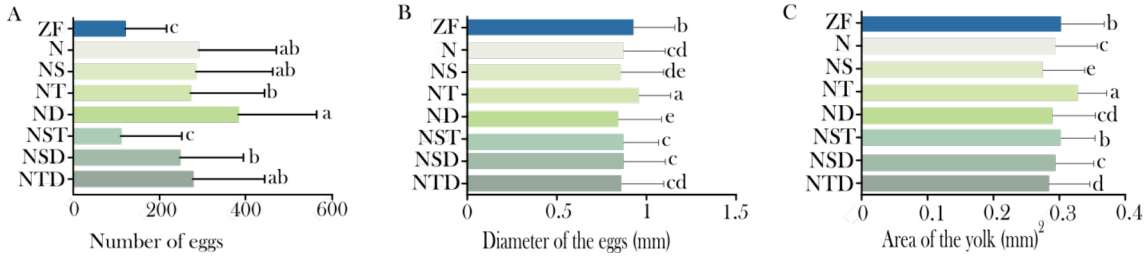


Figure 1: The effect of female broodstock dietary supplementation on egg quality, namely on (A) number of laid eggs; (B) diameter; and (C) yolk area.

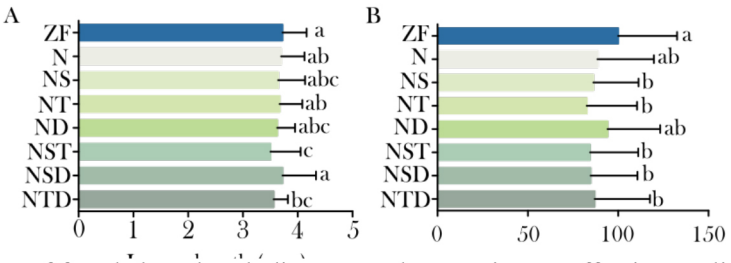


Figure 2: The effect of female broodstock dietary supplementation on offspring quality, namely on (A) larvae length and (B) operculum mineralization.

Discussion

This study investigated the effect of maternal diet supplementation with valuable microalgae species through rotifer enrichment. High-quality eggs are essential for proper offspring development, as they contain the nutritional reserves for embryo development. We observed that maternal diet influenced offspring quantity and quality. Females fed rotifers enriched with ND produced a higher number of eggs, which can be related to the *n*-3/*n*-6 ratio (1.34) from the diet. It was previously observed that low ratios of n-3/n-6 maximize the number of laid eggs[6,7] and that a ratio of 0.8 promotes high breeding success. The treatment with the higher number of laid eggs was also the one with the lowest egg area but without a negative impact on larvae size. Females fed NT enriched rotifers displayed eggs with significantly higher yolk and area, which can be influenced by the higher amount of total lipids in the diet. Therefore, these results indicate that ND supplementation is beneficial for reproduction, number of eggs and larvae size. This could also be explained by the higher amount of DHA, known to improve zebrafish female reproductive performance and larval quality[8]. The optimization of the *n*-3/*n*-6 ratio improved the levels of ARA, EPA and DHA and their transfer to eggs, also improving hatching success and larval quality. Overall, our results showed an improvement in the number of laid eggs using rotifers enriched with ND, working as a functional dietary supplement. The inclusion of these microalgae species represents a possible strategy for improving the reproductive capacities of other species produced in aquaculture. In this work, it was possible to verify that zebrafish represents an important biological model for testing products and ingredients for future applications in fish production.

References

1. Lawrence *et al*. (2015). Zebrafish 12, 366–371.
2. Kolb, A. *et al*. (2018). Zebrafish 15, 445–453.
3. Monteiro, J. F.*et al*. (2018). J. Dev. Biol. 6, (2018).
4. Conceição, L. E.C. *et al*. (2010). Aquac. Res. 41, 613–640.
5. Tarasco, M. *et al*. (2017). Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 197, 45–52
6. Jaya-Ram, A. *et al*. (2008). Aquaculture 277, 275–281.
7. Meinelt, T. *et al*. (2000) Comp. Med. 50, 43–45.
8. Pavlov. (2004) Oxford: Blackwell Publishing. P. 129-C203.

APPLICATION OF GARLIC AND CINNAMON COMPOUNDS ON GROWTH PERFORMANCE AND IMMUNE PARAMETERS OF WHITELEG SHRIMP (*Litopenaeus vannamei*)

M. de Jong[1*], K. Wilson[1], O. Jintasataporn[2], and A. van der Aa[1]

[1]Orffa Additives B.V. Vierlinghstraat 51, 4251 LC Werkendam, The Netherlands
[2]Department of Aquaculture, Kasetsart University, Bangkok, Thailand
Email: wilson@orffa.com

Introduction

As the demand for eco-friendly farming practices and products in aquaculture continues, the industry has shifted to focus on improved maintenance of fish and shrimp health to enhance performance. This focus on the development of novel additives are increasingly applied in aquaculture, including plant-based products, phytogenic feed additives (PFA). A common usage of PFA's is to decrease bacterial pressure of pathogens including *Vibrio parahaemolyticus* and improve growth performance during challenge. This can be achieved because PFA's, specifically garlic and cinnamon, contain several bioactive molecules that can exert multiple effects on the gastrointestinal (GI) tract. This includes an antimicrobial effect by disrupting the cellular membrane of pathogens and the enhancement of the host immunity which aid in anti-inflammatory and antioxidant reactions results in the redirection of energy to maintain performance. The application rate of garlic and cinnamon can be a challenge in aquaculture as therapeutic concentrations during a bacterial challenge can be different for animals in healthy conditions. Therefore, the objective of the experiment was to 1) evaluate the efficacy of a blend of garlic and cinnamon in whiteleg shrimp (*Litopenaeus vannamei*) based on growth performance and immunity and 2) investigate immunity and growth changes after a *V. parahaemolyticus* challenge.

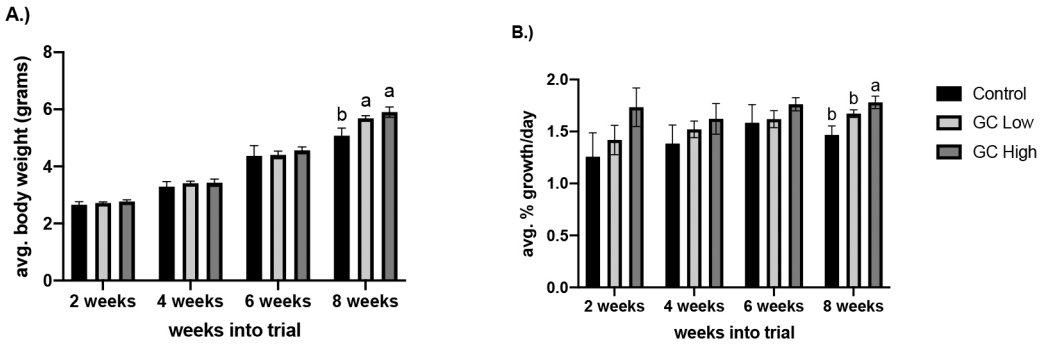


Figure 1. Average body weight (A) and specific growth rate (B) in whiteleg shrimp. Means within weeks with different superscripts are significant at $p \leq 0.05$.

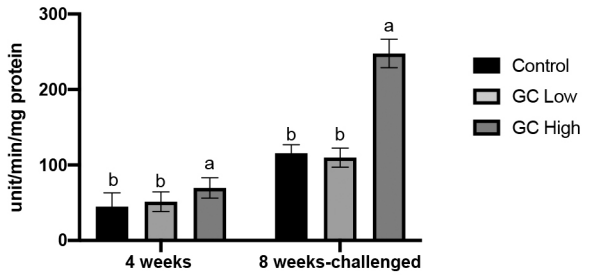


Figure 2. Average phenoloxidase activity in normal conditions and challenged conditions in whiteleg shrimp. Means within weeks with different superscripts are significant at $p \leq 0.05$.

(Continued on next page)

Materials and Methods

A total of 300 juvenile whiteleg shrimp (2.21 ± 0.06 g) were placed in one of the following three treatments: Control- basal diet with 1000 ppm of calcium carbonate as a placebo; GC Low – basal diet with 500 ppm of garlic and cinnamon blend (Excential Alliin Plus; Orffa Additives B.V.) and 500 ppm calcium carbonate and; GC High –basal diet with 1000 ppm of garlic and cinnamon blend. The experiment was carried out in 15 aquariums as a complete randomized design (n=5) with each of 240 L capacity that contained 120 L of 15 ppt saline water. The juvenile whiteleg shrimp were stocked at a density of 150 shrimp/m^3 (20 individual/aquarium). Water in each of the aquariums were changed 20% every three days. Aeration was applied into all experimental unit for maintaining DO > 5 mg/L in a semi-closed system. Feed were presented in pelleted form and placed in the aquariums three times a day at 2.5-3.0 % of cumulative body weight for eight weeks. One hour after feeding, remaining feed was removed to calculate feed intake. Growth performance including weight gain, specific growth rate, feed conversion ratio (FCR) and mortality were measured. Shrimp health and immune status was determined in normal/healthy conditions four weeks into the experiment by total hemocyte count, hemolymph protein and phenoloxidase activity. At eight weeks, each of the shrimp were challenged via injection with *V. parahaemolyticus.* Twelve hours later, each of the immune parameters from 4-weeks were repeated in addition to the record of the activities of lysozyme, super oxide dismutase and glutathione peroxidase.

Results and Discussion

The inclusion of garlic and cinnamon regardless of dosage resulted in an increased body weight gain by 20.9 % in GC Low and 30.0 % in GC High at the final week of the trial ($p < 0.05$) relative to the control group (Figure 1A) which also is represented in the increased specific growth rate difference in GC High (Figure 1B). While feed intake did not significantly change during the trial, the FCR lowered every collection point but became significant ($p < 0.05$) at 8 weeks with a 30-point and 39-point decrease in GC Low and GC High compared to the control, respectively (Control = 1.524; GC Low = 1.272; GC High = 1.183). The significant reduced FCR and weight gain may be attributed to the improved immune status of the GC High treatment at the time of the *Vibrio* challenge. Figure 2 shows that phenoloxidase activity before challenge at 4 weeks resulted in GC High having elevated activity which can act as defense enzymes against invading pathogens. Once the bacterial challenge occurred, there was an overall elevation ($p < 0.05$) between times of collection but only the GC High was significantly elevated among treatments. Lysozyme activity was also elevated in GC High (610 ± 148.44 unit/mL) relative to both GC Low (323.33 ± 73.11 unit/mL) and Control (226.67 ± 40.96 unit/mL).

Conclusions

Phytogenic feed additive garlic and cinnamon blend can create an environment within the host to maintain and improve weight gain and feed efficiency. However, an elevated dose at 1000 ppm can enhance innate host immunity during bacterial challenge effectively and maintain elevated weight gain during production.

MICROALGAE AS A SOURCE OF ANTI-INFLAMMATORY COMPOUNDS

Victória del Pino[1], Patricia Dogo[1], Alexandre Rodrigues[1], Mariana Carneiro[1], Hugo Pereira[2], João Navalho[1]

[1] Necton SA, Olhão, Portugal
[2] GreenCoLab, University of Algarve, Faro, Portugal
* Correspondence: vdelpino@necton.pt

The control of inflammation through nutrition is a growing trend in both humans and animals. In aquaculture, this dietary improvement is particularly relevant since traditional diets (e.g. soybean meal) include ingredients known to promote inflammation, thus reducing fish welfare and overall biological performance [1–3]. Consequently, bioprospection of novel sources of anti-inflammatory compounds for food and feed is a essencial. Macro- and microalgae possess valuable metabolites with relevant properties such as antioxidant and anti-inflammatory bioactivities [4,5]. The project Algae4IBD aims to develop new functional ingredients to prevent and treat Inflammatory Bowel Disease (IBD) using aquatic natural biological resources. In this framework, the project will take advantage of the vast alga biodiversity to find the most promising species with adequate bioactive compounds. After an intensive species screening, state-of-the-art cultivation, extraction, and processing technologies will be employed to obtain the target metabolites. Since the final goal is to develop new functional food and ingredients to prevent and treat inflammation, *in vitro*, *in vivo,* and *ex vivo* trials will be performed, including in the zebrafish model. The zebrafish model will be used to understand the effect of the selected algae *in vivo* in the diversity of the gut microbiome. The project includes four valuable microalgae species currently produced and commercialized by Necton, namely *Tisochrysis lutea*, *Skeletonema costatum*, *Phaeodactylum tricornutum,* and *Nannochloropsis oceanica*. These species present a valuable nutritional profile for fish and invertebrate nutrition, being particularly relevant for bivalve and shrimp aquaculture [6,7]. In addition, anti-inflammatory activities have been previously observed in these microalgae, increasing their value as an anti-inflammatory feed[4,8].

References

1. Perera E & Yufera M (2016) Soybean Meal and Soy Protein Concentrate in Early Diet Elicit Different Nutritional Programming Effects on Juvenile Zebrafish. Zebrafish 13, 61–69.
2. López Nadal A, Ikeda-Ohtsubo W, Sipkema D, et al. (2020) Feed, Microbiota, and Gut Immunity: Using the Zebrafish Model to Understand Fish Health. Front. Immunol. 11.
3. Martinez-rubio L, Morais S, Evensen Ø, et al. (2012) Functional Feeds Reduce Heart Inflammation and Pathology in Atlantic Salmon (*Salmo salar* L .) following Experimental Challenge with Atlantic Salmon Reovirus (ASRV). 7.
4. Bigagli E, Ambrosio MD, Cinci L, et al. (2021) A Comparative *In Vitro* Evaluation of the Anti-Inflammatory Effects of a *Tisochrysis lutea* Extract and Fucoxanthin.
5. Lauritano C, Andersen JH, Hansen E, et al. (2016) Bioactivity Screening of Microalgae for Antioxidant, Anti-Inflammatory, Anticancer, Anti-Diabetes, and Antibacterial Activities. Front. Mar. Sci., 68.
6. McCausland MA, Brown MR, Barrett SM, et al. (1999) Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile pacific oysters (*Crassostrea gigas*). Aquaculture 174, 323–342.
7. Lestari DP & Ekawati AW (2014) Dried *Skeletonema costatum* in Feed Formulation for the Growth of Vaname Shrimp (*Litopenaeus vannamei*). 4.
8. Cardoso C, Pereira H, Franca J, et al. (2019) Lipid composition and some bioactivities of 3 newly isolated microalgae (*Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.). Aquac. Int. Aquaculture International.

Acknowledgments
Work funded by ALGAE4IBD-101000501 H2020-EU.3.2.4.1

DISTINGUISHING THE IMPACTS OF REARING DENSITY VERSUS TANK VOLUME ON *Sparus aurata* DURING THE PREONGROWING PHASE

Z. Dellacqua*[1,3], C. Di Biagio[1,4], A. Martini[2], M. Barata[3], L. Ribeiro[3], P. Pousão-Ferreira[3], A. Fabris[5], C. Boglione[2]

[1] PhD Programme in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome 'Tor Vergata', Via Cracovia Rome (Italy)
[2] Dept. of Biology, University of Rome 'Tor Vergata', Rome (Italy)
Intituto Portugues do Mar e Atmosfera, Parque Natural da Ria Formosa em Marim, Olhão (Portugal)
[4]Laboratory of Evolutionary Developmental Biology, University of Ghent, Karel Lodewijk Ledeganckstraat 35, Ghent (Belgium)
[5] Associazione Piscicoltori Italiani, Via del Perlar 37/A 37135 Verona (Italy)
Email: zacharyjoseph.dellacqua@students.uniroma2.eu

Introduction

The production of Gilthead seabream (*Sparus aurata*), together with European seabass (*Dicentrarchus labrax*), is the second most important aquaculture industry in the European Union (STECF, 2018) consisting of large companies as well as numerous small-medium sized companies which require a strong concentration on business development. However, the economic performance of these companies is generally rather poor mostly due to inefficiency and profitability issues. 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 combination of Large Volumes (ϕ>30m^3) and low densities (≤ 16 larvae/L) has been demonstrated (Koumoundourous *et al*., 2004; Boglione *et al*., 2009; Prestinicola *et al*., 2013) to augment the survival rate and the morphological quality of hatchery-reared seabream. The aim of this study is to individuate which between 'large volume' and 'low density' is the main driver in attaining high quality seabream subadults. The design envisages to test the effects during the pre-ongrowing phase of seabream, carried out at a commercial scale of: A) larger and smaller tank volumes; and B) higher and lower stocking densities.

Methods

Experimental rearing was conducted at the Intituto Portugues do Mar e Atmosfera facilities in Olhão Portugal. Seabream with an average weight of 6.7 ± 2.5g and length of 7.8 ± 1.1cm were selected based on external inspection carried out by experienced technicians and the 'non-deformed' fish were stocked at 3 different densities (LD: 5kg/L; MD: 10kg/L; HD: 20kg/L) in both 500 L and 1000 L cylindrical tanks with a replacement rate of 100% tank volume/hour of natural seawater. Oxygen diffusers were placed at the bottom of tanks in order to maintain a O_2 saturation level above 70%. Light regime was based on natural light and photoperiod during the summer months of August and September in Southern Portugal. Tanks were shadowed with a mesh tarp. The water temperature in the tanks ranged 19-29° C throughout the ~60 days of rearing upon reaching an average final weight of ~55g/individual. The tanks were regularly controlled and managed in order to maintain the experimental densities throughout the experiment. Fish were fed 3% of their body weight/day with AquaGold 5 pellets (AquaSoja, Ovar, Portugal).

A sample of 161 seabream of those classified as 'non-deformed' used to begin the experimental rearing (T_0) were lethally anesthetized (MS-222) and fixed in 4% PFA and X-rayed (Gilardoni CPX 160/4 System, Unleaded film Kodak Mx 125, 55 Kv, 4 mA) for anatomical evaluation of skeletal elements. After ~60 days of experimental rearing (T_F), Over 100 seabream were sampled from each experimental condition, euthanized and X-rayed with a digital DXS Pro X-ray (Bruker).

Results and Discussion

There were no significant differences in survivorship between the conditions. Significant (Kruskal-Wallis, Dunn's *post hoc* with Bonferroni correction, $p<0.05$) smaller total lengths were found in the seabream reared in HD conditions with respect to LD seabream, regardless of the tank volumes (Fig. 1a). Significant differences were also found in the Fulton's condition factor (Fig. 1b) of HD versus LD seabream regardless of the tank volumes. Taking into consideration that the final weights were not significantly different, the metrics enhanced the point that seabream in the HD conditions were characterized by a positive allometric growth.

(Continued on next page)

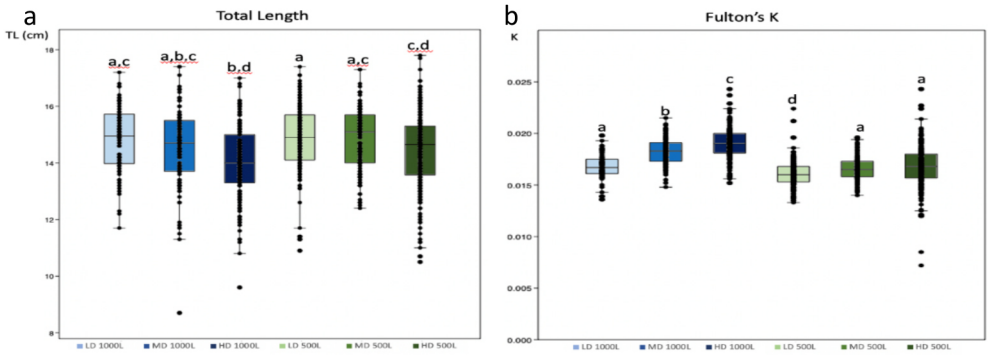


Fig. 1 Total Length (a) & Fulton's condition factor K (b) for individual Seabream fingerlings at T_F ($p<0.05$, Kruskal-Wallis, Dunn's *post hoc* with Bonferroni correction). Different letters highlight significant differences.

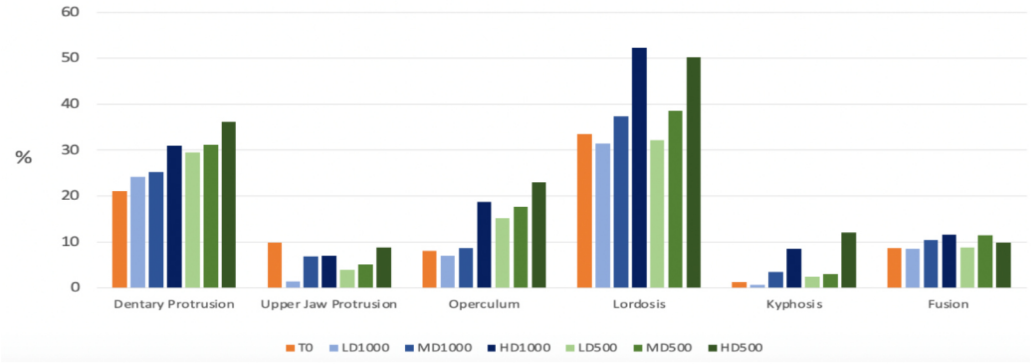


Fig. 2 Prevalence of individuals affected by severe anomaly types from T_0 (at the beginning of the experimental rearing) from T_F (final sampling) for the 6 density and volume conditions.

X-ray images showed a progressive augmentation of lordosis, kyphosis, jaw and opercular anomalies with increasing density, independently from the volume (Fig. 2). Consequently, stocking density seems to be a primary driver in increasing the frequency of these skeletal anomalies rather than the tank volume. When considering differences among fish reared in different tank volumes, the prevalence of particular anomaly types appeared different: i.e., at the same stocking density, the 1000 L rearing conditions yielded a lower prevalence of opercular and jaw anomalies and higher prevalence of both lordosis and vertebral body fusions, when compared to the 500 L conditions of the same density.

Lastly, although the initially selected seabream were labeled as 'non-deformed' upon external inspection; the X-rayed images tell a different story as the T_0 seabream did in fact have many skeletal deformities (Fig 2).

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.

A NUMERICAL STUDY OF SHELLFISHERIES AQUACULTURE AND OFFSHORE RENEWABLE ENERGY CO-LOCATION IN THE IRISH SEA

Jonathan Demmer*; Matthew Lewis; Simon P. Neill

Address: School of Ocean Sciences, 4 Askew St, Menai Bridge LL59 5EG
Email: osp816@bangor.ac.uk

Introduction

The worldwide increase of demand for aquaculture and energy requires developing efficient tools to study the possibility of multi-use platforms at sea (MUPS) and reduce the anthropic pressure on space availability. Offshore renewable energy (ORE) in the Irish Sea will occupy approximately 14% (6,564 km^2) of the space in a close future. Blue mussels industry (*Mytilus edulis L.*) in North wales represents one third of the UK production and could be impacted by the development of other industries (Hambrey & Evans, 2016). Several studies defined a sustainable index (SI) for the co-location of aquaculture and offshore wind farms, which includes physical (i.e. wind velocity, depth range, tidal current) and biological (i.e. sea surface temperature, chlorophyll-a concentration) factors (Di Tullio *et al*., 2018). However, no studies took into account larvae dispersal as a factor for SI to determine the best location to catch shellfish. In order to determine the best suitable area for co-location, we used a lagrangian particle tracking model (PTM) coupled to hydrodynamic model to qualify and quantify: (1) the density distribution of mussel larvae; and (2) the connectivity between aquaculture (6 sites) and ORE (10 sites).

Material and methods

Telemac-2D depth average model with mesh density varying from 30 m to 5,000 m. The domain covered an area of 165,000 km2, which correspond to the whole Irish Sea as previous studies show that larvae can potentially travel up to 300 km (Van der Molen *et al*., 2007). A Lagrangian PTM was developed to predict mussel larvae dispersal from 6 sites for a pelagic larvae duration (PLD) of 45 days. Simulations were performed for two larvae behaviour (i.e. dispersal at the surface and at mid-water depth) during spring of two contrasted year (i.e. 2014 and 2018 showed different wind patterns).

Results

Density distribution showed the same results during spring 2014 and 2018 when larvae travel at mid-water depth. However, the density distribution varied during the PLD and according to the site of release.

Density distribution varied inter-annually when larvae travelled at the surface. Results also showed a difference between release sites and during the PLD.

Connectivity results showed that the site leasing 1 is the most connected with released sites for all simulated years and behaviour (Figure 1). However, the connectivity with leasing 1 site varied through the PLD (Figure 1). Furthermore, results showed that during spring 2014, aquaculture sites are connected with offshore wind farms located in the eastern Irish Sea (i.e. North Wales and North of England) whereas during 2018 they are connected with ORE located in the western Irish Sea (i.e. Dublin and North of Ireland) (Figure 1).

Discussion

The simulated larvae behaviours results showed that when larvae travel at the surface they encountered stronger currents (e.g. wind driven currents), which increased their dispersal. Results observed when mussel larvae travel at the surface are correlate with observations made by mussel farmers during the year 2014 and 2018. The results highlight the importance of vertical position of larvae in the water column to study potential multi-use platforms at sea. However, the leasing site 1 is potentially the best area to develop MUPS in the Irish Sea. This study shows the importance of considering larvae dispersal for the development of sustainable index to define best area for MUPS.

(Continued on next page)

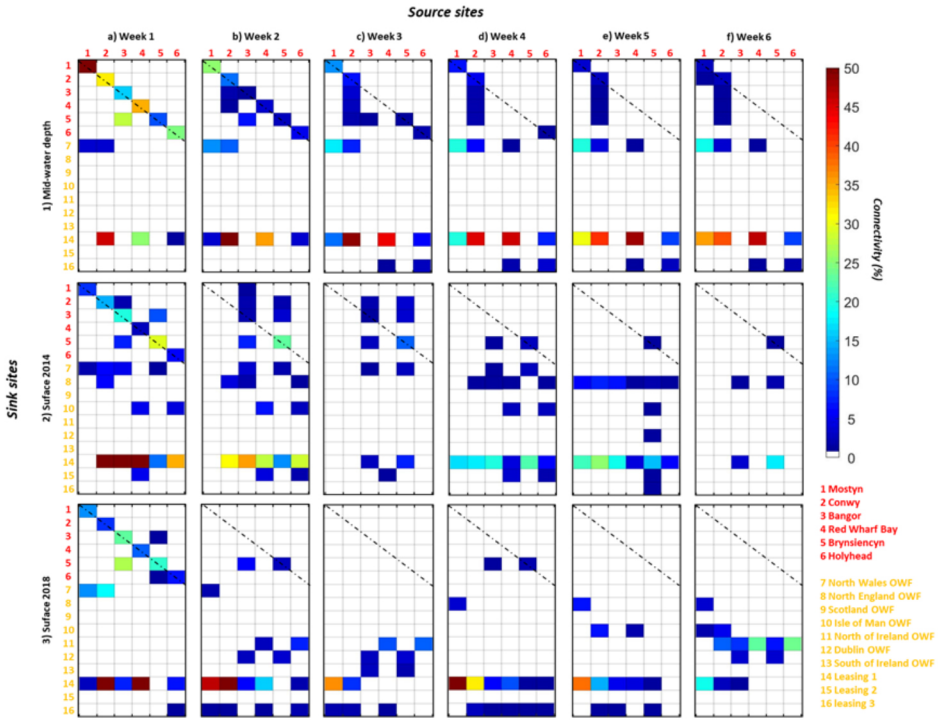


Figure 1: Connectivity matrices for particles when released from 6 sources site (1-6) at: 1) midwater depth; 2) surface during March and April in 2014; and 3) surface during March and April in 2018. Results are presented per week: (a) week 1; (b) week 2; (c) week 3; (d) week 4; (e) week 5; and (f) week 6. Connectivity between larvae from a source (column) with a sink (row) is highlighted by colour scale with high connectivity in red, low connectivity in blue and no connectivity in white. Self-recruitment (e.g. retention within the release site) is indicated by cells that cross the diagonal dashed line. Sites are colour coded as: red = source and sink sites and orange = sink sites only.

References

G.R. Di Tullio, P. Mariani, G. Benassai, D. Di Luccio & L. Grieco (2018). "Sustainable use of marine resources through offshore wind and mussel farm co-location". *Ecological Modelling*, 367: 34-41.

J. Hambrey & S. Evans (2016). "SR694 Aquaculture in England, Wales and Northern Ireland: an analysis of the economic contribution and value of the major sub-sectors and the most important farmed species," *Seafish*.

J. Van der Molen, S.I. Rogers, J.R. Ellis, C.J. Fox & P. McCloghrie (2007). "Dispersal patterns of the eggs and larvae of spring-spawning fish in the Irish Sea, UK," *Journal of Sea Research*, 58(4): 313-330

EFFECTS OF SYNBIOTIC DIETS ON RE-ESTABLISHMENT OF THE ATLANTIC SALMON GUT MICROBIOTA AFTER DYSBIOSIS TRIGGERED BY MEDICATED FEED

Anusha K.S. Dhanasiri[1*], Alexander Jaramillo-Torres[1], Abdelaziz A. A. Mohammed[1], Yanxian Li[1], Torunn Forberg[2], Åshild Krogdahl[1], Trond M. Kortner[1]

[1]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), Oslo, Norway
[2]Biomar RD, Trondheim, Norway
Email: anusha.dhanasiri@nmbu.no

Introduction

Salmon aquaculture industry has rapidly expanded over the last decades with Norway and Chile being the main contributors. With this rapid growth, it has encountered alarms over environmental impacts and sustainability. Among the sustainability issues are factors affecting fish welfare and health including sea lice and disease outbreaks leading to chemical and antibiotic usage in some regions of global salmon farming. Antibiotic usage could lead to issues and concerns over the development of antibiotic resistance and undesirable effects on the host. Several studies have reported the presence of antibiotic resistant bacteria and/or antibiotic resistant genes in intestine of medicated fish and healthy fish, farming and non-farming sites indicting the significance of the antibiotic resistance related to salmon aquaculture industry. Antibiotics can trigger perturbations in host gut microbiome resulting in dysbiosis, leading to detrimental health effects. Even though, a few studies have reported the perturbations in gut microbiota of Atlantic salmon caused by some antibiotics, in depth understanding of antibiotic-induced dysbiosis and related health and physiological effects are lacking.

Synbotic, a mixture of probiotic and prebiotic agents, exerts beneficial affects to the host by increasing the survival and activity of probiotics and indigenous health promoting bacteria in the gut. Several previous studies described the beneficial effects of synbiotics on salmonoids. Mammalian studies showed concomitant use of probiotics, prebiotics and synbiotics with antibiotics could re-establish gut microbiota and prevent antibiotic resistance and antibiotic associated gastrointestinal disorders. However, the use of pre- pro- or synbiotics to assess similar effects in fish are scanty.

The present study attempted to address those research gaps by evaluating the effects of synbiotic diets on re-establishment of the Atlantic salmon gut microbiota after dysbiosis triggered by medicated feeds.

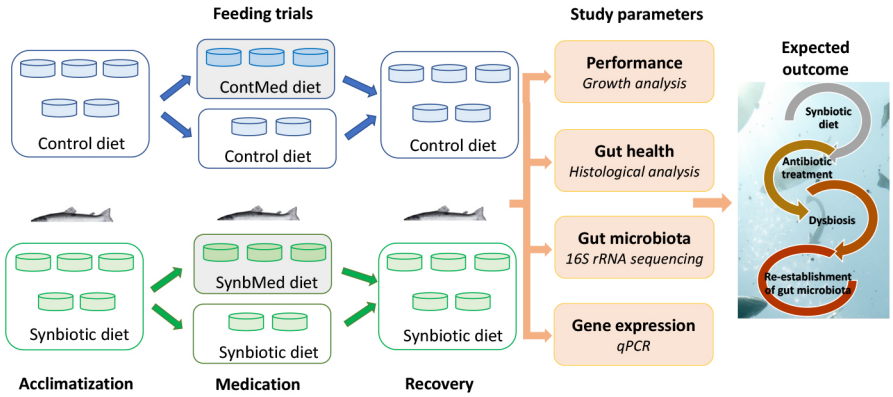


Figure 1. Systematic representation of experimental design used to evaluate the effect of synbiotic diets on re-establishment of the Atlantic salmon gut microbiota after dysbiosis triggered by medicated feeds.

(Continued on next page)

Materials and methods

Systematic representation of experimental design, experimental procedure and expected outcome is illustrated in the Figure 1. Post-smolt Atlantic salmon were fed with a control commercial diet and a synbiotic diet in which the commercial diet was supplemented with 0.04% *Pediococcus acidilactici* and 0.1% scFOS, for a period of five weeks. Each of the feed group had five pens. After the acclimatization period, fish in the three out of five pens in each of the groups were fed with medicated feeds containing either control diet + florfenicol (3.5ppm) or synbiotic diet + florfenicol (3.5ppm) for a period of two weeks. Then those fish were re-fed with their respective non-medicated diets i.e control or synbiotic diets, for another six weeks. The fish in the remaining two pens from each group were continuously fed with control or synbiotic diets until the end of the feeding trial.

Fish used for microbiota analysis were pit-tagged and digesta samples were collected at the start and end of the medicated feeding and end of the re-feeding period. Microbiota analysis was performed with 16S rRNA sequencing and subsequent bioinformatics analysis were carried out using QIIME2. Growth performance, histomorphology and transcriptomic changes were also evaluated.

Results and Discussion

Treatment with antibiotics negatively affected the thermal growth coefficient in both the control and synbiotic diet fed groups. However, the reduction in growth was less in the synbiotic group compared to the control group. Analysis of microbiota data is currently in progress and will be presented in the conference. We are expected to see that antibiotic treatment related perturbations in digesta-associated microbiota in both the groups. Moreover, we hypothesized that the fish fed with synbiotic feed would indicate rapid re-establishment of intestinal microbiota within a period of six weeks or earlier. Further, it was also hypothesized that this rapid maintenance of intestinal microbiota may positively impact in the reduction of the growth loss observed in those fishes. The knowledge generated in this study could be further explored to improve antibiotic related dysbiosis in gut microbiota and related physiological impacts in Atlantic salmon aquaculture industry.

CAN MEAGRE (*Argyrosomus regius*) BENEFIT FROM HIGH DIETARY PROTEIN HYDROLYSATE LEVELS AT EARLY DEVELOPMENTAL STAGES?

Jorge Dias[a*], Wilson Pinto[a], Maria Morais[a], Sara Castanho[b], Ana Candeias-Mendes[b], Florbela Soares[b], Pedro Pousão Ferreira[b] and Luís E.C. Conceição[a]

[a]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[b]IPMA – EPPO, Portuguese Institute for the Ocean and Atmosphere (IPMA, IP), Aquaculture Research Station, Av. 5 de Outubro s/n. 8700-305 Olhão, Portugal

*E-mail: jorgedias@sparos.pt

Introduction

Meagre (*Argyrosomus regius*) is an emerging fish species with potential for aquaculture diversification in Southern-European countries. During the early developmental stages, meagre larvae are extremely voracious and have exceptionally high growth rates. These features primarily suggest the need for an abundant supply of dietary amino acids (AA) for growth and energetic purposes (Conceição et al., 2011). On the other hand, such characteristics favour the opportunity for an early introduction of inert microdiets in meagre feeding regime. However, microdiets are largely composed by complex proteins that offer a reduced digestibility in comparison with live-prey. This holds true particularly at the onset of exogenous feeding, when larval digestive tract is still undergoing maturation. Dietary protein complexity in microdiets can be attenuated through the inclusion of protein hydrolysates, composed in a large fraction by di and tri-peptides with a high potential for bioactive properties. Such inclusion contributes for a higher amino acid absorption efficiency, favours the maturation of larval digestive tract and potentially positively affects stress and disease resistance (Conceição et al., 2011). Nevertheless, optimal inclusion levels for protein hydrolysates in microdiets for first feeding larvae are not yet determined. In fact, several studies have shown that inadequate inclusion levels may lead to detrimental effects on larval performance (Cahu et al., 1999; Kolkovski and Tandler, 2000). To this end, this study aimed at evaluating the effect of moderate and high dietary protein hydrolysate levels on the growth performance and survival of first-feeding meagre larvae.

Materials and methods

Meagre larvae were reared at IPMA facilities (Olhão, Portugal) according to standard zootechnical procedures from 3 to 31 days after hatching (DAH). To this end, larvae were initially distributed by 9 tanks of 200L at a density of 60 larvae/L. During the experimental period, larvae fed on one of the following microdiets (dietary treatments): MOD – moderate protein hydrolysate levels; and HIGH: high protein hydrolysate levels. Both diets were produced by fluid-bed agglomeration and contained 60% crude protein and 17% crude lipids. Additional ingredients to the protein hydrolysates included in both microdiets were as follows: fishmeal, squid meal, crustacean meals, wheat gluten, fish oil and soy lecithin. From 3 to 20 DAH larvae from both treatments were also co-fed rotifers and Artemia following IPMA feeding regime. Fish were sampled at 3, 16 and 31 DAH for determination of biometrical parameters and survival (endpoint only).

Results

At 16 days after hatching (DAH) the total length of larvae was not significantly different (Figure 1) between treatments. However, at 31 DAH meagre larvae fed with MOD diet presented a significantly higher total length than those fed the HIGH diet (20.5 vs 23.85 mm). At the end of the trial no significant differences were observed in regards to meagre larval survival (varying from 23.7 to 24.5 %) in the MOD and HIGH treatments.

Discussion

This study showed that meagre larvae did not benefit from the inclusion of high dietary protein hydrolysate levels after 2 weeks of development. At the end of this period, results did not show significant differences in larval performance of both MOD and HIGH treatments. However, at the end of the experimental period, larvae from the MOD treatment showed a significantly higher total length than larvae from the HIGH treatment. These findings suggest that high dietary protein hydrolysate levels may lead to detrimental performance results following two weeks of meagre development. Similar findings were previously reported for Senegalese sole (Canada et al., 2017) and gilthead seabream (Nunes, 2019) larvae. These results, obtained in different species, may be related to the increasing ability of larval digestive tract to deal with complex protein whilst maturating. In this sense, older larvae may be able to benefit from slow digestion and absorption of complex dietary proteins. In contrast, short peptides in protein hydrolysates may have a faster flow through the gut wall, potentially creating amino acid imbalances and increasing catabolism (Kolkovski and Tandler, 2000). Moreover, survival and growth results do not suggest benefits from eventual bioactivities of the used protein hydrolysates. Overall, high dietary protein hydrolysate levels do not seem recommended following the first two weeks of development of meagre and other marine fish species.

(Continued on next page)

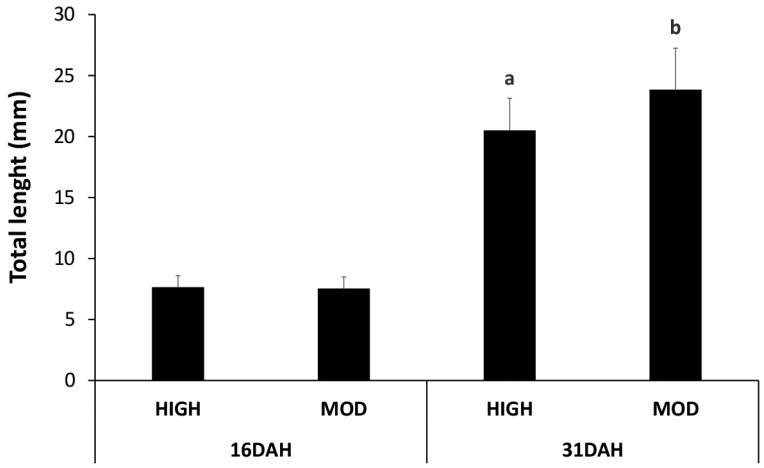


Figure 1. Total length of meagre larvae fed with microdiets containing different quantities of hydrolysates in their composition.

References

Cahu, C.L., Zambonino Infante, J.L., Quazuguel, P., Le Gall, M.M., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture 171, 109-119.

Canada, P., Conceição, L.E.C., Mira, S., Teodósio, R., Fernandes, J.M.O., Barrios, C., Millán, F., Pedroche, J., Valente, L.M.P., Engrola, S., 2017. Dietary protein complexity modulates growth, protein utilisation and the expression of protein digestion-related genes in Senegalese sole larvae. Aquaculture 479, 273-284.

Conceição, L.E.C., Aragão, C., Rønnestad, I., 2011. Proteins. In: Holt, J. (Ed.), Larval Fish Nutrition. John Wiley & Sons, Inc., UK, pp. 83-116.

Kolkovski, S., Tandler, A., 2000. The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. Aquacult Nutr 6, 11-15.

Nunes, B., 2019. Development of a microencapsulated prototype to deliver low molecular weight proteins at the onset of exogenous feeding of marine fish larvae. Master Thesis. Politecnic Institute of Beja, 59 pp.

Acknowledgements

Funding from project 39948_FeedMi, supported by Portugal and the European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020.

EVALUATION OF THE POTENTIAL OF TWO STRAINS OF *Bacillus subtilis* AS ORAL ADJUVANTS IN RAINBOW TROUT (*Oncorhynchus mykisS*)

F. Docando[1], C.R. Serra[2], P. Arense[1], N. Nuñez-Ortiz[1], P. Enes[2], A. Oliva-Teles[2], C. Tafalla[1] and P. Díaz-Rosales[1*]

[1]Animal Health Research Center (CISA-INIA-CSIC), Madrid, Spain
[2]CIIMAR, University of Porto, Porto, Portugal
E-mail: diaz.patricia@inia.es

Introduction

Oral vaccines are highly demanded by the aquaculture sector, which requests alternatives to labor-intensive injectable vaccines that involve individual handling of fish, provoking stress-related immunosuppression and handling mortalities. Despite this, most attempts to obtain effective oral vaccines have failed in fish and mammals. In this context, the search for mucosal adjuvants that when orally delivered along with antigens could help circumvent intestinal tolerance, and thus induce an adequate immune response to the antigen, is an important step for the development of effective oral vaccines. In the current work, we performed a series of experiments with rainbow trout (*Oncorhynchus mykiss*) aimed at establishing the adjuvant potential of two *Bacillus subtilis* spore-forming strains isolated from farmed fish, designated as FI99 and FI162.

Materials and methods

The effect of the two strains of *Bacillus subtilis* was first evaluated *in vitro* and *ex vivo* at transcriptional level. Thus, rainbow trout intestinal epithelial cell line RTgutGC and gut tissue sections, were incubated with different concentrations of FI99 and FI162 for 24 h and the expression of genes involved in immune response evaluated by real-time PCR. Moreover, the capacity of both strains to adhere to RTgutGC cells was evaluated by flow cytometry. Finally, the FI99 strain was selected to undertake an *in vivo* experiment. In this case, a single dose of the bacteria was orally administered (by force feeding) and the transcriptional effects studied in the spleen, kidney and gut after 1, 3 and 7 days.

Results

Although both strains had the capacity to modulate the transcription of several genes related to innate and adaptive immune responses, it was the FI99 strain that provoked more immune effects, also exerting a higher binding capacity to intestinal epithelial cells. Consequently, we selected this strain to establish the transcriptional effects in the spleen, kidney, and gut of rainbow trout at 1, 3, and 7 days after a single oral administration of the bacteria. In the gut, genes related to T cell activity as well as IgD were modulated by the bacteria, whereas in systemic tissues, genes involved in inflammation were the ones that were mostly affected, along with immunoglobulin and antimicrobial genes. Overall, the transcriptional effects of FI99 *in vivo* were more evident in spleen and kidney, than in the gut.

Discussion and conclusions

Although *Bacillus* spp. are well known for their probiotic properties, their potential as mucosal adjuvants had not yet been studied in depth in fish. Our results demonstrate that *B. subtilis* modulates the levels of transcription of a wide range of immune genes in the rainbow trout gut. Interestingly, significant differences were found in between the two strains tested in both their immunostimulatory and their binding capacity. Finally, we have established that a single oral administration of the FI99 strain is capable of regulating immune gene transcription locally as well as in systemic immune tissues, pointing to this probiotic strain as a good mucosal adjuvant candidate that would help to increase the immune response mounted against orally-delivered antigens.

Acknowledgements

This work was supported by the Spanish Ministry of Science, Innovation and Universities (project AGL2017-85494-C2-1-R) and by the *Comunidad de Madrid* (grant 2016-T1/BIO-1672). Technical support of Lucía González is also greatly acknowledged.

ZEBARFISH AS AN EXPERIMENTAL MODEL IN FISH NUTRITION STUDIES: ASSESSMENT OF MACRO- AND MICROALGAE EXTRACTS EFFECT ON IMMUNE RESPONSE AND GROWTH

M. Monteiro[1,2], A.S. Lavrador[1], F. Rangel[1,2], A. Oliva-Teles[1,2], A. Couto[1,2], A.P. Carvalho[1,2], P. Enes[1,2] and P. Díaz-Rosales[1,3*]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, Edifício FC4, Porto 4169-007, Portugal
[3]Inmunología y Patología de Peces, Centro de Investigación em Sanidad Animal (CISA, INIA), Carretera de Algete a El Casar s/n, 28130, Madrid, España
E-mail: mailingmarta@gmail.com

Introduction

Enhancing host defense mechanisms with natural immunotherapeutic agents has become increasingly important for the treatment and prophylaxis of farmed fish diseases. Due to their immunotherapeutic potential, algae-derived molecules have been a focal point for aquaculture research (Harikrishnan et al., 2011). In fact, previous studies concerning the role of algal extracts as immunostimulants reported an improvement of innate immune responses in fish fed algal supplemented diets (del Rocío Quezada-Rodríguez & Fajer-Ávila, 2017). Thus, the present research intends to explore novel immunotherapeutics produced by macro- and microalgae and use such bioactive compounds as functional ingredients in diets for aquaculture using zebrafish larvae and juveniles as an experimental model.

Materials and methods

Methanolic (methanol/water, 50:50 v/v) extracts from the macroalgae *Fucus vesiculosus* and *Ulva rigida*, and ethanolic (ethanol/water, 80:20 v/v) extract from microalgae *Nannochloropsis gaditana* were obtained as described in Monteiro *et al*. (2019). Diets for larvae and juveniles zebrafish were formulated, and supplemented with the algal extracts. Thus, a plant-based diet was used as a control (diet C), and seven other diets were prepared similar to diet C, supplemented with 1g Kg^{-1} of each algal extract (*Fucus vesiculosus*, F; *Nannochloropsis gaditana*, N and *Ulva rigida*, U), or a combination of extracts (diets FN, FU, NU, FNU). A fishmeal-based diet was also included as a reference in zebrafish growth trial (C+). First, the effect of the dietary treatments on zebrafish larvae in a short-feeding trial was assesed by evaluating the immune response and intestinal morphology, by gene expression and histological analysis, respectively. Secondly, dietary treatments effect on zebrafish juveniles' growth performance, intestinal integrity and immune status was evaluated through growth and immune- related genes expression in the muscle and intestine, respectively, as well as, through histological analysis.

Results

In zebrafish larvae, most promising results were obtained with methanol extract from *F. vesiculosus*, which exerted an anti-inflammatory action when incorporated alone into zebrafish diets and promoted immune activation, by inducing the expression of pro-inflammatory cytokines, TNF-α and IL-1β when combined with the other extracts. Moreover, dietary inclusion of the extracts improved intestinal morphology compared to that of fish fed the control diet.

Regarding zebrafish juveniles, diet C+ outperformed the remaining dietary treatments, growth-wise. None of the extracts improved diet C negative effects. However, the inclusion of *U. rigida* and *N. gaditana* extracts promoted an immunomodulatory action after 1 week of trial, by upregulating cytokine expression. This effect subsided after 30 days, suggesting tolerance may be developed over time. In contrast, effects on growth-related genes were still observed after 5 weeks of feeding F, N, U, and FN diets. Algal extracts dietary inclusion did not compromise intestinal integrity.

(Continued on next page)

Discussion and conclusions

One major limitation of plant-based products is the presence of antinutritional factors, which fish are not able to metabolize and, when present in the diets, can cause several effects associated with digestive physiology and metabolism, impacting on fish welfare and resulting in reduced productivity (Oliva-Teles et al., 2015). This study showed that, although zebrafish is an omnivorous species, a plant-based diet (C) reduced zebrafish growth compared with a fishmeal based diet (C+). Although the C+ group performed better growth-wise, there was no specific pattern in growth-related gene expression that could explain the major differences observed in growth performance. Therefore, the exact mechanisms underlying the effects of dietary plant protein sources and algal extract supplementation on myogenic regulation related expression in fish remain unclear, and more studies should be conducted in the future.

The extracts effects on cytokine expression were only observed up to a week of feeding, suggesting that tolerance to the extract effect may be developed over time (Bricknell and Dalmo, 2005). Differences in gene expression observed among larvae and juveniles could be associated with the developmental stage and maturity of zebrafish immune system (Ito et al. 2008). Adding to the fact that extract supplementation does not compromise intestinal integrity, a short administration of *F. vesiculosus* extract to larvae and *U. rigida* and *N. gaditana* extracts to juveniles could stimulate periodically the fish immune system, defending fish from eventual disease risk, while reducing the costs of supplement feeding.

Acknowledgements

The authors would like to thank BUGGYPOWER for the kind supply of the *Nannochloropsis gaditana* species used in this study. M. Monteiro, A.S. Lavrador and F. Rangel were supported by grants SFRH/BD/114995/2016, ZEBRALGRE_BM_2019 and SFRH/BD/138375/2018 respectively, from FCT – Foundation for Science and Technology, under the POCI program. A. Couto and P. Enes are supported by national funds through FCT. This research was partially supported by the Strategic Funding to UID/Multi/04423/2019 (POCI-01-0145-FEDER-007621) through national funds provided by FCT under the project PTDC/CVT-WEL/5207/2014 (POCI-01-0145-FEDER-016797).

References

Bricknell, I., & Dalmo, R. A. (2005). The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol 19(5), 457-472.

del Rocío Quezada-Rodríguez, P. & Fajer-Ávila, E.J. (2017) The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). *J Appl Phycol*, 29:423–431.

Harikrishnan, R., Balasundaram, C. & Heo, M. S. (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture*, 317(1-4): 1-15.

Ito, K., Takizawa, F., Yoshiura, Y., Ototake, M., & Nakanishi, T. (2008). Expression profile of cytokine and transcription factor genes during embryonic development of zebrafish *Danio rerio*. *Fish Sci*, 74(2): 391-396.

Monteiro, M., Santos, R. A., Iglesias, P., Couto, A., Serra, C. R., Gouvinhas, I., Barros, A., Oliva-Teles, A., Enes, P. & Díaz-Rosales, P. (2019). Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro- and microalgae extracts. *J App Phycol*, 1–14.

Oliva-Teles, A., Enes, P., & Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Feed and Feeding Practices in Aquaculture (pp. 203– 233). Elsevier.

IMMUNE EFFECTS OF DNA VACCINATION AGAINST VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

A. Arrogante, F. Docando, L. González, P. Díaz-Rosales* and C. Tafalla

Animal Health Research Center (CISA-INIA-CSIC), Madrid, Spain
E-mail: diaz.patricia@inia.es

Introduction

DNA vaccination has proved as an effective method for inducing a potent and enduring protection against different fish viruses, especially rhabdoviruses such as viral hemorrhagic septicemia virus (VHSV). The VHSV DNA vaccine consists in a eukaryotic expression plasmid that codes for the G glycoprotein. When intramuscularly injected, this vaccine is able to confer a strong and long-lasting protection to vaccinated fish. Nevertheless, the immune mechanisms through which this vaccine exerts its effects are still not clear. In this context, the main objective of this work was to increase our understanding of how DNA vaccines work in fish, by studying how leukocytes from vaccinated rainbow trout respond to a secondary encounter with the virus.

Materials and methods

Fish of approximately 50 g were intramuscularly injected with 10 μg of a VHSV DNA vaccine in 100 μl of saline solution. Control groups included fish injected with the empty plasmid or the same volume of saline solution. At days 14 and 28 days post-vaccination, fish from the different immunization groups were sacrificed and spleen and head-kidney leukocytes isolated. Leukocytes were then exposed *in vitro* to heat-inactivated virus or left untreated. The proliferation of IgM^+ B cells and the levels of transcription of different immune genes was evaluated.

Results

Head kidney leukocytes obtained from vaccinated fish proliferated when exposed to the inactivated virus *in vitro* to a greater extent than leukocytes obtained from unvaccinated fish. This proliferation was detected after 28 days but not at earlier time points or in splenic leukocytes. Regarding the transcriptional analyses conducted, spleen and head kidney leukocytes responded to inactivated VHSV by increasing the transcription of different immune genes, including pro-inflammatory cytokines and antimicrobial peptides. However, in most cases, no significant differences were observed between the responses of vaccinated and control groups. Notably, head kidney leukocytes obtained from vaccinated fish transcribed significantly higher levels of the innate receptor MDA5 in response to re-stimulation with the inactivated virus than leukocytes from control fish.

Discussion and conclusions

The fact that the differential proliferative response of B cells to VHSV *in vitro* found in head kidney leukocytes obtained from vaccinated fish was observed at late time points strongly suggests that it is at this point when B cells that specifically recognize VHSV (memory B cells) can be found in the head kidney. Additionally, the increased MDA5 transcription observed when leukocytes from vaccinated fish encounter the virus, strongly suggests that viral recognition is primed in vaccinated fish.

Acknowledgements

This work was supported by the Spanish Ministry of Science, Innovation and Universities (project AGL2017-85494-C2-1-R) and by the *Comunidad de Madrid* (grant 2016-T1/BIO-1672).

GENETIC VALIDATION OF THE XRAY METHOD FOR PHENOTYPING FEED INTAKE IN ATLANTIC SALMON

Gareth F. Difford*[1], B. Hatlen[1], B. Gjerde[1], O. H. Romarheim[1], G Baeverfjord[1], K Heia[1], A. Norris[2], A.K. Sonesson[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-9291 Tromsø, Norway
[2]MOWI Genetics AS, Bergen, Norway
Email: gareth.difford@nofima.no

Introduction

Feed plays a crucial role in the economic and environmental performance of Atlantic salmon production with the cost of feed accounting for over 50% of the total production cost (http://www.fiskeridir.no) and 73-80% of the carbon footprint (Winther et al., 2020). Improving the feed efficiency of Atlantic salmon has potential to be a win-win situation for improving profitability and resource efficiency. Direct selection for improved feed efficiency requires records of feed intake on replicated family groups in tanks through daily recording of the given and the wasted feed in each tank. Due to the large number of tanks required this is both costly and labour demanding and accesses half of the genetic variation. An alternative method is to feed with pellets containing radio opaque markers and use Xray radiography to count the total markers consumed by individual fish. The Xray method for daily feed intake has been shown to be heritable in rainbow trout (Kause et al., 2006) and chinook salmon (Walker et al., 2012), however estimates for Atlantic salmon are lacking. The advent of genomic selection and advances in digital radiography and image analysis offer the possibility to improve the feasibility of obtaining individual genomic breeding values for feed intake and feed efficiency of the breeding candidates. The objective of the present studies was to 1) estimate genomic parameters for Xray feed intake records and 2) validate individual Xray feed intake records with the "gold standard" tank feeding method at family level in replicate tanks.

Materials and methods

A total of 2450 Atlantic salmon parr from 35 nucleus families of the MOWI Genetics AS (MOWI ASA, ***Øyerhamn***, Norway) were PIT tagged and transported to Nofima's Research Station for Sustainable Aquaculture, Sunndalsøra. All fish were genotyped with a customized 65K SNP chip. The PIT-tagged fish were sorted by family and divided into two parallel trials; the first with a total of 700 fish equally split over two replicated tanks with an equal number of fish from all families in each of the two tanks (Xray trial) and the second trial with 1750 fish split into 70 tanks with two tanks per family and 25 fish per family (tank feeding trial). The tanks were divided in two different rooms in the same building.

In the **Xray trial**, fish were acclimated to the two tanks for three weeks. During this period fish were transitioned to a feeding regime where they received the entire daily feed ration over a six hour period. A day prior to Xray, the feed was switched to an identical dietary formulation including glass beadlets. After consuming the daily ration fish were anesthetised and Xrayed. Beadlets were counted using a customised image analysis software developed by Nofima. Number of recorded beadlets per fish were converted to the amount of feed intake (g) per fish (FI_{Xray}) using a calibration standard based on feed samples of known mass. FI_{Xray} was analysed by a mixed linear animal model using DMU (Madsen and Jensen, 2014) including the fixed effects of tank (with two levels), a fixed regression on time within the Xray recording day, a random animal additive genetic effects captured by the genomic relationship matrix. The heritability was estimated from variance components ($h^2 = Va/Vp$). The average feed intake per fish, per family and tank (FI_{XrayF}) was analysed by a linear model including the fixed effect of tank (with two levels) and a random effect of family. The variance components for the family effect was expressed relative to the total variance ($H^2 = Vf/Vp$).

In the **tank feeding trial**, daily feed rations and refusals were collected over 50 days. At day 50 the trial, fish were euthanized and recorded for body length and weight. The average family biomass per tank was modelled as a function of time throughout the trial and daily feed intake records (FI_{TF}) were matched to the Xray feed intake records of the Xray trial (FI_{Xray}) on an equal average family body weight basis. The accumulated feed intake per tank (FI_{TF}) was analysed by a linear model including the fixed effect of room (with two levels) and the random effect of family ($H^2 = Vf/Vp$). A bivariate analysis was run FI_{XrayF} and FI_{TF} with the fixed effects of tank (with two levels) for FI_{Xray} and the fixed effect of room for FI_{TF}, and the random effect of family for both traits. The covariance between random effects of family used to compute the correlation between for families between methods.

(Continued on next page)

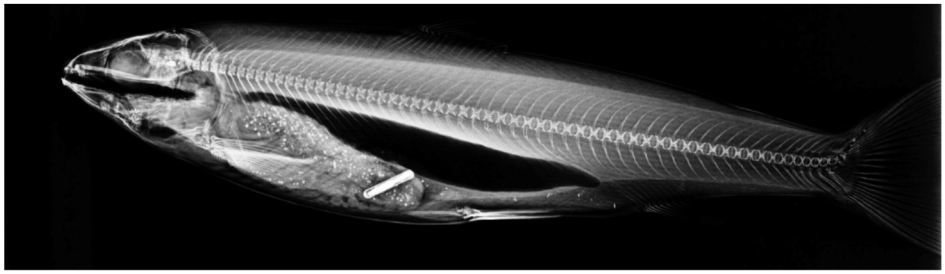


Figure 1. A digital Xray radiograph of an Atlantic salmon with radiopaque beadlets.

Table 1. Descriptive statistics for FI_{Xray} of individual records and family means as well for FI_{GS} of family means.

Trait	N	Level	Mean* ± SD	ICC† ± SE
FI_{Xray}	685	Individual	1.56 ± 0.28	0.22 ± 0.05
FI_{XrayF}	68	Family	1.56 ± 0.13	0.38 ± 0.02
FI_{TF}	70	Family	1.51 ± 0.41	0.37 ± 0.02

* Indicates average feed intake in grams per fish at measurement. † is the intraclass correlation coefficient for heritabilties, level indicates if the ICC is narrow sense heritability (h^2) or broadsense heritability (H^2).

Results

Results of the feed intake at individual and family level are presented in Table 1 along with their respective intraclass correlation coefficients. The heritability (h^2) of FI_{Xray} was 0.22 ± 0.05. The family means of FI_{Xray} explained 38% of the total variation which was very similar to the family effect (37%) of the total variation in the accumulated FI_{TF}. Crucially, the rank correlation between the families FI_{XrayF} and FI_{TF} was high and positive (0.85 ± 0.25).

Conclusion

For the first time, significant heritability of feed intake using the Xray method has been reported in Atlantic salmon. Validation at the family shows that a substantial proportion of the variation in feed intake among the families are co-explained by both methods. This demonstrates the feasibility of the Xray method for recording feed intake in Atlantic salmon and lays down the foundation for research into feed efficiency in Atlantic salmon.

Funding

The Xray trial was developed by the Nofima internal project "PrecisionVision", the gold standard trial was part of the EU project "NewTechAqua" (H2020 No 862658) and the EU project AquaImpact (H2020 No 818367).

References

Kause, A., Tobin, D., Dobly, A., Houlihan, D., Martin, S., Mäntysaari, E.A., Ritola, O., Ruohonen, K., 2006. Recording strategies and selection potential of feed intake measured using the X-ray method in rainbow trout. Genet. Sel. Evol. 38, 389–409. https://doi.org/10.1051/gse:2006011

Madsen, P., Jensen, J., 2014. A user's guide to DMU, version 6, release 5.0. Cent. Quant. Genet. Genomics Dept. Mol. Biol. Genet. Univ. Aarhus Res. Cent. Foulum Box 50, 8830 Tjele Denmark.

Walker, S.P., Ingram, M., Bailey, J., Dodds, K.G., Fisher, P.J., Amer, P.R., Symonds, J.E., 2012. Chinook salmon (Oncorhynchus tshawytscha) feed conversion efficiency : evaluation and potential for selection. Proc. New Zeal. Soc. Anim. Prod. 72, 227–230.

Winther, U., Hognes, E.S., Jafarzadeh, S., Ziegler, F., 2020. Greenhouse gas emissions of Norwegian seafood products in 2017.

NEW COMMERCIAL MICROALGAE DIETS FOR BIVALVES APPLIED TO PORTUGUESE OYSTER (*Crassostrea angulata*)

Patrícia Diogo[1], Gonçalo Bastos[1], Cristiana Gastão[2], João Carneiro[2], Ana Marreiros[3,4], Alexandre Rodrigues[1], António Falcão[2], Victória del Pino[1] and João Navalho[1]

[1]Necton, S.A., Belamandil, 8700-152 Olhão, Portugal, [2]Viveiros Rio Mira LDA, Roncanito-Algoceira, 7630-013 Odemira, Portugal, [3]Department of Biomedical Sciences and Medicine, University of Algarve, 8005-139, Faro, Portugal, [4]Algarve Biomedical Center, Campus Gambelas, 8005-139, Faro, Portugal.
E-mail: patricia.diogo@necton.pt

Introduction

Few commercial multialgal diets formulated for bivalve's are available and generally lack important microalgae difficult to produce industrially, such as *Tisocrysis lutea* and *Skeletonema costatum*. Microalgae production in hatcheries is expensive (30-50% of total costs)[1], labour intensive and subjected to productivity fluctuations. Therefore, commercial microalgae diets are a major asset. The objective of a nursery is to achieve high larvae quantities of good quality and improve juvenile growth. Good quality nutrition is essential for broodstock maturation in order to generate high quality gametes and offspring, and to optimize juvenile growth. Oysters (*Crassostrea* spp.) depict the world's most produced mollusc species. Portuguese oyster (*Crassostrea angulata*) has a high potential for aquaculture, although its natural populations suffered a decline in the past decades. Therefore, *C. angulata* production is important for aquaculture, being simultaneously relevant for natural populations restocking and conservation. Commercial microalgae diets can improve aquaculture management. This work aims to develop commercial diets for bivalves, formulated with a blend of microalgae species commonly used in aquaculture for oyster nutrition applied to Portuguese oyster broodstock and juveniles.

Material and methods

Pilot liquid concentrated products (8% of dry weight-DW) of industrially produced microalgae were formulated. Diet 1 contained *Tetraselmis* sp., *Skeletonema* sp., *Tisocrysis lutea* and *Pavlova* sp. (8/16:6/16:1/16:1/16), while diet 2 was formulated with *Skeletonema* sp., *Tisocrysis lutea* (T-ISO clone) and *Tetraselmis* sp. (11/16:5/6:1/16). Diets´ biochemical analysis was performed. Controls were based on live algae: microalgae for broodstock were produced on site (*Tisocrysis lutea*, *Tetraselmis* sp. and *Skeletonema* sp.); juveniles from the control group were grown in Mira river (Odemira, Portugal) feeding on the naturally occurring microalgae. Animals were fed daily with an amount equivalent to 4% of the oyster dry meat (g) in DW of microalgae (mg). A preliminary experiment was performed in *C. angulata* broodstock, where the control and Diet 2 groups were conditioned in duplicate (each replica n=20) at 20±1°C, 20‰, being evaluated the oysters' weight, biometry and condition index. Gametes were extracted by gonadal incisions. Oocytes from females of control (n=2) and diet 2 treatment (n=2) were fertilized *in vitro* with sperm from a single control male in triplicate, to compare oocyte quality between treatments in this preliminary approach. Sperm concentration was quantified with a Neubauer chamber. The evaluation of oocyte fertilization and larvae survival (48 hours post fertilization) was conducted. Juveniles were directly placed in nets on the natural environment as control in duplicate (n=250 oysters per replica) or conditioned indoors and fed with Diet 1 and 2 for 3 months (duplicate, n=250). Juveniles were sampled (n=50) throughout time for measurements (survival, weight, length and width) (total n=3175) and collected for condition factor evaluation (n=266). The environmental conditions were daily monitored. IBM SPSS Statistics 26.0 software was used for statistical analysis, one-way ANOVA was used to compare differences between juvenile groups and Student´s t-test for *in vitro* fertilization data ($p<0.05$). A cluster analysis was applied to juvenile data and, subsequentially, a decision tree approach allowed to understand juvenile's growth pattern in the different size groups of oysters in the treatments.

Results

Proximal composition showed the proteins, lipids and ashes content of Diet 1 (23, 10.9, 63%) and Diet 2 (9.9, 2.1, 82.2%) respectively. The broodstock established in control group was heavier and had higher length (94.4±15.6g, 83.2±7.2mm) compared to Diet 2 treatment (56.8±18.5g, 69.7±7.5mm) in the beginning of the experiment. Preliminary results of oocytes obtained by the broodstock fed with Diet 2 showed 86.3±10.6% of fertilization rate, which was significantly lower compared to control (93.9±8.6%). For juvenile there were no significant differences in the survival rate in all treatments. Juveniles fed with Diet 2 showed higher and less variable weight and length gain (R^2=0.8542, R^2=0.7219) than Diet 1 (R^2=0.3101, R^2=0.4233) and control (R^2=0.1469, R^2=0.3733). Even though oysters in the control started with higher weight and biometric measurements, at the end of the trial there were no significant differences in all treatments regarding the wet weight and height (n=3175). To the group of juveniles collected for condition factor index evaluation, a K-means cluster

(Continued on next page)

analysis was applied to aggregate oysters by size groups (large, medium and small size groups). A decision tree approach was applied through a CHAID method to the clusters obtained, with wet weight as dependent variable (n=266). In small size oyster cluster group, Diet 1 and control promoted the highest wet weight below 40.6mm, while above this length, Diet 2 improved oyster's weight. In medium size oysters cluster group below 40.6m (33.5-34.8mm) Diet 1 showed the highest wet weight. In large size oysters cluster group below 40mm, Diet 1 and control had the highest weight, while in oysters above 40mm Diet 2 showed the highest value.

Discussion

Preliminary tests in *C. angulata* broostock showed that oysters fed on Diet 2 had an equivalent weight to the control group. Obtained oocytes with Diet 2 treatment exhibited high fertilization rate and larvae survival, although significantly lower than the control, which can be explained by the low number of animals. Therefore, in the future, a large-scale test will be performed with Diet 2 in oysters maturation and larvae quality. The growth of juvenile oysters with diets is more constant throughout time than in control, which suggests that natural environmental fluctuations promote variability in microalgae species and abundance, impacting oyster growth. In oysters below 40.6 mm, Diet 1 promoted equivalent weight to the natural environment. Higher protein and lipids content in Diet 1 can support the high requirements for growth of smaller oysters. Prototype Diet 2 promoted high and steady growth in Portuguese oyster juveniles. This is a balanced diet based on the microalgae species and proportions used in oysters hatcheries[2]. To enhance wet weight gain in Portuguese oysters a dietary protocol based on Diet 1 until 40.6 mm and Diet 2 after this length can be used. In conclusion, the formulation of microalgae diets, such as Diet 2, specifically developed for bivalves can simplify and support nurseries management.

References

1.Willer, D. & Aldridge, D. C. Microencapsulated diets to improve bivalve shellfish aquaculture. R. Soc. Open Sci. 4, (2017).

2.Anjos, C. et al. Broodstock conditioning of the Portuguese oyster (*Crassostrea angulata*, Lamarck, 1819): influence of different diets. Aquac. Res. 48, 3859–3878 (2017).

NEW MICROALGAE FORMULATIONS FOR GREEN WATER TECHNIQUE: CASE STUDY OF MEAGRE (*Argyrosomus regius*) LARVAL REARING

Patricia Diogo[1*], Gonçalo Bastos[1], Ana Coelho[1], Sara Castanho[2], Ana Mendes[2], Diogo Esteves[2], Laura Ribeiro[2], Cátia Marques[2], Alexandre Rodrigues[1], Florbela Soares[2], Victória del Pino[1], Pedro Pousão[2] and João Navalho[1]

[1]Necton, S.A., Belamandil, 8700-152 Olhão, Portugal, [2]Aquaculture Research Station, Portuguese Institute for the Ocean and Atmosphere (IPMA), Olhão, Portugal.
*E-mail: patricia.diogo@necton.pt

Introduction

Microalgae are used in marine larval rearing in green water technique with synergistic effects beneficial for larvae. Marine fish show altricial larvae that hatch with immature organs such as eyes and gut essential to capture and digest preys. Microalgae in the water column applied through green water technique have beneficial effects in larvae since they allow the refraction of light and improve the observation of live prey, maintaining rotifers simultaneously enriched. Additionally, microalgae are known to vector beneficial probiotics that colonize larvae gut, improving environmental water quality, fish welfare and stress resistance. Industrially produced microalgae are a major asset in aquaculture, although still underused mainly due to the lack of specific product development and application protocols specifically adapted to the rearing systems. In marine larviculture the use of live microalgae produced within the facilities is still a common practice despite being labour intensive and prone to productivity fluctuations. There have been major advances in biotechnology improving the quality of microalgae biomass and increasing the number of microalgae strains available commercially. However, the application of this high-quality biomass in aquaculture presents some important challenges such as microalgae precipitation and stability in the water column, which may impair water quality. This disadvantage can be mitigated by the use of beneficial probionts through the modulation of the microbiota in the tank[1]. The application of probiotics as water conditioners has been thoroughly studied and it is a common practice in animal production, including aquaculture. The objective of this study was to develop new formulations for green water technique with industrially produced microalgae strains and probiotics using meagre (*Argyrosomus regius*) larval rearing as a case study.

Material and methods

The selection of microalgae strains and its typology was performed according to cellular dimensions, stability in water column and a technoeconomic study, after which *Nannochloropsis* sp. and *Chlorella* sp. spray dried were selected. Pilot product A (PPA) contained only microalgae and pilot product B (PPB) contained microalgae along with probiotic bacteria. An exploratory approach was performed to find adequate indicators of the formulation mixture quality to find mixing quality indicators adequate to the formulation. The hydration and the blending times were optimized with a set of tests. The colour of the blends was evaluated according to the RAL scale and the precipitation time was monitored after 24h in salt water. To investigate the effects of the selected conditions, an experimental design was set up with a control composed by live *Nannochloropsis* sp., a microalgae formulation and a formulation containing microalgae and probiotics. The experiment was performed in IPMA aquaculture research station (EPPO, Olhão) in open cylindroconical tanks (200L) in triplicate. Newly hatched larvae were introduced (43.25 larvae/L) with a water temperature of 21±1ºC, 37‰, a photoperiod of 10-14 D:L. Rotifers where introduced at mouth opening, artemia and microdiet (Caviar, Bernaqua®) where introduced at 10 days after hatching (DAH) (100-200µm) increasing the granulometry through development and fed *Ad libitum*. Environmental parameters were monitored (pH, salinity, oxygen, temperature, ammonia, nitrates). The microalgae present in the water column and its precipitation was monitored spectrophotometrically three times a week (1h, 2h and 3h after microalgae introduction). Larvae survival and growth was evaluated (n=15 per replica per time point) through total length and dry weight analysis. Microbiota was monitored in water and larvae (TSA, TCBS). A final stress challenge was applied in all treatments with a transport of 250 km. The environmental parameters were monitored (temperature, oxygen, pH, ammonia and nitrates), survival was evaluated on the arrival and 8 days afterwards.

Results and Discussion

The products revealed an adequate mixing rate of 3 min (129 rpm), being selected also an hydration time of 10 min followed by 5 min of blend. The mixture was sieved with a 50 µm mesh to remove the foam and microalgae agglomerates. The colour of the products according to RAL scale was for PPA 6028 and for PPB 6024. The quantities to be applied in the tanks were adjusted according to the turbidity measured with a probe (5±1 FTU). The microalgae dry weight in the tanks range from 0.01 to 0.04g/L.

(Continued on next page)

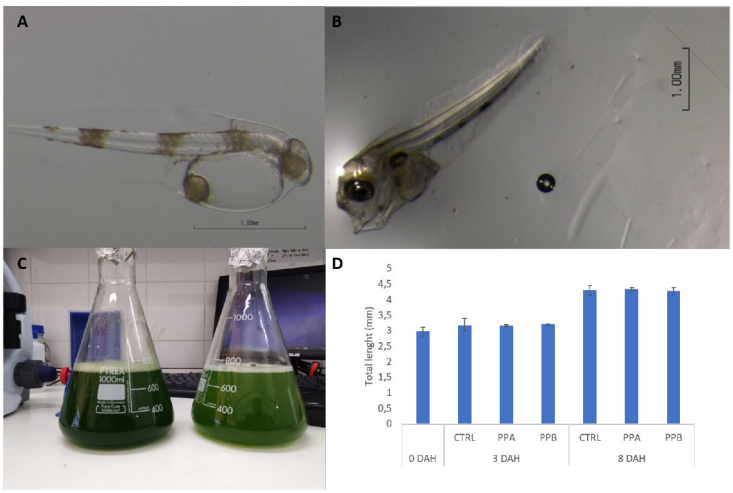


Fig 1– Meagre (*Argyrosomus regius*) larvae at A) hatching and B) 8 DAH; C) products suspension analysis A (left), pilot product B (right) and D) Total length (mm) of meagre larvae until 8 DAH.

Preliminary results showed similar growth of meagre larvae reared in green water with live microalgae or with formulated products. The combination of probiotics and microalgae allowed a higher stability of the microalgae in the water column compared with the product containing only microalgae. One hypothesis for this result is the generation of biosurfactant metabolites by the probiotics[2] contributing for the reduction of surface tension. This work will allow to understand the effect of formulated microalgae and probiotic products for green water technique applied in meagre larviculture and its effects on larvae and water bacterial communities, larvae welfare and stress resistance.

References

1.Tarnecki, A. M., Wafapoor, M., Phillips, R. N. & Rhody, N. R. Benefits of a Bacillus probiotic to larval fish survival and transport stress resistance. Sci. Rep. 9, 1–11 (2019).

2.Al-Ajlani, M. M., Sheikh, M. A., Ahmad, Z. & Hasnain, S. Production of surfactin from *Bacillus subtilis* MZ-7 grown on pharmamedia commercial medium. Microb. Cell Fact. 6, 1–8 (2007).

Acknowledgements

Supported by Project Allarvae - 069971 supported by CRESC Algarve, Portugal 2020 and European Union

FUCOXANTHIN ANALYSIS IN INDUSTRIAL CULTURE OF *Tisochrysis lutea* THROUGH SPECTROPHOTOMETRY TO IMPROVE BIOMASS QUALITY FOR AQUAFEEDS

Patricia Diogo[1], Clémence Tennevet[1,2], Gonçalo Bastos[1], Ana Coelho[1], Alexandre Rodrigues[1], Mariana Carneiro[1], Fengzheng Gao[3], Hugo Pereira[4], Victória del Pino[1], João Navalho[1]

[1] Necton SA, Olhão, Portugal
[2] Université de Rouen, Normandie, France
[3] Wageningen University & Research, Wageningen, Gelderland, Netherlands
[4] GreenCoLab, University of Algarve, Faro, Portugal
[5] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
* Correspondence: patricia.diogo@necton.pt

Introduction

Microalgae industrially produced for aquaculture purposes have several advantages over other biomass sources since they contain high variability of ingredients, different species present distinct nutritive profiles, being also possible to induce the production of target metabolites through the modulation of its cultivation methodology1. Fucoxanthin is a carotenoid produced by microalgae species with high aquaculture application such as Tisochrysis lutea, Phaeodactylum tricornutum, Chaetoceros muelleri or Thalassiosira pseudonana2. It is the most abundant xanthophyll in macro- and microalgae due to its celular photoprotection capacity. The interest in fucoxanthin for food and feed application is growing due to its advantageous properties such as antioxidant anti-proliferative, anti-obesity, anti-diabetic, anti-inflammatory and anti-angiogenic activities, that are beneficial for aquafeeds formulations, to improve fish nutrition and health3–4. Tisochrysis lutea is considered a valuable, premium microalgae species for live feed, fish and invertebrate nutrition, particularly due to its balanced biochemical profile that contains relevant fatty acids for fish nutrition such as EPA and DHA and adequate protein content to support animal growth. It has no cellular wall, being therefore more digestible than microalgae with cellulosic cell walls, although its nutritional benefits must yet be validated in the aquacultured species. Therefore, the production of Tisochrysis lutea biomass with high fucoxanthin content would add value to this microalga, to ultimately improve fish welfare. Hence, the present study aimed to validate a protocol to analyse spectrophotometrically the fucoxanthin content in Tisochrysis lutea industrial culture by a straightforward method previously developed5 for other microalga species. The validation of the protocol was performed by comparing the fucoxanthin levels obtained between this protocol and highly accurate methods to measure this pigment (HPLC).

Materials and methods

The fucoxanthin analysis was performed according to the methodology established by Wang et al.[5]especially diatoms and Chrysophyta. Recently, it has been shown to have anti-inflammatory, anti-tumor, and anti-obesityactivity in humans. Phaeodactylum tricornutum is a diatom with high economic potential due to its high content of fucoxanthin and eicosapentaenoic acid. In order to improve fucoxanthin production, physical and chemical mutagenesis could be applied to generate mutants. An accurate and rapid method to assess the fucoxanthin content is a prerequisite for a high-throughput screen of mutants. In this work, the content of fucoxanthin in P. tricornutum was determined using spectrophotometry instead of high performance liquid chromatography (HPLC in *Tisochrysis lutea* cultures. For that purpose, outdoors flat pannel green walls where inoculated at diferent concentrations: Low (L) (0.67±0.04 g/L), Medium (M) (1.07±0.05 g/L) and High (H) (1.50±0.05 g/L) cell concentration (n=2 each treatment). The cultures were monitored for three days, with 2 daily sample collections (morning and afternoon) to analyse fucoxanthine by two diferent methods. The validation of the spectrophotometric protocol was performed by comparing the pigment quantification through spectrophotometry[5]especially diatoms and Chrysophyta. Recently, it has been shown to have anti-inflammatory, anti-tumor, and anti-obesityactivity in humans. Phaeodactylum tricornutum is a diatom with high economic potential due to its high content of fucoxanthin and eicosapentaenoic acid. In order to improve fucoxanthin production, physical and chemical mutagenesis could be applied to generate mutants. An accurate and rapid method to assess the fucoxanthin content is a prerequisite for a high-throughput screen of mutants. In this work, the content of fucoxanthin in P. tricornutum was determined using spectrophotometry instead of high performance liquid chromatography (HPLC to HPLC (High Performance Liquid Chromatography) method according to Gao et al.[6]. Algal strain *Tisochrysis lutea* produced locally at Necton S.A. was grown photoautotrophically with Nutribloom® Plus. *Tisochrysis lutea* samples were analysed spectrophotometrically for cell concentration using a previously established calibration curve. For fucoxanthin analysis the cells were centrifuged at 4000x g for 10 min, the supernatant discarded and then rinsed with ddH_2O (1:1; v/v) for 3 min. Cells were recollected by centrifugation and pellets were suspended in ethanol (ethanol:algae culture volume = 1:1; v/v) for pigment extraction. Absorbance values were read

(Continued on next page)

at 445 nm and 663 nm (A_{445} and A_{663} range from 0.2 to 1) within 5 min. In parallel, a volume of culture was centrifuged and the cells diluted with culture medium Nutribloom® Plus (medium:algae culture volume = 1:1; v/v) for 3 min, and the absorbance measured at 750 nm. (A_{750} ranges from 0.1 to 0.8). The concentration of fucoxanthin in algal cells was determined through measuring the absorbance of cells suspended in culture medium at 750 nm and algal cell suspension in ethanol at 445 nm and 663 nm through the following Formula:

$$C\textit{fuc}' = 6.39 \times A_{445} - 5.18 \times A_{663} + 0.312 \times A_{750} - 5.27.$$

IBM SPSS Statistics 26.0 software was used for statistical analysis and data is expressed as average±SD. Independent t-test was used to compare differences between spectrophotometric and HPLC results ($p<0.05$).

Results

All data was normalized according to the same conditions, namely the quantification in both methodologies are expressed in relation to microalgae dry weight (including ash), since the spectrophotometric method includes the salt content of the culture and microalgae. The preliminary results of the present study showed that the levels of fucoxanthin in Low concentration culture measured through spetrophotometric method has no significant differences from the pigment quantification performed through HPLC (table 1).

Table 1- Comparison of fucoxanthin content in *Tisochrysis lutea* culture inoculated in Low concentration treatment measured through spectrophotometric (SPM) and HPLC method during 3 days of culture.

	Fucoxanthin (mg/g) SPM	**Fucoxanthin (mg/g) HPLC**
Day 1	11.25±1.84	10.21±0.68
Day 2	6.84±1.59	5.8±0.43
Day 3	6.13	5.74±1.81

Discussion

The use of HPLC analysis is considered to be the most accurate method to measure microalgae bioative compounds, such as the fucoxanthin pigment. However, for industrial purposes, a simple method for cultured-microalgae pigment analysis was previouly proposed for *Phaeodactylum tricornum* through spectrophotometry. The present study aimed to validate this methodology in *Tisochrysis lutea* produced industrially at Necton S.A. It was possible to observe that there were no significant differences on the fucoxanthin concentration evaluated through both methods in cultures with low cell concentration (L) (0.67±0.04 g/L). The evaluation of the remaining treatments (M, H) will allow a full validation of the protocol application for this microalgae species. Once the protocol is fully validated, the fucoxanthin levels in *T. lutea* will be investigated throughout its biomass processing steps, to understand the maintenance of the pigment in industrial biomass processing stages.

References

1.McClure, D. D., Luiz, A., Gerber, B., Barton, G. W. & Kavanagh, J. M. An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae *Phaeodactylum tricornutum*. Algal Res. 29, 41–48 (2018).

2.Bigagli, E. et al. A Comparative In Vitro Evaluation of the Anti-Inflammatory Effects of a *Tisochrysis lutea* Extract and Fucoxanthin. (2021).

3.Cao, W. et al. Transcriptomic analysis reveals effects of fucoxanthin on intestinal glucose transport. J. Funct. Foods 49, 205–213 (2018).

4.Mohamadnia, S., Tavakoli, O., Faramarzi, M. A. & Shamsollahi, Z. Production of fucoxanthin by the microalga *Tisochrysis lutea*: A review of recent developments. Aquaculture 516, 734637 (2020).

5.Li, F. L. et al. A rapid method for the determination of fucoxanthin in diatom. Mar. Drugs 16, 1–13 (2018).

6.Gao, F., Teles (Cabanelas, ITD), I., Ferrer-Ledo, N., Wijffels, R. H. & Barbosa, M. J. Production and high throughput quantification of fucoxanthin and lipids in *Tisochrysis lutea* using single-cell fluorescence. Bioresour. Technol. 318, 124104 (2020).

Aknowledgements

Work funded by MAGNIFICENT-745754 project BBI-RIA - Bio-based Industries Research and Innovation action, ZEBRABLOOM-ALG-01-0247-FEDER-039896, ALLARVAE ALG-01-0247-FEDER-069971

AQUACULTURE VIRTUAL CAREER DEVELOPMENT PLATFORM FOR THE SOUTH BALTIC REGION – INITIAL RESULTS AND FUTURE PERSPECTIVES

B. Dmochowska*, A. Bischoff-Lang, H. Łądkowska, N. Nika, A. Sutnikas

AquaVIP INTERREG SB project, communication: University of Gdańsk, Bażyńskiego 8, Gdańsk, 80-309, (Poland)
Email: b.dmochowska@ug.edu.pl

Introduction

Recognizing the significant demand for aquaculture professionals and a need for progress in innovative technologies in the South Baltic Region AquaVIP – Aquaculture Virtual Career Development Platform for the South Baltic Region, a three-year project led by Klaipeda Science and Technology Park, accompanied by University of Rostock, University of Gdańsk and Klaipeda University has undertaken a number of educational and research activities to meet the objective – to boost aquaculture labor market within the South Baltic region by fostering human resources capacity through cross-border training and networking.

The project focuses 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. AquaVIP target group includes stakeholders along the aquaculture value chain: 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.

Materials and methods

AquaVIP project offer is carried out through AquaVIP experiments, AquaYouth, AquaProfi, and AquaTION services.

AquaVIP experiments performed in partners' facilities are foreseen as core activities for the training and networking. The research topics of the experiments include: 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.

AquaYouth – "Aquaculture Youth career development" service includes summer schools on innovative aquaculture technologies such as recirculating aquaculture systems, aquaponics, and integrated systems, study visits in modern, innovative farms and AquaVIP facilities, students' panels during branch events, and guide in aquaculture career with jobs catalogue, jobs presentations, films on success stories and farms presentations.

The summer schools delivered by the University of Gdańsk and Klaipeda University in May and June/July 2021 were both successful and 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 RAS in research facilities, and partner aquaculture companies. Reports and presentations form the summer schools are available at the project website: aquavip.edu.pl.

AquaProfi – "Aquaculture Professionals' success support" service includes aquaculture professional trainings at Rostock University and is dedicated on the one hand for fish farmers aiming at becoming master fish farmers, but also for aquaculture professionals who are generally looking for further training opportunities. Thanks to the theoretical contents and practical training farmers upgrade their skills in innovative solutions which increase their business capacity and expand employability in the sector.

AquaTION – "Aquaculture innovation – boosting education and business capacity" is an e-learning platform, which will be developed on the basis of state-of-the-art knowledge, experience form previous aquaculture projects, and experimental and training activities of the AquaVIP project. 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 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.

(Continued on next page)

Results

In the framework of the AquaVIP project, we put a strong base for strengthening aquaculture programs in the universities, provide hands-on-experience for academic communities and create conditions for changes in the labor market. Innovative aquaculture will bring benefit to businesses in our region and society in general – as it provides healthy, secure and regionally produced high quality food. The use of innovative environmentally friendly production technologies will also open new and international markets, providing further new jobs and blue-green growth in the South Baltic area.

ACLIMATATION OF MACROALGAE *Ulva ohnoi* IN DIFFERENT SALINITIES IN THE BIOFLOC SYSTEM

Ana Paula Mariane de Morais, Leila Hayashi, Felipe do Nascimento Vieira*

Universidade Federal de Santa Catarina – UFSC
E-mail: felipe.vieira@ufsc.br

Introduction:
Macroalgae are commonly used in IMTA systems due to their ability to absorb nitrogen compounds and phosphate (TROELL et al., 2009). The *Ulva* genus stood out due to its ability to reach high biomasses with high protein content (ROBERTSON-ANDERSSON 2003), in addition to being found all over the world and abundant in estuary environments. (PEDERSEN, 1995; RIVERS E PECKOL, 1995).

There are several environmental factors that affect Ulva growth and nutrient absorption. Among them, salinity is considered the most important, due to its reduction negatively affecting the growth and absorption of macroalgae nutrients (FONG et al. 1996, MARTINS et al. 2001), therefore presenting a tolerance to salinities of 20 to 40 ‰ (WU et al. 2018). In this way, it is possible that macroalgae that tolerate high concentrations of salinity have different strategies for the growth and absorption of nutrients. Green algae have the ability to withstand variations in salinity and opportunistic growth, so they are capable of rapid colonization and growth when conditions are favorable (LITTLER, 1980; MSUYA; NEORI, 2008).

Given the importance of aquaculture, the BFT system stands out for its increased production, however, it generates an excess of organic matter, nitrogen compounds and phosphorus. Therefore, due to its ability to absorb nitrogen compounds, and phosphate, it is expected that the macroalgae will be able to consume the nitrogen excess compounds and phosphorus, which they tend to accumulate in the production environment. The objective of the work is to acclimatize the *Ulva onhoi* macroalgae in different salinities in the biofloc system to be integrated in a multitrophic system.

Material and methods:
The experiment was carried out in the Laboratory of Marine Cameroon (LCM) of the Federal University of Santa Catarina (UFSC). The macroalgae of the species (*U. ohnoi*) were collected from the sedimentation pond of the UFSC marine mollusk laboratory, taken to the LCM where cleaning was performed manually with seawater to remove epiphytes and adhered animals.

The experimental design consisted of four treatments with three repetitions each, totaling 12 experimental units named: S15: salinity 15 ‰; S20: 20 ‰ salinity; S25: salinity 25 ‰ and S30: salinity 30 ‰. The experimental units consisted of white square tanks with 40 liters of useful volume for macroalgae production, allocated in a greenhouse. To acclimatize the macroalgae in the units, 25% filtered biofloc water (Bidim Bag filter) was inserted and filled with seawater to reach the initial salinity of 30 ‰ for all treatments. The reduction of salinity was performed daily by total changes of water in the tanks according to the salinity for each treatment (15, 20, 25, and 30 ‰).

The macroalgae were populated at a density of 2 g L^{-1} with an individual heating and aeration system. The nutrients came from the biofloc and it was considered a photoperiod of 10 - 12 hours and natural irradiance, weighed weekly to accompany the growth, when it exceeded the initial weight of 80 g, the excess was collected to calculate the final biomass at the end of the experiment. The experiment lasted 3 weeks.

Results:
The results show that there was no significant difference in the increase in biomass of macroalgae between treatments ($P > 0.05$). All reached biomass of more than 1500 g. Thus, the macroalgae adapted to the four salinities tested without mortality and there was an increase in biomass in all treatments.

Conclusion:
The macroalgae grew in all the tested salinities, demonstrating that it can integrate the multitrophic system in the salinities in any of the four salinities tested, opening the possibility of integrating them with species that support a lower salinity.

(Continued on next page)

Financial support:
FAPESC (2020TR728).

Bibliographic references:

FONG, P., BOYER, K. E., DESMOND, J. S. & Zedler, J. B. 1996. Salinity stress, nitrogen competition, and facilitation: what controls seasonal succession of two opportunistic green macroalgae? J. Exp. Mar. Biol. Ecol. 206:203-221.

LITTLER, M.M., 1980. Morphological form and photosynthetic performances of marine macroalgae: test of a functional/ form hypothesis. Bot. Mar. 22, 161–165

MARTINS, I., PARDAL, M. A., LILLEBØ, A. I., FLINDT, M. R. & MARQUES, J. C. 2001. Hydrodynamics as a major factor controlling the occurrence of green macroalgal blooms in a eutrophic estuary: a case study on the influence of precipitation and river management. Est. Coast. Shelf Sci. 52:165-177.

MSUYA, F. and Neori, A. Effect of Water Aeration and Nutrient Load Level on Biomass Yield, N Uptake and Protein Content of the Seaweed Ulva lactuca Cultured in Seawater Tanks. Journal of Applied Phycology, 20, 1021-1031. 2008 http://dx.doi.org/10.1007/s10811-007-9300-6

PEDERSEN, M.F., 1995. Nitrogen limitation of photosynthesis and growth: comparison across aquatic plant communities in a Danish estuary (Roskilde Fjord). Ophelia 41, 61–272.

RIVERS, J.S., PECKOL, P., 1995. Summer decline of Ulva lactuca (Chlorophyta) in a eutrophic embayment: interactive effects of temperature and nitrogen availability. J. Phycol. 31, 223–228.

ROBERTSON-ANDERSSON. The cultivation of Ulva lactuca (Chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. MSc Dissertation, University of Cape Town, South Africa. 2003

TROELL, M.; Joyce, A.; Chopin, T.; Neori, A.; Buschmann, AH; Fang, JG. Engenharia ecológica na aquicultura - Potencial para aquicultura multi-trófica integrada (IMTA) em sistemas marítimos offshore. Aquicultura, 297 (1-4): 1-9. 2009

WU, H.; SHIN, S. K.; JANG, S.; YARISH, C.; KIM, J. K. Growth and nutrient bioextraction of Gracilaria chorda, G. vermiculophylla, Ulva prolifera, and U. compressa under hypo- and hyper-osmotic conditions. Algae. 2018. doi. org/10.4490/algae.2018.33.11.13

EFFECTS OF MICROALGAE ADDITION AND FISH FEED SUPPLEMENTATION IN THE INTEGRATED REARING OF PACIFIC WHITE SHRIMP AND NILE TILAPIA USING BIOFLOC TECHNOLOGY

Felipe Vieira[1], Vitor Silva[1], Patriula Pereira[1], Mateus Martins[1], Marco Lorenzo[1], Herculano Cella[1], Rafael Lopes[1], Roberto Derner[1], Paola Magallón[2]

[1] Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Florianópolis, SC, Brazil
[2]CONACYT-Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, México
*E-mail: felipe.vieira@ufsc.br

Introduction

The integration of different species aims at optimizing the use of nutrients generated by a primary fed species by employing other species that can take advantage of the excess nutrients, thus improving the use of resources of the overall production system (Chopin et al., 2001). Two species with the potential to be integrated are Pacific white shrimp (*Litopenaeus vannamei*) and Nile tilapia (*Oreochromis niloticus*). Poli et al. (2019) evaluated different Nile tilapia densities (10, 20, 30% of the biomass relative to the shrimp biomass) and found gains in yield and nutrient recovery, along with a reduction in the sludge: biomass relationship as the fish densities increased. In this work, the fish were fed at the rate of 1% of their biomass and the feed conversion ratio (FCR) observed by the authors was 0.2, indicating a relevant contribution of the bioflocs in the nutrition of the fish. However, there are no studies assessing if reared fish without feed supplementation can still maintain adequate performances impacting the overall integrated system, which would allow a reduction in the system inputs.

The microalgae *Scenedesmus obliquus* is freshwater green algae rich in protein and lipid contents, in addition to the carotenoid lutein. Its inoculation along with *Chlorella* sp. in a biofloc system benefitted the immune system of the fish (Jung et al., 2017). As consequence, the inoculation of microalgae species in biofloc-based integrated systems may enhance the biofloc attractiveness and nutritional composition, increasing natural food consumption by the fish and improving their growth performance.

Therefore, this study evaluated the supplementation of *S. obliquus* and fish feed in a Nile tilapia and Pacific white shrimp integrated system using biofloc technology and their effects on growth performance, production of solids and water microbiology

Material and methods

A two-factor 62-day experiment was conducted, in which each factor had two levels, addition or no addition of the microalgae *Scenedesmus obliquus* (5 mg L^{-1} twice a week) and the provision or no provision of fish feed (1% of the fish biomass), adding up to four treatments that were evaluated in quadruplicate. In all cases, shrimp were fed four times a day, according to a feeding table. The experimental units consisted of 800 L (useful volume) tanks for the shrimp and 90 L (useful volume) tanks for the tilapia, maintained under continuous recirculation. The shrimp (2.16 ± 0.01 g) were stocked under a density of 400 shrimp m^{-3} (320 shrimp $tank^{-1}$) and the fish (1.53 ± 0.12 g) were stocked under a density of 522 fish m^{-3} (47 fish $tank^{-1}$). Growth performance, sludge production and water microbiology were evaluated.

Results and discussion

Shrimp achieved a final mean weight of 12.1 ± 0.6 g and a survival of 78.3 ± 7.1%, with no significant differences between treatments. Fish that were fed exhibited final mean weight and final biomass 58% higher when compared with unfed fish. Fish survival was higher in treatments with the addition of microalgae (93.9 ± 1.8%) compared with the treatments of no microalgae addition (86.2 ± 7.6%). Fish final biomass was also 14% higher when microalgae was added (Tabela 1). The overall system yield was higher in the treatments with the provision of fish feed (4.2 ± 0.2 kg m^{-3}) compared with no addition (3.9 ± 0.2 kg m^{-3}). Sludge production did not differ significantly between treatments (1.0 ± 0.1 kg $tank^{-1}$), the same occurring with the count of total heterotrophic bacteria and *Vibrio*.

Conclusion

This work suggest that fish feed supplementation at the rate of 1% of the biomass improved fish growth performance and system yield, without affecting sludge production and water microbiology. The addition of *S. obliquus* under the concentration of 5 mg L^{-1} $tank^{-1}$ twice a week improved fish survival and final biomass. However, it did not significantly affect the overall system performance, sludge production and water microbiology.

Table 1. Nile tilapia (*Oreochromis niloticus*) growth performance when reared in integration with Pacific white shrimp (*Litopenaeus vannamei*) using biofloc technology for 62 days, evaluating the addition of microalgae and fish feed in a factorial design.

Variables	Fish feed addition	Microalgae addition		*Mean*
		No	Yes	
Final mean weight (g)	No	11.06 ± 0.18	11.23 ± 0.51	***11.15 ^{b} ± 0.37***
	Yes	16.49 ± 1.65	17.86 ± 2.65	***17.18 ^{a} ± 2.17***
	Mean	***13.77 ± 3.09***	***14.55 ± 3.96***	
SGR (% day^{-1})	No	3.09 ± 0.20	3.31 ± 0.29	***3.20 ^{b} ± 0.18***
	Yes	3.82 ± 0.11	3.97 ± 0.36	***3.90 ^{a} ± 0.25***
	Mean	***3.46 ± 0.43***	***3.64 ± 0.41***	
Survival (%)	No	81.91 ± 7.86	94.14 ± 1.06	***88.03 ± 8.35***
	Yes	90.42 ± 5.06	93.61 ± 2.45	***92.02 ± 4.06***
	Mean	***86.17 ^{B} ± 7.62***	***93.88 ^{A} ± 1.77***	
FCR[1]	No	-	-	-
	Yes	0.24 ± 0.01	0.23 ± 0.02	-
	Mean	-	-	
Biomass (kg)	No	0.42 ± 0.04	0.49 ± 0.02	***0.46 ^{b} ± 0.05***
	Yes	0.69 ± 0.04	0.78 ± 0.11	***0.74 ^{a} ± 0.09***
	Mean	***0.56 ^{B} ± 0.15***	***0.64 ^{A} ± 0.17***	

Data presented as mean ± standard deviation. * Indicates significant differences by two-factor ANOVA ($p < 0.05$). Values followed by lowercase letters (a or b) in the same column indicate significant difference by Tukey's test ($p < 0.05$) when considering only the effect of fish feed addition. Values followed by uppercase letters (A or B) in the same row indicate significant difference by Tukey's test ($p < 0.05$) when considering only the effect of microalgae addition. SGR: Specific growth rate. FCR: Feed conversion ratio.

Financial support:
Aquavitae project (Horizon 2020, grant number 818173).

References

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche□González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J. Phycol. 37, 975–986.

Poli, M.A., Legarda, E.C., Lorenzo, M.A., Martins, M.A., Vieira, F.N., 2019. Pacific white shrimp and Nile tilapia integrated in a biofloc system under different fish-stocking densities. Aquaculture, 498: 83-89.

Jung, J.Y., Damusaru, J.H., Park, Y., Kim, K., Seong, M., Je, H.W., Kim, S., Bai, S.C., 2017. Autotrophic biofloc technology system (ABFT) using Chlorella vulgaris and Scenedesmus obliquus positively affects performance of Nile tilapia (Oreochromis niloticus). Algal Res. 27, 259–264.

GROWTH PERFORMANCE AND LIVER GENE EXPRESSION IN SEABREAM (*Sparus aurata*) FED ECO-EFFICIENT AQUAFEEDS

Pereira, G. V. [1*], A.M. Fernandes[1], B. Silva[1], J. Dias[1], B. Buck[2,3], J. Johansen[4], L.E.C. Conceição[1]
J. Calduch-Giner[5], J. Pérez-Sánchez[5]

[1]SPAROS, Lda. Olhão, Portugal
[2]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany
[3] University of Bremerhaven, Appl. Mar. Biol., Bremerhaven, Germany
[4]Salten Havbrukspark AS, Nygårdsjøen, Norway
[5] Nutrigenomics Group, Institute of Aquaculture Torre de la Sal, IATS-CSIC, Spain
*Email: gabriellapereira@sparos.pt

Introduction

New emerging ingredients have risen in recent years in order to follow the world shift towards sustainable aquaculture. These alternative ingredients (e.g., insect products, by-products of fisheries, aquaculture and animal production, bacterial biomasses and algae-based products) tend to replace those commonly used up to date (e.g. fish meal, fish oil, soybean meal) due to sustainability issues (Gasco et al., 2018, Oswald et al., 2019). The results here presented show what effects on performance and fish metabolism may be expected when formulations based on emerging ingredients are used in gilthead seabream. This work is part of the GAIN project (H2020 grant 773330, European Union) which aims to develop a new generation of sustainable fish feeds specifically designed to facilitate aquaculture eco-intensification through increased circularity and resource utilization.

Material and Methods

Growth performance and liver gene expression was assessed in *Sparus aurata* fed with four different diets: 1) a control diet containing fish meal and traditional soy products (CTRL); 2) a diet rich in processed animal proteins derived from farm animal by-products (PAP); 3) a diet with alternative ingredients without the inclusion of PAP (NoPAP); and 4) a diet containing a mixture of alternative ingredients (MIX). This nutritional trial was performed with four replicates, each one with 55 fish with an initial weight of 30g, at a temperature of 22±0.35ºC, during 9 weeks. Liver samples were analyzed by PCR-array for expression of 42 genes.

Results

At the end of the trial, no statistical differences were found in final body weight (FBW), relative growth rate (RGR) and voluntary feed intake (VFI). However, fish fed control diet showed higher protein efficiency ratio (PER), when compared to fish fed with other formulations. Additionally, fish fed with control and NoPAP diets presented a lower feed conversion ratio (FCR) when compared with fish fed PAP and MIX diets. When comparing between groups, the NoPAP group showed up-regulation of lipid metabolism-related genes (*elovl6*, *fads2*, *scd1a*, and *scd1b*) and downregulation of the lipolytic transcription factor pparα, when compared to the control group (Figure 1). In contrast, fish fed PAP diet presented down-regulation of performance-related genes (*ghr-i*, *igf-i*), lipid metabolism-related genes (*elovl4*, *elovl5*, *scd1a*, *fads2* and *hl*) and antioxidant defence-related genes (*cpt1a* and *cs*).

Discussion and Conclusions

The increased expression of *scd*, *fads2* and *elovl6* enzymes in NOPAP group is a typical characteristic feature of a reduced supply of n-3 LC-PUFA. On the other hand, the hepatic expression of fish fed the PAP diet was mostly opposite with a marked down-regulated expression of elongases (*elovl4, elovl5*), having PUFA as main substrates. Therefore, the new sustainable formulations tested in this trial did not affect much the fish growth performance, but liver gene expression suggests several adaptations to the different feed formulations, in particular for lipid metabolism, GH/IFG system and antioxidant defence. Taking together these results the PAP diet may lead to lower performance in the long-term.

(Continued on next page)

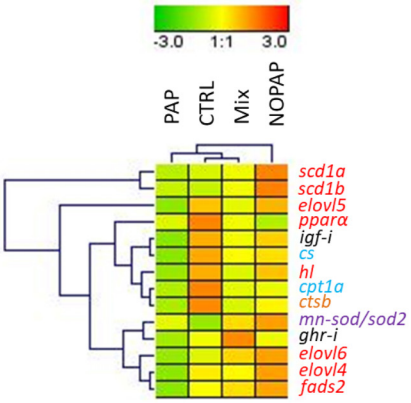


Figure 1: Heat map of liver gene expression profile after filtering for most nutritionally regulated genes.

References

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., Caruso, G., 2018. Fishmeal Alternative Protein Sources for Aquaculture Feeds, in: Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., Caruso, G. (Eds.), Feeds for the Aquaculture Sector: Current Situation and Alternative Sources, SpringerBriefs in Molecular Science. Springer International Publishing, Cham, pp. 1–28. https://doi.org/10.1007/978-3-319-77941-6_1

Oswald, A. T., Ishikawa, M., Koshio, S., Yokoyama, S., Moss, A. S., & Serge, D. (2019). Nutritional evaluation of Nannochloropsis powder and lipid as alternative to fish oil for kuruma shrimp, Marsupenaeus japonicus. Aquaculture, 504, 427-436.

GROWTH AND MICROBIOME MODULATION IN NILE TILAPIA (*Oreochromis niloticus*) FED DIETS WITH VARIABLE PROTEIN SOURCES

G. V. Pereira[1], A. T. Gonçalves[1,2], M. Viegas[1], C. Teixeira[4], P. Rema[3] J. Dias[1]

[1] SPAROS Lda., Olhão, Portugal.
[2]GreenCoLab – Associação Oceano Verde, Faro, Portugal
[3]Universidade de Trás os Montes e Alto Douro (UTAD), Vila Real, Portugal
[4] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal.
*E-mail: gabriellapereira@sparos.pt

Introduction

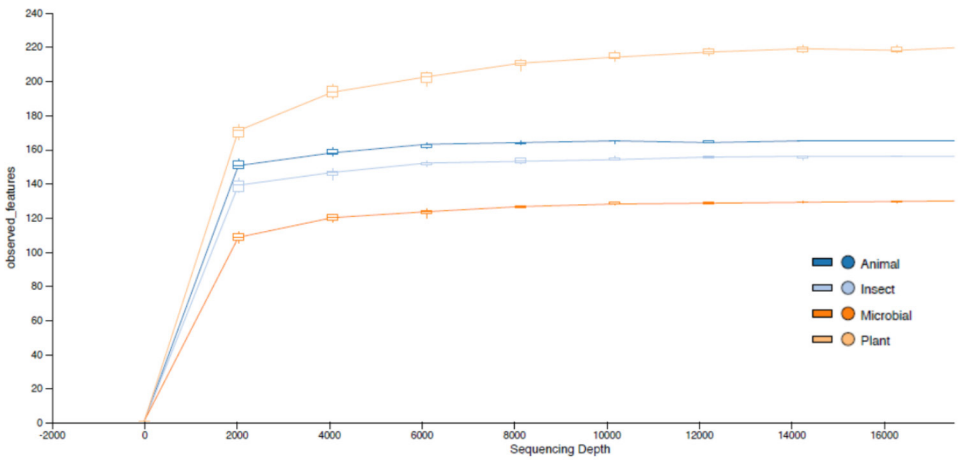
Among the major objectives of a more sustainable aquaculture, within aquaculture nutrition it is important to highlight the replacement of animal protein from marine origin for other protein alternatives such as: microalgae, seaweeds, microbial biomasses and insects. Those ingredients are rising as new emerging and sustainable ingredients, however not much is known about their effects in fish growth performance, health, and even less is known about effects in fish gut microbiome and metabolism in general. The objective of this trial was to assess the effect of different dietary protein sources on the growth performance and modulation of microbiome of Nile Tilapia (*Oreochromis niloticus*).

Materials and methods

A total of 180 Nile tilapia (*O. niloticus*) with initial body weight (IBW) of 12.14 ± 0.26 g, were transferred to 12 units (tanks of 90 L) totalling 15 fish per tank. The four diets were tested in triplicate and consisted on different protein sources such as: 1) a diet containing animal products (ANIMAL); 2) high levels of insect meal (INSECT); 3) two sources of bacterial biomass (MICROBIAL); and 4) plant products (PLANT). Fish were fed by hand, in two daily meals to satiation over 46 days. Fish were bulk weighed at the end of the trial and three fish per replica were sampled for intestinal microbiome analysis. Fish intestines were sampled following aseptically conditions to avoid contamination. DNA extraction was made using the DNA extraction High Pure PCR Template Preparation Kit (Roche, Portugal) preceded by a lysozyme lysis step as explained elsewhere (Falcinelli et al 2015). Illumina Sequencing by 16S rRNA gene amplification of the V1-V2 region was performed by Genoinseq (Centre for Neuroscience and cell Biology, Coimbra, Portugal) as previously described (Pereira et al., 2019). Sequences were filtered, denoised and merged using DADA2 (Callaham et al 2016) and downstream bioinformatic analysis was performed using Qiime2 version 2020.11 (Bolyen et al 2019).

Results

Final body weight (FBW) and standard growth rate (SGR) were higher in fish fed MICROBIAL and ANIMAL diets when compared to fish fed PLANT and INSECT diets. Additionally, feed conversion factor (FCR) was lower in fish fed ANIMAL diet when compared to fish fed PLANT and INSECT diets. Fat digestibility was higher in MICROBIAL and INSECT diets when compared to other diets. Regarding the microbiome analysis, it was possible to observe a reduction in abundance of Proteobacteria in fish fed INSECT, MICROBIAL and PLANT diets when compared to the reference diet (ANIMAL). Furthermore, PLANT diet influenced in the increase of observed features (Figure 1) when compared to ANIMAL diet, while MICROBIAL and INSECT decreased the bacterial diversity.



(Continued on next page)

Discussion and conclusion

Microbiota community modulation in Nile tilapia's intestines was evident after feeding with diets with alternative protein sources. Diets with higher fat digestibility were associated with the most relevant alterations in gut microbial modulation with a reduction in Proteobacteria abundance and overall, less diversity (MICROBIAL and INSECT). However, it is interesting that only in MICROBIAL group these changes are correlating with a better performance output. The most abundant phyla in Nile tilapia intestines were Fusobacteria and Bacteroidetes, mainly due to the strong presence of the *Cetobacterium* and *Paludibacter* from phyla respectively. These genera have also been found to be dominant in the gastrointestinal tracts of other freshwater species and have a positive impact in the digestion efficiency and metabolism of the hosts. Although no significant changes on the abundance of these phyla was observed, the reduction of Proteobacteria promoted several low-magnitude modulations in the microbial composition that might be involved in the observed performance differences. This study highlights the impact of different protein sources on tilapia's gut microbiome and provide insights on the axis diet-host-gut microbiome dynamics.

Acknowledgements

This work is part of project 47175_FICA, supported by Portugal and the European Union through FEDER/ERDF, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

References

Falcinelli S, et al (2015) *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Sci Rep 5, 9336, DOI:10.1038/srep09336

Pereira G. et al (2019) Autochthonous probiotic bacteria modulate intestinal microbiota of Pirarucu, *Arapaima gigas*. Journal of World Aquaculture Society 50(6), DOI:10.1111/jwas.12638

Callahan BJ, et al (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 13:581-583 DOI:10.1038/nmeth.3869

Bolyen E, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotec, 37:852-857 DOI:10.1038/s41587-019-0209-9

OVERVIEW OF POLYPHENOLS AND PIGMENTS FROM TEN DIFFERENT ORGANIZAMS FROM THE ADRIATIC SEA

A. Dobrinčić[a*], L. Čižmek[b,c], K. Van Hayelwick[c], Z. Zorić[a], R. Čož-Rakovac[b,c] and V. Dragović-Uzelac[a]

[a]Faculty of Food Technology & Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia
[b]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
E-mail:lcizmek@irb.hr
[c]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[d]Faculty of Bioscience Engineering, University of Gent, Onderbergen 1, Belgium

Introduction

Marine environment has been known as a rich source of bioactive compounds, such as proteins, amino acids, polysaccharides, fatty acids, vitamins, minerals, dietary fibers, sterols, pigments and polyphenols (Mišurcová et al., 2014), that have a huge functional and nutraceutical potential. Among these compounds, polysaccharides are recognized as one of the most promising sources of anti-inflammatory, antioxidant, antimicrobial, antiviral, anti-coagulant, and antitumor activities. Polysaccharides extraction is preceded by pretreatment with acetone and ethanol in order to remove compounds like polyphenols and pigments that are considered undesirable in polysaccharides extraction so those extracts are usually discarded. However, polyphenols and pigments are valuable biological resources of high exploitation potential. They are produced when these organisms are adapting to extreme environmental conditions (high salt concentration, low light, lower temperatures) they live in (Freile-Pelegrín and Robledo, 2013).

This study aimed to characterize and quantify polyphenols and pigments (spectrophotometric and HPLC analysis) in acetone and ethanol extracts from 10 different marine species from the Croatian coast of the Adriatic Sea: 2 green algae (*Ulva lactuca* and *Codium bursa*), 5 brown algae (*Fucus virsoides, Padina pavonica, Cystoseira barbata, Halopteris scoparia*, and *Cystoseira compressa*), 1 coral (*Eunicella cavolini*), 1 sea squirt (*Aplidium conicum*) and 1 sponge (*Chondrosia reniformis*).

Materials and methods

Ten marine organisms were collected from the coastal area of Croatia, washed in seawater and then rinsed with distilled water, frozen at -60 °C, freeze dried (CoolSafe lyophilizer, Model: 55-9 PRO, Labogene, Denmark) and milled. Extraction was performed in two successive steps: first 18 hours at room temperature with acetone and then 4 hours at 70 °C with 96 % ethanol. Extracts were filtered and stored at 4 °C until analysis.

Extracts were analyzed for total polyphenols (TP) content using spectrophotometric Folin-Ciocalteu method at 765 nm while chlorophyll-a (C_a), chlorophyll-b (C_b) and total carotenoids ($C_{(x+c)}$) content were measured at 470, 644.8, 648.6, 661.6 and 664.1 nm depending on the solvent used for extraction and quantified with appropriate equations (Lichtenthaler and Buschmann, 2001). HPLC system (Agilent Infinity 1260 system) equipped with Agilent photo-diode array detector (DAD) 1260 an automatic injector, Chemstation software and Zorbax Eclipse XDB-C18 column (4.5 x 250 mm, 5 um) (Agilent) was used for separation, identification and quantification of polyphenols according to Fecka and Turek (2008). The same HPLC system with C30 column Develosil, 5µm (250×4.6 mm I.D.) (Phenomenex, USA) was used for separation, identification and quantification of carotenoid and chlorophylls according to Castro-Puyana et al. (2016).

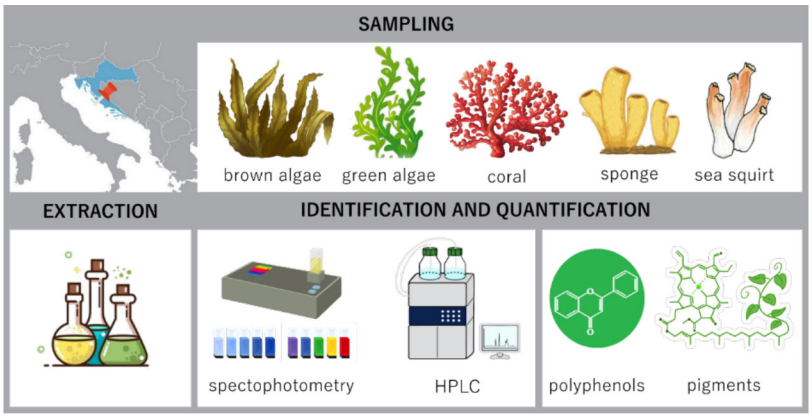


Figure 1. Schematic overview of the experimental design used in this research

(Continued on next page)

Results

The highest TP content in acetone and ethanol extracts were achieved in brown algae *C. barbata*, *C. compressa* and *F. virsoides*. Green algae *U. lactuca* and brown algae *F. virsoides* had the highest C_a and $C_{(x+c)}$ content among acetone extracts and *C. compressa* among ethanol extracts. The highest C_b content was achieved in green algae *U. lactuca*.

HPLC analysis also confirmed that brown algae have higher content of phenols than green algae. Detected phenols could be classified as hydroxybenzoic acid, ellagic acid and flavan-3-ols. Fucoxantine was predominant carotenoid in all brown algae, in acetone and ethanol extracts, followed by β-carotene, zeaxantine and lutein. These algae had significant amount of C_a but C_b was not detected. Opposite to the brown algae, green algae had significant amount of C_b and low amount of carotenoids. None of the carotenoids and chlorophylls were detected in coral *E. cavolini* and sponge *C. reniformis* while sea squirt *A. conicum* had only traces of C_a, fucoxantine and zeaxantine.

Discussion and conclusion

Different organisms have shown to have a diverse content of polyphenols and pigments and besides inherent characteristics of each species, environmental factors to which organisms are exposed, such as light, temperature, and salinity, may also affect their polyphenol and pigment contents (Osório et al., 2020). In general, results of our study showed that polyphenols and pigments content was significantly higher in brown algae, especially from *Cystoseira* and *Fucus* genus, while coral, sponge and sea squirt had very low content of polyphenols and pigments.

Brown algae contain high amount of phlorotannins, the most studied group of phenolic compounds from algae which possess a unique structure that is not found in terrestrial plants (Freile-Pelegrín and Robledo, 2013). Although the content of chlorophylls was higher than carotenoids in brown algae, the presence of fucoxanthin overlaps the presence of chlorophylls and other carotenoids in a macroscopic point of view and thus it is responsible for their brown color (Osório et al., 2020). Chlorophyll b is present only in plants and green algae what was confirmed by HPLC analysis.

References

Castro-Puyana, M., Pérez-Sánchez, A., Valdés, A., Ibrahim, O.H.M., Suarez-Álvarez, S., Ferragut, J.A., Micol, V., Cifuentes, A., Ibáñez, E., and García-Cañas, V. 2017. Pressurized liquid extraction of *Neochloris oleoabundans* for the recovery of bioactive carotenoids with anti-proliferative activity against human colon cancer cells. Food Research International 99, 1048-1055.

Fecka, I., and Turek, S. 2008. Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques. Food Chemistry, 108, 1039-1053.

Freile-Pelegrín, Y., and Robledo, D. 2013. Bioactive phenolic compounds from algae. In: Bioactive compounds from marine foods: Plant and animal sources. Hernandez-Ledesma, B., Herrero, M., Eds., John Wiley & Sons: Chichester, UK, pp. 113-129.

Lichtenthaler, H.K., and Buschmann, C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry, F4.3.1-F4.3.8.

Mišurcová, L., Orsavová, J., and Ambrožová, J.V. 2014. Algal Polysaccharides and Health. In: Polysaccharides. Springer International Publishing, pp. 1-29.

Osório, C., Machado, S., Peixoto, J., Bessada, S., Pimentel, F.B., Alves, R.C., and Oliveira, M.B.P.P. 2020. Pigments content (Chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. Separations 7, 1-14.

A COMPARISON OF TWO STRAINS OF ATLANTIC SALMON REARED IN RAS AND FLOW THROUGH PRODUCTION SYSTEMS

Jamie Downes[1*], Fintan Egan[2], Tom McDermott[1], Suzanne Kelly[1], Katie Thomas[1], Jack D'Arcy[1], Aideen Kearney[1], Liz Ryder[2], Alan Drumm[2], Neil Ruane[1]

[1]Marine Institute, Rinville, Oranmore, Co. Galway, Ireland
[2]Marine Institute, Furnace, Newport, Co. Mayo, Ireland
*Jamie.downes@marine.ie

Recirculating Aquaculture Systems (RAS) are a proven technology increasingly employed in the freshwater stage of Atlantic salmon production. Over the last decade many salmon producing regions have already begun to take advantage of the potential benefits of RAS technology in order to increase production and the overall size of Atlantic salmon smolt produced. The experience from a number of commercial farms is showing that smolts produced in RAS show better production performance in terms of growth and/or survival in sea cages than smolts from FT systems, however there is a need to verify these observations in a controlled manner (Ulgenes et al., 2008; Kolarevic et al 2014).

This study describes the design and performance of a small research recirculation system used in the production of Atlantic salmon smolts. Two commercial strains of Atlantic salmon were reared from first feeding to smolt in this RAS unit, the growth and performance was compared with fish reared in a conventional flowthrough system. The final weight achieved by the RAS stocks was 280g – Stock 1, 347g – Stock 2 and the flowthrough system, 89g – stock 1, 110g stock 2. After smoltification the fish were transferred to the Lehanagh Pool Marine Research Site, Marine Institute and the performance was monitored through four months post transfer.
*Jamie.downes@marine.ie

Acknowledgement:
This project is funded by the European Maritime and Fisheries Fund under the BIM knowledge Gateway fund 17/KGS/009.

References:
Ulgenes, Y., Attramadal, K., Terjesen, B.F., 2008. The motivation for using recirculation in Norway — a shift from the need for restricted water consumption to better control of the culture water environment and higher product quality. Aquacultural Engineering Society's Fourth Issues Forum. Aquacultural Engineering Society, Roanoke, Virginia, pp. 80–93.

Kolarevic, J., Baeverfjord, G., Takle, H., Ytteborg, E., Reiten, B. K. M., Nergård, S., & Terjesen, B. F. (2014). Performance and welfare of Atlantic salmon smolt reared in recirculating or flow through aquaculture systems. Aquaculture, 432, 15-25.

A LONG-TERM DEVELOPMENTAL STUDY OF DEFORMITIES IN ATLANTIC SALMON: THE RECOVERY OF HYPER-RADIODENSE VERTEBRAE

Lucia Drábiková*, Per Gunnar Fjelldal, Adelbert De Clercq, M. Naveed Yousaf, Thea Morken, Charles McGurk, P. Eckhard Witten

Evolutionary Developmental Biology, Biology Department, Ghent University, Ghent, Belgium.
E-mail: Lucia.Drabikova@ugent.be

Introduction

Hyper-radiodense vertebrae (HDV) represent a frequently observed radiological deformity in Atlantic salmon *Salmo salar*, L. (Helland et al., 2006; Fraser et al., 2019). Increased radiodensity is caused by ectopic cartilage replacing adipose tissue in bone marrow spaces. In some cases, there are bent bone trabeculae due to anterior-posterior compression of the vertebral centrum (Helland et al., 2006). Animals have been observed with a single or up to four individual HDV. While HDV are found in freshwater stages, studies postulate that the deformity either disappears (Baeverfjord et al., 2009; Berge et al., 2009; Fjelldal et al., 2016) or exacerbates in seawater stages (Baeverfjord et al., 2009). Based on Helland et al. (2006) HDV is suggested to be related to mineral deficiency.

The specific fate of HDV in individual animals and factors inducing this deformity remain to be elucidated. This study comprised a seawater follow-up trial of animals previously subjected to deficient and excessive dietary phosphorus (P) in the freshwater (parr) stage (Drabikova et al., 2021). The study analysed HDV in individual PIT-tagged animals at (i) pre-smolt stage, (ii) seven months post seawater transfer, and (iii) at harvest size.

Materials and Methods

Atlantic salmon were studied from the parr stage (average weight 13.49 ± 1.50 g) until harvest size (average weight 4480.87 ± 1259.28 g). Parr were fed a diet with one of three levels of available P: low P (LP) (2.6 g/kg), regular P (RP) (5.9 g/kg), and high P (HP) (9.1 g/kg) for 11 weeks. Animals were subsequently PIT-tagged and fed regular commercial diets until harvest. Deformities were assessed on x-rays prior to smoltification, after seven months in seawater, and at harvest size. From each diet group, 45 animals (total no. 135) were x-rayed and assessed for HDV to progress or regress. A detailed analysis of vertebral anatomy and microstructures was conducted by Alizarin red S staining of mineralised bone and through serial histological sections.

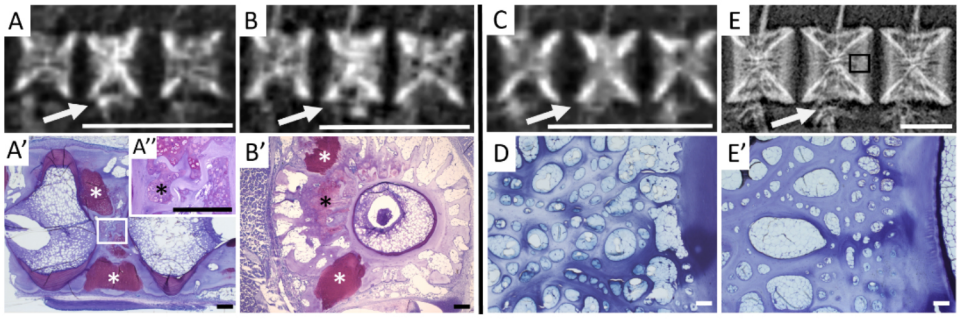


Fig 1. **Hyper-radiodense vertebrae (HDV)**. X-ray images (A-C,E, scale bar = 0.5 cm) and histological sections (A'-B',D,E', Toluidine blue staining, scale bar = 250 µm) of vertebrae from abdominal vertebral column region. HDV representatives (arrow in A-C; A'-B'), a regular (i.e. non-deformed) vertebra (D), and a fully recovered vertebral centrum with a HDV history (arrow in E,E'). (A-C) HDV with a characteristic increased radiodensity and anterior-posterior compression. (A') A parasagittal section of HD vertebra shown in (A) with ectopic cartilage tissue in bone marrow spaces. Intervertebral ligaments and spaces remain intact. (A'') Magnification of the white square in (A') shows detailed image of bent bone trabeculae and ectopic cartilage (asterisk). (B') Cross-section of HD vertebra shown in (B) with unilateral ectopic cartilage (left side) (black asterisk). The right side remains non-deformed. Regular cartilage at the bases of the vertebra is also present (white asterisks in A',B'). (C) HD vertebra present in an animal at the pre-smolt stage and (E) the same vertebra at harvest size. The black square in (E) shows the approximate location of (E').

(Continued on next page)

Results and Discussion

At the pre-smolt stage, HDV were detected in 30 out of 135 animals of which 26 had a LP diet history. Despite HDV, intervertebral spaces and ligaments remain intact (Fig. 1A-C).

All identified HDV had fully recovered seven months post seawater transfer with no traces of the previous deformity (Figure 1E-E'). All recovered HDV remained non-deformed for another six months until harvest. Bone trabeculae in recovered HDV were comparable with regular, non-deformed, vertebrae (Fig. 1 D,E').

HDV were observed to be uni-lateral, affecting the left side of the vertebra (Figure 1B'). This possibly relates to the uni-directional swimming in tanks with a small diameter (0.6 m). Bent bone trabeculae, a characteristic feature of HDV, are likely associated with soft, low-mineralised bone in animals with a LP diet history (Helland et al., 2006). This soft bone does not fracture under an increased compression load (Witten et al., 2019). Equally, bone trabeculae in HDV do not fracture but bend and are surrounded by ectopic cartilage (Fig. 1A'').

Funding: EU-H2020-MSCA-766347.

References

Baeverfjord, G., Helland, S., Hough, C., 2009. Control of malformations in fish aquaculture: science and practice. Federation of European aquaculture producers. RapidPress, Luxembourg, 149 pages.

Berge, G.M., Witten, P.E., Baeverfjord, G., Vegusdal, A., Wadsworth, S., Ruyter, B., 2009. Diets with different n–6/n–3 fatty acid ratio in diets for juvenile Atlantic salmon, effects on growth, body composition, bone development and eicosanoid production. Aquaculture 296, 299–308.

Drábiková, L., Fjelldal, P.G., De Clercq, A., Yousaf, M.N., Morken, T., McGurk, C., Witten, P.E., 2021. Vertebral column adaptations in juvenile Atlantic salmon *Salmo salar*, L. as a response to dietary phosphorus. Aquaculture 736776.

Fjelldal, P.G., Hansen, T.J., Lock, E.J., Wargelius, A., Fraser, T.W.K., Sambraus, F., El-Mowafi, A., Albrektsen, S., Waagbø, R., Ørnsrud, R., 2016. Increased dietary phosphorous prevents vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 22, 72–90.

Fraser, T.W.K., Witten, P.E., Albrektsen, S., Breck, O., Fontanillas, R., Nankervis, L., Thomsen, T.H., Koppe, W., Sambraus, F., Fjelldal, P.G., 2019. Phosphorus nutrition in farmed Atlantic salmon (*Salmo salar*): Life stage and temperature effects on bone pathologies. Aquaculture 511.

Helland, S., Denstadli, V., Witten, P.E., Hjelde, K., Storebakken, T., Skrede, A., Åsgård, T., Baeverfjord, G., 2006. Hyper dense vertebrae and mineral content in Atlantic salmon (*Salmo salar* L.) fed diets with graded levels of phytic acid. Aquaculture 261, 603–614.

Witten, P.E., Fjelldal, P.G., Huysseune, A., McGurk, C., Obach, A., Owen, M.A.G., 2019. Bone without minerals and its secondary mineralization in Atlantic salmon (*Salmo salar*): the recovery from phosphorus deficiency. J. Exp. Biol. jeb.188763.

LOW SPERM TO EGG RATIO FOR *IN VITRO* FERTILISATION OFFERS A SOLUTION FOR THE REPRODUCTIVE CONTROL OF CULTURED SOLE (*Solea senegalensis*).

Sandra Ramos-Júdez[1]=, Wendy Ángela González-López[1]=, Jhons Huayanay Ostos[1], Noemí Cota Mamani[2], Carlos Marrero Alemán[1], José Beirão[3], Neil Duncan[1]*

1 IRTA, Sant Carles de la Ràpita, C. Poble Nou km. 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain.
2 Dirección General de Investigaciones en Acuicultura, Instituto del Mar del Perú (IMARPE), Lima, Peru
3 Faculty of Biosciences and Aquaculture, Nord University, NO-8049 Bodø, Norway
= Authors made an equal contribution, *neil.duncan@irta.cat

Introduction

Cultured Senegalese sole (*Solea senegalensis*) reared from egg to adult in captivity do not spawn spontaneously. Implementation of *in vitro* fertilisation procedures would appear to be a solution to this problem (Rasines et al., 2012). However, cultured sole produce small amounts of poor-quality sperm (González-López et al., 2020) that have been considered inadequate for *in vitro* fertilisation procedures. The sperm to egg ratio in fish has been observed to vary from 1,000s to 100,000s of sperm per egg (Beirão et al., 2019), with flatfish generally exhibiting the lowest sperm to egg ratios. The present study aimed to define the sperm to egg ratio required to achieve high levels of fertilisation on an experimental scale and using commercially relevant numbers of eggs.

Materials and Methods

The cultured Senegalese sole used were hatched and reared entirely in captivity (females of 1.53 ± 0.28 kg and males of 1.05 ± 0.25 kg). Fish were maintained in 10,000 L tanks in RAS (IRTAmar®) and fed a diet of polychaetes, mussels and pellets (Broodfeedlean, SPAROS). Photoperiod was natural and water temperature was constant 16 ± 1 ºC. Eggs were obtained by inducing ovulation by administering 5 µg/kg of GnRHa to females with mean oocyte diameter ≥ 600 µm. The females were held in total darkness until ovulation. Sperm was stripped from untreated culture males (González-López et al., 2020). Sperm concentration was measured (Thoma chamber) and motility assessed with ImageJ CASA. In a sperm to egg ratio experiment, sperm (n=5 males) was serially diluted with modified Leibovitz to obtain eight dilutions: 1:4; 1:19; 1:79; 1:319; 1:959; 1:2879; 1:5759; 1:11519 and triplicate fertilisations of 0.5 mL of eggs (n=5 females) were completed. After 24 hours of incubation (16ºC) the development of ≥ 50 eggs were determined per replica. In a proof of concept experiment > 100,000 eggs were fertilised with ≥ 150 µL of motile sperm in female – male pairs (n=7). Eggs were incubated and hatching rate assessed. In an egg viability experiment, three batches of eggs were stored at room temperature and fertilised at collection and at 30 - 60 minute intervals after collection. Egg development was assessed after 24 h. In a sperm collection experiment, the sperm quality (n = 13 males) was assessed when collected directly into a syringe containing Leibovitz (1:4), diluted in Leibovitz (1:4) 4 ± 2 minutes after collection and undiluted sperm.

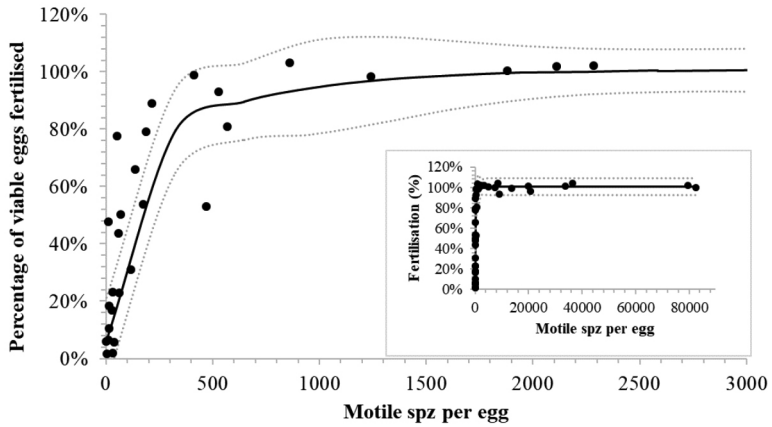


Figure 1. The percentage of viable eggs fertilised in relation to the number of motile spermatozoa (spz) per viable egg for Senegalese sole (*Solea senegalensis*). The insert figure shows the entire data set up to over 80,000 spz per egg and the large figure shows a close up of the data up to 3,000 motile spz per egg. The continuous line shows a non-linear regression and the dotted lines indicate 95% confidence intervals.

(Continued on next page)

Results and Discussion

Twenty eight males (60.9 % of 46 checked) had adequate sperm quantity and quality for the experiments. Eight (62% of 13 induced) females ovulated good quality eggs (82.6 ± 9.2 % fertilisation) that were used in the experiments. The mean time from GnRHa administration to ovulation was 41:57 ± 1:46 h, mean fecundity was 130,789 ± 36,723 eggs/fish or 87,174 ± 24,378 eggs/kg of body weight. In the sperm to egg ratio experiment the relationship between percentage fertilisation and sperm per egg was described by a non-linear regression based on an equation for an exponential rise to a maximum with double, five parameters ($R = 0.93$, $P < 0.0001$) (Fig. 1). Only 649 motile sperm fertilised 90 ± 13% (± 95% CI) of viable eggs and 1617 motile spz fertilised 99 ± 12% (± 95% CI) of viable eggs. In addition, percentage motility was negatively correlated to the number of sperm required to fertilise a viable egg ($R = -0.93$). However, there was no correlation between motile sperm required and percentage of viable eggs. In the proof-of-concept experiment the mean percentage hatch was 70 ± 14 % to produce 131,540 ± 34,448 larvae per fertilisation. The sperm from a single cultured male, mean volume of 145 ± 50 µL ($8 \pm 6.8 \times 10^8$ spermatozoa) was used to fertilise 190,512 ± 38,471 eggs, which gave a ratio of 592 ± 611 motile sperm per egg. The egg viability experiment indicated that the percentage of fertilised eggs gradually decreased with storage time with 81 ± 26% (± 95% CI) fertilisation after 30 minutes and 57 ± 20% (± 95% CI) after an hour. The sperm extraction experiment demonstrated that the percentage motility was significantly ($P < 0.05$) higher in samples collected directly into modified Leibovitz (33.4 ± 3.5%) compared to dilution 4 ± 2 minutes after collection (6.6 ± 1.6%) or undiluted sperm (2.9 ± 1.2%).

Senegalese sole was amongst the lowest sperm to egg ratio recorded in fish (Beirão et al., 2019). The possible relationship between low sperm to egg ratio and sole reproductive characteristics such as paired-spawning, gamete fertilisation in close proximity with no sperm competition will be discussed. Eggs should be fertilised as soon as possible after ovulation and sperm quality was improved with early dilution in modified Leibovitz. The low sperm to egg ratio was tested and shown to fertilise large amounts of eggs (>100,000) with the sperm from a single cultured male (approx., 200 µL of sperm per 100 mL of eggs).

Acknowledgements: The authors thank IRTA staff and Josep Lluis Celades and Ignacio Giménez (RARA-AVIS SL), Mario Villalta Vega (IES Alfacs) and Alex Rullo Reverté (IES Alfacs) for technical assistance. The study was funded by INIA-FEDER project nº RTA2014-0048 and supported by the project 038433_REARLING, Portugal and the European Union through ERDF, COMPETE 2020, Portugal 2020, coordinated by Isidro Blanquet (Sea8 Group, Portugal). PhD grants funded the participation of WGL (CONACYT, Mexico) and SRJ (AGAUR, Catalonia).

References

Beirão J, Boulais M, Gallego V, O'Brien JK, Peixoto S, Robeck TR, Cabrita E. 2019 Sperm handling in aquatic animals for artificial reproduction. Theriogenology 133, 161–178. (doi:10.1016/j.theriogenology.2019.05.004)

González-López WÁ, Ramos-Júdez S, Giménez I, Duncan NJ. 2020 Sperm contamination by urine in Senegalese sole (Solea senegalensis) and the use of extender solutions for short-term chilled storage. Aquaculture 516, 734649. (doi:10.1016/j.aquaculture.2019.734649)

Rasines, I., Gómez, M., Martín, I., Rodríguez, C., Mañanós, E., Chereguini, O., 2012. Artificial fertilization of Senegalese sole (*Solea senegalensis*): hormone administration methods, timing of ovulation and viability of eggs retained in the ovarian cavity. Aquaculture 326–329: 129–135.

IMPLEMENTATION OF *IN VITRO* FERTILIZATION TECHNIQUES IN A SENEGALESE SOLE (*Solea senegalensis*) HATCHERY ON AN INDUSTRIAL LEVEL

I. Blanquet[1], D. Rosado[1], M. Martins[1], F. Silva[1], C. Vilafranca[1], W. Gonzalez-Lopez[2], Sandra Ramos-Júdez[2], F. Chauvigné[3], J. Cerdà[3], I. Giménez[4] and N. Duncan[2]

[1]SAFIESTELA-SUSTAINABLE AQUA FARMING INVESTMENTS, S.A., 4570-275 Porto, Portugal
Email: isidroblanquet@sea8.eu
[2]IRTA-Sant Carles de la Ràpita, 43540 Tarragona, Spain
[3]IRTA-Institute of Biotechnology and Biomedicine (IBB), Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
[4]Rara Avis Biotec, S.L., Calle Moratín 17, 4º, 46002 Valencia, Spain

Introduction

The sustainable aquaculture production of Senegalese sole (*Solea senegalensis*) has been frustrated by the failure of hatchery produced sole to spawn fertilised eggs. The present work has implemented *in vitro* fertilisation techniques on an industrial level to secure closing the biological cycle and sustainable aquaculture production. The project REARLING, funded by the Portugal 2020 program, Compete 2020, and the European Union through FEDER/ERDF lead by Safiestela Ltd. (www.sea8.eu) aimed to test and implement on an industrial scale technologies using *in vitro* fertilisation protocols, which had been demonstrated on a laboratory scale (Ramos-Júdez *et al*., 2021) and the application of recombinant gonadotropins (Rara Avis Biotec, https://www.raraavis-bio.com) to increase sperm production (Chauvigné *et al*., 2018).

Materials and Methods

The Senegalese sole females (0.9 ± 0.1 kg) used for industrial level *in vitro* fertilisation were hatched and reared entirely in Safiestela Ltd. The cultured females were the 1st generation reared in captivity from eggs spawned naturally in Safiestela Ltd facilities from wild sole captured in northern Portugal. Wild males (0.8 ± 0.1 kg) were used to provide sperm. Sole were maintained in 5000 L tanks in RAS and fed pelleted diets (Broodfeedlean, SPAROS), and fresh food. Photoperiod was natural and water temperature was constant 17 ± 1 ºC. For the data presented here a total of 165 cultured females and 171 wild males were used. The females were induced in groups from 2 to 25 females. Eggs were obtained by inducing ovulation with the administration of 25 µg kg^{-1} of GnRHa to females that had a mean oocyte diameter ≥ 600 µm. The females were held in total darkness until ovulation. Sperm was stripped from wild males. Sperm concentration and motility was assessed subjectively zero, low, medium and high. Sperm with high concentration and motility was used. A total of 0.1 mL of sperm was used for each 100 mL of eggs. Sperm was obtained first and stored over ice until the females ovulated and eggs were obtained. Eggs were incubated in large industrial egg incubators and percentage fertilisation and number of larvae obtained were assessed.

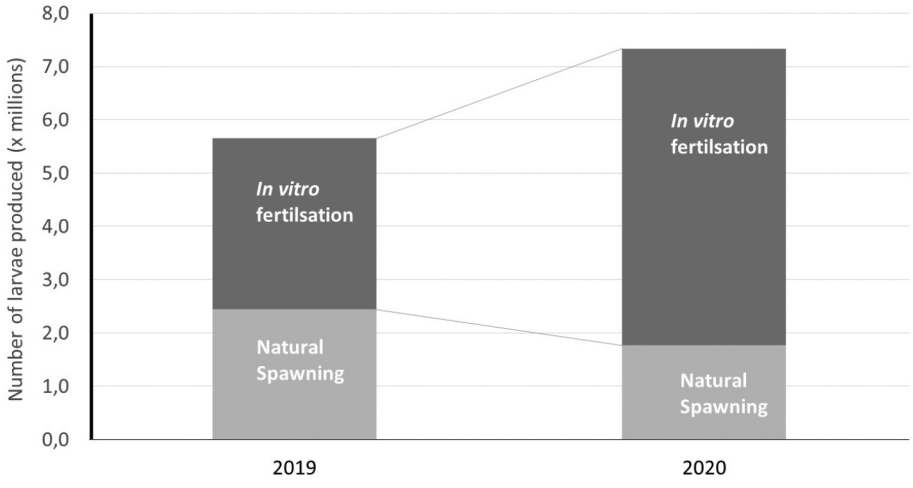


Figure 1. Number of Senegalese sole (*Solea senegalensis*) larvae produced from *in vitro* fertilisation (dark grey) and natural spawning (light grey) during the two years, 2019 and 2020, of the Rearling project coordinated by Safiestela Ltd.

(Continued on next page)

Results and Discussion

Approximately half of the males checked were rejected as sperm quantity or quality was insufficient. The average quantity of highly concentrated motile sperm obtained from a male was 0.1 mL. A total of 14 groups of females ranging from 2 to 25 females were induced. The mean time for females from GnRHa administration to ovulation was 40 h, and the mean volume of eggs obtained from a female was 100 mL. Approximately 12 males were required to fertilise a litre of eggs. Eggs from groups of females were incubated together. The mean percentage fertilisation of the 14 groups of eggs was 40.0 ± 18.1 %. Large numbers of larvae were produced for the larval rearing facility (Fig. 1).

In the first year (2019) of the project, 3.21 million larvae were produced using the *in vitro* fertilisation technology implemented in Safiestela Ltd. In comparison, 2.44 million larvae were obtained from naturally spawning wild broodstock. In the final year of the project (2020), the success of *in vitro* fertilisation enabled a large increase in the production of larvae with 5.57 million larvae produced from *in vitro* fertilisation and less than a quarter of all the larvae (1.77 million) being provided from naturally spawning wild broodstock. The *in vitro* fertilisation technology gave control over reproduction that has ensured that Safiestela Ltd has both increased production of the entire company and initiated a genetic breeding program based on desirable performance characteristics of the Safiestela Ltd stock.

In addition to the implantation of *in vitro* technologies, Safiestela Ltd used recombinant gonadotropins produced by the company RARA AVIS BIOTEC SL to increase good quality sperm from cultured Senegalese sole by approximately 10-fold. This gives the potential to ensure good quality sperm from all males avoiding discarding half of males checked for sperm and, therefore, both reducing time needed to evaluate males and the wasteful practice of growing male breeders that produce small qualities of poor sperm.

Taken together, the project has implemented *in vitro* fertilisation technologies and methods to enhance sperm production in the Senegalese sole. This has removed the reliance on sometimes unpredictable spawns from wild broodstocks, and Safiestela Ltd now has full control to program the timing and quantity of fertilised eggs that are produced.

Acknowledgements: The study was funded by project 038433_REARLING, Portugal and the European Union through ERDF, COMPETE 2020, Portugal 2020, coordinated by Isidro Blanquet (Sea8 Group, Portugal).

References

Chauvigné, F., W. González, S. Ramos, C. Ducat, N. Duncan, I. Giménez, J. Cerdà. 2018. Seasonal-and dose-dependent effects of recombinant gonadotropins on sperm production and quality in the flatfish *Solea senegalensis*. Comparative Biochemistry and Physiology, Part A 225: 59–64

Ramos-Júdez S, González-López WÁ, Huayanay Ostos J, Cota Mamani N, Marrero Alemán C, Beirão J, Duncan N. 2021 Low sperm to egg ratio required for successful *in vitro* fertilization in a pair-spawning teleost, Senegalese sole (*Solea senegalensis*). R. Soc. Open Sci. 8: 201718. https://doi.org/10.1098/rsos.201718

VALORISATION OF EUROPEAN AQUACULTURE SIDE STREAMS AND BY-PRODUCTS – A COST-BENEFIT ANALYSIS

Kreiss, C.M.[1], Edebohls, I.[2*]., Brüning, S.[2], Bruckner, C.[3], Vázquez, J.A.[4], Baarset, H.[5], Micallef, G.[6]

[1]Thuenen Institute of Fisheries Ecology, Herwigstr. 31, 27572 Bremerhaven, Germany
Email: cornelia.kreiss@thuenen.de
[2]Thuenen Institute of Sea Fisheries, Herwigstr. 31, 27572 Bremerhaven, Germany
[3]Salten Havsbrukpark, 8120 Nygårdsjøen, Norway
[4]Instituto de Investigaciones Marinas - Spanish National Research Council (IIM-CSIC), 36208 Vigo, Spain
[5]Waister AS, 3170 Sem, Norway
[6]Gildeskål Research Station AS (GIFAS), 8140 Inndyr, Norway

Against the background of the stagnating EU aquaculture sector growth in contrast to the increasing sector size at world level (including Norway), it is important to address prevailing hindering factors such as import pressure or social pressure due to actual and perceived environmental impacts. Within the H2020 project Green Aquaculture Intensification (GAIN) innovative production tools based on the principles of circular economy were developed and evaluated in order to facilitate the paradigm shift of eco-intensification in European aquaculture. While the valorisation of side-stream products is beneficial for a more sustainable production, related changes in production practices, may impact capital investment, labour input or energy demand, all of which affect costs. We applied an established benchmarking approach to contrast today's economic performance of "typical farms" using the conventional ensilage method with their profitability when processing mortalities within a drying unit. Further, in-depth cost-benefit analyses were conducted for a theoretical decoupled algae aquaponics system (*Ulva lactuca*) utilizing disposal water from smolt RAS production; as well as an input-output based economic analysis for the valorisation of aquaculture fish by-products (FBP) on industry scale. The results reveal a promising economic balance for various market opportunities such as selling dried mortalities to the pet food sector or *Ulva lactuca* to the Asian market with the side-effect of reducing the nutrient load of European smolt waste water and potentially increasing smolt production. Processing various FBP into secondary products also proved to be promising approaches for fish protein hydrolysate, peptones and their further use as medium e.g. for lactic acid bacteria. The profitability of the examined pathways were based on accessible market prices. Further key aspects for future marketing success will also include market access and consumer demand.

GENETIC ANALYSIS OF SEVEN NATURAL POPULATIONS OF TENCH (*Tinca tinca* L. 1758.) IN HUNGARY – ESTABLISHMENT THE BIOLOGICAL BASIS OF SELECTIVE BREEDING

Al Fatle Fatema Ali[1,4], Tamás Molnár [1,2], Erika Edviné Meleg[1*], Gergely Szabó[1], Gábor Fekete[1], Zoltán Sallai[3], Balázs Kovács[2] and István Lehoczky[1]

[1]Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary, Isaszegi St. 200, 2100
[2]Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences Gödöllő, Hungary, Páter Károly St. 1, 2100
[3]Vaskos csabak Bt, Békésszentandrás, Hungary hrsz 0153/6
[4]Doctoral School of Biological Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, Páter Károly St. 1, 2100
e-mail: lehoczky.istvan@nbgk.hu

Introduction

Tench (*Tinca tinca* L. 1758.-) is a medium sized *Cyprinid* species with Euro-Siberian distribution and the species is considered native in the Danube catchment area in Hungary. The species main habitats are small shallow lakes, oxbow lakes, calm bays and streams with slow currents and with well warmed waters in summer and with plenty of submerged vegetation and muddy bottoms. As an eurythermal species the tench can survive even 37°C of water temperature for short periods of time, although the lethal temperature is 35.3°C for the species. During its evolution tench developed the ability to survive with extremely low levels of oxygen, which means that the species will survive with less than half the requirement of the common carp. These characteristics qualify the tench to successfully face the effects of climate change. Nowadays the production of the species is marginal in Hungary but with the changing environment the tench may become more important in Hungarian Aquaculture. In order to support the development of tench production, the development of a selective breeding programme is necessary for the species to utilize its genetic reserves. As a basic step of the selective breeding programme in the present study we studied the genetic variability of seven populations of the species living in natural habitats in Hungary using 12 microsatellite DNA markers.

Materials and methods

Fin samples were taken from 178 tench individuals from seven different natural populations in the country's eastern, central, and western regions. The simple salting out technique, as reported by Miller et al. 1988, was used to isolate DNA. The quality and quantity of the isolated DNA were assessed by the NanoDrop™ spectrophotometer. As described in the original publications, PCR amplification was performed using 12 microsatellite DNA markers (MTT-1, MTT-2, MTT-3, MTT-5, MTT-6, MTT-8, MTT-9, MT-3, MT-6, MT-8, CypG24, and MFW1). The PCR products were analyzed using an automated ABI Prism 3130 Genetic Analyzer. The length of fragments was assessed using the Genotyper 4.0 software. GenAlEx6.501 was used for the main statistical analysis. To identify genotyping errors, allele dropouts, and null alleles, MICRO-CHECKER version 2.2.3 was employed. A Bayesian clustering analysis was performed to assess the genetic relationship between populations and individual assignments using the statistical software STRUCTURE version 2.3.3.

Results

The results of the microsatellite analysis showed that the genetic variability of the examined seven tench populations is moderate. The populations deviated from Hardy-Weinberg equilibrium in case of 0 to 3 markers. The average number of alleles (Na) per locus ranged from 2.5 (Derecske) to 4.25 (Fertő Lake) while the allelic richness ranged from 2.06 (Derecske) to 3.32 (Fertő Lake) respectively. Altogether the lowest diversity was observed in the Derecske population, both the heterozigosity and private allelic richness was significantly lower in case of this stock and this population showed the highest F value. Fertő Lake and Szaporca (Drava River) represent the highest diversity in the study. The basic population genetic characteristics of the stocks are listed in **Table 1.** Four genetic clusters were detected by the Structure analysis (k=4). The first cluster is frequent in the North-West region (Fertő Lake), the second in the Middle-Eeast region (Kolon Lake, Derecske), the third in the Middle and South-West region (Csörnöc, Szaporca) and the fourth in the Eastern region (Cibakháza) of Hungary. Tisza Lake stock is a mixed population with equal frequency for all clusters (most probably as a result of human impact – tench is stocked regularly in this water body).

(Continued on next page)

	Fertő-Lake	Kolon-Lake	Csörnöc	Derecske	Cibakháza	Tisza-lake	Szaporca
Na	4.25 ± 2.17	3.83 ± 1.69	3.25 ± 1.35	2.5 ± 1.16	3.33 ± 1.37	3.00±1.12	2.91±1.16
Neff	2.23 ± 0.77	1.81 ± 0.57	1.97 ± 0.63	1.39 ± 0.31	1.81 ± 0.64	1.96±0.52	1.96±0.70
Ho	0.43 ± 0.20 ab	0.32 ± 0.16 ab	0.45 ± 0.23 b	0.17 ± 0.14 a	0.37 ± 0.20 ab	0.36±0.18 ab	0.47±0.32 b
uHe	0.50 ± 0.18 a	0.40 ± 0.20 ab	0.44 ± 0.20 ab	0.25 ± 0.17 b	0.40 ± 0.18 ab	0.47±0.17 ab	0.43±0.25 ab
F	0.12 ± 0.18 ab	0.15 ± 0.18 ab	-0.03 ± 0.15 a	0.28 ± 0.31 b	0.04 ± 0.18 ab	0.17±0.30 ab	-0.11±0.31 a
AR	3.32 ± 1.32	2.74 ± 1.02	2.67 ± 1.03	2.06 ± 0.71	2.56 ± 0.92	2.86±1.00	2.81±1.09
AR_p	0.40 ± 0.42 a	0.12 ± 0.22 ab	0.16 ± 0.21 ab	0.02 ± 0.06 b	0.12 ± 0.31 ab	0.12±0.31 ab	0.10±0.29 ab

Table1. Basic population genetic characteristics of the stocks (Na:number of alleles; Neff:number of effective alleles; Ho:Heterozigosity observed; uHe: unbiased expected heterozigosity; F: Fixation index; AR: allelic richness; ARp: private allelic richness)

The pairwise Fst values between population pairs were low or moderate. The Nei's genetic distance ranged between 0.015 and 0.139 between population pairs. The 73% of molecular variance was found within individuals while 20% among individuals and only 7% of variance was found among populations.

Discussion and conclusion

The genetic variability of the local stocks ranges from low to moderate and the variability levels are lower compared to other *Cyprinids* such as Common carp (Kohlmann et al 2003), however they are similar and comparable to variability data described in Tench by Kohlmann et al. 2007. In order to start a successful selective breeding programme the inclusion of the greatest possible genetic diversity is necessary. Based on the results it is suggested to involve Fertő Lake and Szaporca populations in such a programme together with populations that represent high number of private alleles such as Csörnöc (ARp: 0.16).

Acknowledgement

The work was supported by the VEKOP-2.3.2-16-2016-00012 project.

References

Kohlmann, K., Gross, R., Murakaeva, A., Kersten, P. (2003) Genetic variability and structure of common carp (Cyprinus carpio) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. Aquatic living resources 16(5):421-431.

Kohlmann, K., Kersten, P., Flajšhans, M. (2007) Comparison of microsatellite variability in wild and cultured tench (*Tinca tinca*). Aquaculture. 272(S1):147-151.

TURNING BIOGAS DIGESTATE RESIDUES INTO C PHYCOCYANIN (C-PC)

S.S.W. Ende[1*], J. Meyer[1], C. Elle[2], A. Noke[3], A. Stelling[4], M.J. Slater[1] and J. Henjes[1]

[1]Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Sektion Marine Bioökonomie, Am Handelshafen 12, D-27570 Bremerhaven
*Email: stephan.ende@awi.de
[2] Sea & Sun Technology GmbH, Arndtstraße 9-13, D-24610 Trappenkamp
[3] City University of Applied Sciences, Neustadtswall 30, D-28199 Bremen
[4] INPUT Ingenieure GmbH, Freien Str. 25, D-31319 Sehnde

Introduction

Economically viable microalgae companies established in Germany produce food supplements or extracts for cosmetic or pharmaceutical applications. However, these value chains generally do not consider side streams from agricultural production processes and often do not have sustainable energy or quality assurance concepts.

The aim of the planned project that has recently started (June 2021) is to establish an aquatic value chain with the production organism *Arthrospira platensis* predominately based on residue biogas waste streams (liquid digestate and heat) on side of the biogas plant. The main product is C Phycocyanin (C-PC) as a food, feed supplement and cosmetic raw material in organic quality.

Material & methods

Arthrospira platensis will be obtained from different German culture collections. The anaerobic digestate used to test biogas digestate will be obtained from the project partner INPUT who operates a biogas plant near Hannover (Lower Saxony) in an industrial scale with maize silage and animal manure (cattle slurry/manure, dry chicken manure). The operating parameters of the biogas plant are as follows: hydraulic retention time 30 days, organic loading rate 9-11 kg volatile solids (VS)/m3·day, anaerobic sludge concentration 8-9%, temperature 40-45 °C. Before using as a nutrient medium, anaerobic digestate will be processed. First the solids are separated from the liquid. Then, in series 1, digestate was centrifuged (MPW-251, Donserv, 5000 rpm for 5 min) and then pasteurized (30 min, 90 °C). In series 2, digestate was distilled at 100 °C in distillation flasks with the working volume of 200 cm^3.

In the planned experiment, the volume of liquid digestate used to prepare the culture medium constituted from 10 to 50 % of the medium volume. For dilution of the liquid digestate, deionized water was used. As a control, *A. platensis* will be grown on a 100 % synthetic medium (Zarrouk) without adding any liquid digestate (control) and four different digestate residue-based media (chemical conditions to be determined after chemical composition analysis of the digestate) (Figure 1). Growth of *A. platensis* will be determined by dry matter concentration following according to Bundesamt für Verbraucherschutz und Lebensmittelsicherheit [1]. Phycocyanin concentration will be determined following the protocol of Siegelman and Kycia 1978 [2].

Fig. 1: Experimental design of biogas liquid digestate testing for the cultivation of *Arthrospira platensis* and respectively Phycocyanin production.

(Continued on next page)

Expected results to be presented at EAS

At EAS 2021 preliminary growth results and phycocyanin contents of *A. platensis* grown on four different biogas digestate residue-based media will be presented. Experiments are currently conducted and first data will be available and analysed prior to this conference.

References

[1] Lebensmittelsicherheit BfVu. Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Bestimmung der Trockenmasse in Fleisch und Fleischerzeugnissen, ASU L06.00–3. Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB, § 35 Vorläufiges Tabakgesetz, § 28b GenTG 1 (L). 2004.

[2] Siegelman, H., & Kycia, J. (1978). Algal bili-proteins: Handbook of phycological method. Cambridge University Press.

EX VIVO MODELISATION TO DETERMINE THE EFFECT OF A STANDARDIZED DRY GRAPE EXTRACT TO PROTECT SIBERIAN STURGEON *Acipenser baerii* RED BLOOD CELLS FROM OSMOTIC STRESS

P. Engler*, Y. Sakr, G. Le Reste, P. Garsi, S. Calvez and P. Chicoteau

Nor-Feed, 3 rue Amedeo Avogadro, 49070 Beaucouze, France
paul.engler@norfeed.net

Introduction

In aquaculture, oxidative stress can be induced by degraded water parameters such as salinity changes. Previous work showed that a standardized dry grape extract (SDGE) could be used in an *ex vivo* challenge to improve the stability of rainbow trout (*Oncorhynchus mykiss*) red blood cells (RBC) when exposed to an osmotic stress. The aim of this work was to extend the research on another anadromous species of fish, Siberian sturgeon (*Acipenser baerii*).

Material & methods

RBC solutions (RBCS, 25% v/v) were prepared from blood collected on healthy sturgeons, then mixed with phosphate buffer saline (PBS, pH = 7.4) and solutions of SDGE (Nor-Grape® WS, Nor-Feed) at various concentrations (1, 2, 3 and 6g/L in PBS). They were incubated at 56°C for 30 minutes, to induce a hemolysis reaction and were then centrifuged at 5000 rpm for 10 minutes at room temperature. The released hemoglobin was measured by spectrophotometry at 560 nm.

Results

Results evidenced that standardized dry grape extract at the two highest concentrations (3 and 6 g/L) reduced hemolysis of RBC to 32.4% and 35.6% respectively (Figure 1.), compared to the lowest concentration (1g/L, 95.5% hemolysis).

This work showed the positive impact of a SDGE on the stability of RBC from Siberian sturgeon when exposed to an osmotic stress, in a similar way than with rainbow trout RBC.

Discussion

Further research is required to establish if a dietary supplementation with a SDGE in the fish diet could evidence the same effect to manage oxidative stress, induced by the fluctuation of salinity, in order to reduce oxidative damage fish cells.

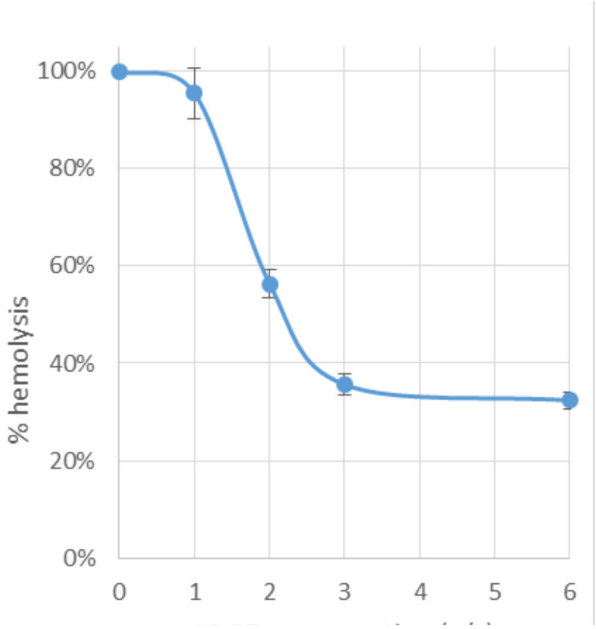


Figure 1. Effect of various SDGE solutions on hemolysis resistance of sturgeon RBC exposed to an osmotic stress.

SEARCH FOR BIOMARKERS OF RESISTANCE IN *R. decussatus* TO *P. olseni* PARASITE INFECTION

João Estêvão[1*], Hugo Osorio[2], Benjamin Costas[1], Andreia Cruz[3], Sergio Fernández-Boo[1]

[1] Animal Health and Aquaculture (A_2S), CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Porto, Portugal
[2] i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal.
[3] Oceano Fresco S.A, Porto de Abrigo, 2450-075 Nazaré, Portugal
Presenting author email: jestevao@ciimar.up.pt

Introduction

The grooved carpet shell (*Ruditapes decussatus*) is a bivalve mollusc species with a distribution from NE-Atlantic coast and Mediterranean Sea. This species is highly desirable and sold at high prices being Portugal the top-seller (FAO, 2020). A decline on the species happened since the 90´s due to biotic and abiotic factors, such as parasite infection and degradation of the environment. Infection by *Perkinsus olseni* parasite is the main biotic factor that causes decrease of populations. It was first diagnosed in the 1980s, after introduction of the invasive species *Ruditapes philippinarum* from Asia for intensive production in Europe (Vilas et al., 2011). It has been observed that *Perkinsus* causes changes in humoral and cellular responses and rise of mortality in infected individuals. Epizootic studies indicates that temperature and salinity influence host-parasite interaction, especially in adults. Despite of lacking an adaptive immune system, this species has demonstrated to thrive in highly affected areas, reaching adult phase and surviving high parasite prevalence (Villalba et al., 2004)some of which are associated with mass mortalities. Life cycle involves vegetative proliferation within the host, by which a cell named trophozoite undergoes successive bipartitioning. Other stages have been observed in vitro or in vivo, depending on the species: hypnospore, zoosporangium and zoospore. Molecular taxonomy supports a close affinity between dinoflagellates and Perkinsus spp. Six species of Perkinsus are currently considered valid: P. marinus, P. olseni, P. qugwadi, P. chesapeaki, P. andrewsi and P. mediterraneus. Histology and, above all, incubation of host tissues in Ray's fluid thioglycollate medium (RFTM.

Several studies focused on host-parasite early interaction and markers of resistance (Fernández-Boo et al., 2016; Hasanuzzaman et al., 2017; Cruz et al., 2020)the effects of longer term infection were assessed in adult clams collected from a P. olseni-affected bed, by comparing moderate to very heavily infected clams with non-infected ones. Haemocyte and plasma proteins were separated by two-dimensional electrophoresis; spot patterns were qualitatively compared between treatments within each experiment and the spots indicating differential protein expression associated with P. olseni challenge or with field infection were processed for protein identification. Fifteen clam proteins (four in haemocytes and eleven in plasma, while none addressed the presence of tolerance/susceptibility signatures in different populations. Thus, this study seeks to identify markers of tolerance/susceptibility in populations of *R. decussatus* affected by *P. olseni* by looking at the haemolymph´s proteome profile of tolerant individuals in comparison with susceptible ones.

Table 1. *Number of identified proteins from the three analyses.*

Analysis	Exclusive Proteins			DE [a] proteins (A. R. [b] ≥ 1.50 and ≤ 0.67)[c]		
	Susceptible	Tolerant	Total	Suscep/ Toler	Toler/ Cont	Total
Susceptible vs. Tolerant	0	2	2	37	4	41
Tolerant vs. Control	12	1	13	25	90	115
Algarve	10	9	19	34	37	71
Naples	255	7	262	181	31	212
Pontevedra	23	1	24	198	12	210
Turkey	10	18	28	57	55	112
Venice	10	50	60	58	67	125

[a] Differentially Expressed. [b] Abundance Ratio. [c] Considering abundance ratios obtained from Proteome Discoverer software (abundance ratio *p*-value was not considered).

(Continued on next page)

Materials and Methods

Five populations with high prevalence of the parasite across Europe namely, Pontevedra (Spain), Algarve (Portugal), Naples (Italy), Venice (Italy), and Izmir (Turkey) were sampled. A *Perkinsus*-free population (Noia, Spain) was used as control.

After *Perkinsus* diagnosis and haemolymph extraction, 5 individuals highly infected (susceptible) and 5 individual non- or low-infected (tolerant) were chosen at each population for a proteomics analysis by LC-MS. Three analyses were considered to identify markers of tolerance/susceptibility: i) Tolerant vs. Susceptible (in all populations); ii) Tolerant (in all populations) vs. Control (Noia population); iii) Tolerant vs. Susceptible (at each population). Expressed and exclusive proteins were identified according to Osório *et al*., 2021. Functional annotation was performed on identified protein from all analyses to pinpoint the expression profiles using the Blast2Go software.

Results and discussion

A substantial number of exclusive proteins was identified in the Naples population, followed by Pontevedra. The same tendency was observed in the differentially expressed proteins, being the number of predominant in the susceptible individuals in both exclusive and differentially expressed proteins (Table 1). These results indicate a higher response on affected individuals from these populations to parasite infection. Functions related to metabolism were most represented in susceptible than in tolerant individuals suggesting a higher production of energy to counteract the infection. Also, six proteins were differentially expressed in all tolerant individuals and seem to be possible markers of tolerance to *P. olseni* infection.

These results point towards a host generated tolerance suggesting an existence of adaptation mechanisms to parasite.

Acknowledgements

This research was supported by the project Tools4Breed – Challenge test and genetic markers for *Perkinsus* as a tool for *Ruditapes decussatus´* selective breeding with reference FA_05_2017_025 financed by Fundo Azul and República Portuguesa. João Estêvão was supported by FA_05_2017_025 and by FCT UI/BD/150906/2021 grants.

References

Cruz, A. *et al*., 2020. Genetic variability in ruditapes decussatus clam combined with Perkinsus infection level to support founder population selection for a breeding program. PeerJ 8, e9728.

FAO, 2020. The State of World Fisheries and Aquaculture 2020, The State of World Fisheries and Aquaculture 2020. FAO.

Fernández-Boo, et al. 2016. Protein expression profiling in haemocytes and plasma of the Manila clam *Ruditapes philippinarum* in response to infection with *Perkinsus olseni*. J. Fish Dis. 39, 1369–1385.

Hasanuzzaman, A.F.M. *et al*., 2017. Transcriptomic profile of Manila clam haemocytes in response to Perkinsus olseni infection. Aquaculture 467, 170–181.

Osório, H. *et al*., 2021. Proteomics Analysis of Gastric Cancer Patients with Diabetes Mellitus. J. Clin. Med. 10, 407.

Vilas, R. *et al*., 2011. Very low microsatellite polymorphism and large heterozygote deficits suggest founder effects and cryptic structure in the parasite Perkinsus olseni. Infect. Genet. Evol. 11, 904–911.

Villalba, A. *et al*., 2004. Perkinsosis in molluscs: A review. Aquat. Living Resour. 17, 411–432.

A CASE STUDY AS A PROOF OF CONCEPT AND VALIDATION OF FEEDING PRACTICES AND STRATEGIES OF THE MEDAID PROJECT

A.Estevez[a], K.Tsakoniti[b] , E. Prieto[c] , J. García[d], B. Basurco[e]

a. IRTA Centre of San Carlos de la Rápita, 43540 Spain
b. Galaxidi Marine Farm, Galaxidi, 33200 Greece
c. Cudomar S.L., Alicante, Spain
d. Dibaq, Segovia, 40260 Spain
e. IAMZ-CIHEAM, Zaragoza, 50059 Spain
alicia.estevez@irta.es

Introduction

The evaluation of aquaculture performance is a difficult task as full-scale aquaculture production is affected by numerous parameters, such as structure and size of culture units, environmental conditions (temperature, water flow, winds, fouling, etc.), husbandry (feed and feedig pracices, fish handling, monitoring, etc.), which are very difficult to be mimicked at reseach scale. The evaluation of performance should be addressed with a multidisciplinary approach, difficult to be mimicked at research scale, and very expensive at a pilot or commercial scale. Consequently, the evaluation of causes behind a poor zootechnical performance is a very difficult objective for aquaculture managers, as the above mentioned parameters are interrelated and the sector operates with low profitability margins and on a highly competitive international scale.

Material and Methods

Two fish farms in two different locations in the Mediterranean Sea - in East Mediterranean (Galaxidi Marine Farm, Phocis, Greece) and in West Mediterranean (Cudomar, Campello, Alicante, Spain)- were selected to carry out this case study.

Same batch of fish (seabream fry from Galaxidi) was selected for the experiment and 82,000 juveniles (3g) for each farm were transported in June 2018 to the 2 owgrowing cage farm locations. Fish were grown in the same type and diameter (16 m) sea cages in both farms. Initially ongrowing in the farms, until the fish reached 100 g average weight, was implemented using each farm standard feeds (provided by Irida in the case of Galaxidi and by Dibaq in the case of Cudomar). From 100 g until harvest (420-445 gr) the farms followed the recommendations designed by MedAID about feed composition and feeding practices obtained in the trials carried out in MedAid WP2:

1. Use of 45/16 Protein/Lipid and 25/16 FM/FO ratios during winter conditions and 44/20 Protein/Lipid and 16/7 FM/FO in summer
2. Use only 1 dose of food per day during ongrowing
3. The feeds were selected (Table 1) following the recommendation 1 and were distributed in only one dose per day (recommendation 2)

Every month fish were sampled to asses their growth in weight. Environmental conditions (temperature, salinity, oxygen, pH) and disease and/or mortality events were also recorded.

At the end of the ongrowing period around 1000 fish were sampled for assesing their final weight, fillet quality and biochemical composition. For that purposes the fish were slaughtered and eviscerated to record visceral weight, abdominal fat weight, heart weight and fillet yield

Table 1. Biochemical composition of the feeds used during the final ongrowing period in the sea cages

	Nutraplus SP	Vital M
Protein (%)	45.0	45.0
Fat (%)	16.0	20.0
Cellulose (%)	2.7	2.8
Ash (%)	6.5	8.0
Moisture (%)	9.0	10.0
Crude Energy (MJ/Kg)	20.2	21.1
Digestible Energy (MJ/Kg)	16.8	17.5
FM/FO	22/7	16/7

(Continued on next page)

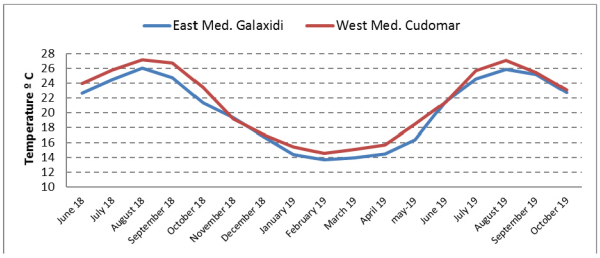


Fig 1. Evolution of monthly average water temperature during the ongrowing study.

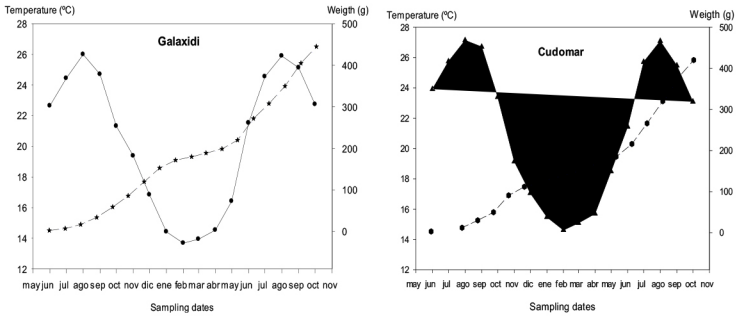


Fig 2. Results of temperature (^{0}C) and growth (g) obtained in Galaxidi and Cudomar sea cages.

Growth rate was evaluated as specific growth rate (SGR) and relative growth rate (RGR) Thermal growth Coefficient (TGC) and Feed Conversion Ration (FCR) were also calculated taken into account the feed provided during the ongrowing period (16 months) and the growth of the fish.

Results

The evolution of monthly average water temperature during the ongrowing study showed a similar pattern in both locations (East Mediterranean and West Mediterranean), with slighty higher temperatures in Alicante, Spain (Figure 1). Oxygen and salinity were also periodically recorded, being 8.19±0.23 mg/l of oxygen in Cudomar and 6.10±0.67 mg/l in Galaxidi and 37-38 ppt salinity in Cudomar and 39-40 ppt in Galaxidi.

FCR in East Med Galaxidi (1.85) was slightly higher in Galaxidi than Cudomar (1.65), although no statistically significant differences (Student's t test, P=0.955) in terms of growth (SGR, Fig. 2) or conversion rate (FCR) (Student's t test, P= 0.667) were found between the two sea cages.

Feeding with a lower cost diet (lower DP/DE and FM/FO) during low temperature months does not affect feed conversion performance. Moreover, our results at pilot scale are sligthy better than other references for commercial feed conversion for marine fish in the Mediterranean, such as that reported by García-García et al. (2016) for seabream (FCR= 2) grown for 18 months in Spanish Mediterranean (Murcia) from 12 g to 450 g in 25m diameter cages.

Althought it was not the purpose of this work to estimate the economic benefit of using a lower cost diet (10% cheaper) during low temperature months (February-June), our estimation showed a 3% reduction on the feed cost. Being the cost of feed the highest cost in seabream production, this measure would certainly be a well-meaning recommendation to be considered by Mediterranean farmers. Proximate and fatty acid composition of the fillet was also analysed and presented in the Congress.

THE VISUAL ANIMAL WELFARE DIAGNOSTICS INTERNET OF AQUACULTURE (IOA)

D. Ewald[1*], M. Vahl [2*], J.V. Apel[1], T. Dolereit[2], V. Edling[1], R. Fisch[1], M. Bögner[3] M.Ramm[1], T. Haug[1], L.J. Arndt[1], C. Dhumasker[1] and U. Freiherr von Lukas[2]

[1]MonitorFish GmbH, Hönower Str. 34, 10318 Berlin (Germany)
*Email: ewald@monitorfish.com
[2]Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Sektion Marine Bioökonomie, Am Handelshafen 12, 27570 Bremerhaven
*Email: stephan.ende@awi.de
[2]Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Sektion Marine Bioökonomie, Am Handelshafen 12, 27570 Bremerhaven

Introduction

In order to implement the 2030 Sustainable Development Goals (SDGs) of the Food and Agriculture Organization of the United Nations (FAO), production capacities in aquaculture must be increased and resources must be conserved. This can only be achieved if fish health is improved and fish death rates are reduced, thus saving feed and water. It is therefore essential that aqua culturists and fish farmers are motivated to use suitable technological solutions such as monitoring systems. An optimally aligned monitoring system can balance farming conditions, animal health and efficient use of resources. These are prerequisites for sustainable aquaculture, the production of quality products and profitable sales. Because the knowledge of animal welfare in general is rudimentary the overall production success is reduced. Animal welfare is not only important from an ethical point of view, it also forms the basis for the harvest and thus, the revenue of each farm. Therefore, the goal is to develop a novel non-invasive monitoring concept and system for selected aquaculture organisms (AKO) with physicochemical sensors and image sensors via the cloud-based software AnFish including the determination of physical and chemical water parameters, activity patterns and the condition of the animals. Through this, we are able to assess stress and health conditions of aquatic organisms in different stages of production from hatching to harvest. This allows real-time optimization of fish production processes. Furthermore, with a software like this we are able to digitally certify farming conditions and aquaculture facilities.

Material & methods

Fishes are stocked in experimental units under various conditions (single housed to commercial conditions in terms of density, size, light, etc…). Numerous cameras are installed above the experimental units to record constantly. Image captures are recorded under various conditions. Further data recorded manually are the number of fish, weight and biomass, mortality, signs of illness and stress.

In addition, the standard built-in sensors continuously record the water quality. Furthermore, the work includes the extension of the software module for camera control around the digital focus, its porting for the new development platform (Raspberry Pi 4, Ubuntu), as well as the elimination of various driver problems.

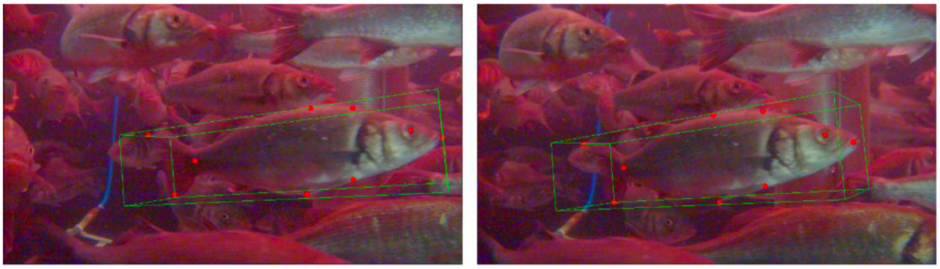


Figure 1 Visualization of a survey result with point features and enclosing 3D cuboid

(Continued on next page)

Results
At EAS 2021, preliminary results of the image analysis and the interaction of various physical/chemical sensors of the recordings will be presented. Currently, the final experiments are being performed and a higher-level analysis/model is being developed from the data, which will be available and analysed before the upcoming conference.

We would like to present:
- Overall experimental setup
- Preliminary results of digital/automated counting of fish primarily coupled to biomass as well as single individuals
- Stereo matching and the 3D reconstruction

Discussion and conclusion
Preliminary final results on automated counting as well as biomass determination of food fish (considered key data for successful software development) will be presented at EAS 2021. Reliability of initial data (number of digital counts vs. manual counts and vs. starvation condition) will be discussed and approaches for detection algorithms comparing metadata to a fish behavior database will be presented.

DIGITAL INDICATORS FOR STRESS

BIOINDICATORS FOR THE EVALUATION OF FISH FARMING, HEALTH AND PRODUCT QUALITY IN DIFFERENT AQUACULTURE SYSTEMS (BIOFIA)

D. Ewald[1*], D.v.Muilekom[2], J. Müller[3,4], S. Starke[5], H. Seibel[3], M. Schlachter[4], C. Schulz[3,4], T. Goldammer[2,6], V. Edling[1], S. Billakuduru[1], A. Kübler[1]

[1]MonitorFish GmbH, Berlin, Germany
[2]Institute for Farm Animal Biology (FBN), Institute of Genome Biology, Fish Genetics Unit, Dummerstorf, Germany
[3]Institute of Animal Breeding and Husbandry, Department for Marine Aquaculture, Christian-Albrechts University, Kiel, Germany
[4]Gesellschaft für Marine Aquakultur mbH (GMA), Büsum, Germany
[5]Microganic GmbH, Melle, Germany
[6]University of Rostock, Faculty of Agricultural and Environmental Sciences, Molecular Biology and Fish Genetics, Rostock, Germany

*Email: ewald@monitorfish.com

Introduction

Optimal husbandry conditions, healthy animals and innovative raw materials are prerequisites for the sustainable aquaculture of animal organisms, the production of quality products and profitable sales. Even if, from a human perspective, fish grow up successfully in aquaculture, knowledge about animal welfare itself is rudimentary and production success is reduced correspondingly.

Aquaculture always involves a high economic risk for the fish farmer, up to and including total loss caused by undetected diseases in the fish or even environmental toxins. Comprehensive monitoring of the fish kept can not only minimize these risks, but also improve the economic yield and ultimately make fish farming more environmentally friendly. To reduce these risks, MonitorFish GmbH has developed innovative software solutions such as ANFISH© and OptoFish©. MonitorFish GmbH is a startup company of the Berlin University of Applied Sciences which is intensively involved in the development of software solutions for the optimization of fish farm production management. Their software works cloud-based and uses artificial intelligence to improve their own analytical precision and speed. Within the project BioFiA a comprehensive approach to analyse stress in Atlantic salmon (Salmo salar) during different microalgae diets and at different salinities is conducted. In this framework, the MonitorFish software is adaptated to and validated within trials.

Material & methods

Biochip-based molecular indicators can be used to measure stress, health and welfare of aquatic organisms at different stages of production from hatching to harvesting, to certify husbandry conditions and aquaculture facilities and thus optimise fish production processes right through to the slaughter process.

Within the framework of the project, the use of such self-developed species-specific molecular biochips is planned for key production stages. Especially the juvenile life stages with high mortalities will be intensively considered. For this purpose, a comprehensive monitoring of the rearing conditions with physicochemical and image sensors (e.g. water parameters, activity patterns, health status) is carried out. This also includes the detection of bacteria and parasites. Big data analyses of the monitoring data are carried out using AI algorithms. The comprehensive sensory analysis allows an early assessment and even prediction of animal welfare in real time. The project focuses on the recording and reduction of negative influences in aquafarming, in particular juvenile development stages and the mechanical processing of fish, the influence of microbiological parameters on the health of aquaculture organisms and microalgae as innovative probiotic feeds. In addition, the product quality as food for humans is tested. The networking of the project with the subproject BaMS-AQUATOR will lead to the testing of industrial usability and feasibility studies.

(Continued on next page)

Blaue Bioökonomie BioFiA

MONITORFISH

Project partners are

- Christian-Albrechts-Universität zu Kiel, Institute for Animal Breeding and Husbandry
- Society for Marine Aquaculture (GMA) mbH
- Research Institute for Farm Animal Biology (FBN), Institute for Genome Biology, Dept. of Fish Genetics
- Max Rubner Institute (MRI), Federal Research Institute for Nutrition and Food
- Microganic GmbH
- MonitorFish GmbH
- University of Hamburg, Department of Microbiology & Biotechnology
- University of Rostock, Faculty of Agricultural and Environmental Sciences, Aquaculture and Sea Ranching
- Molecular Biology and Fish Genetics, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

For the Testings, fish are kept in experimental units under various conditions (single housing to commercial conditions in terms of density, size, light, etc...). Numerous cameras are installed above the experimental units, recording continuously. The image recordings are captured under different conditions. Other data that are collected manually are the number of fish, weight and biomass, mortality, signs of disease and stress.

Stress is identified by gene expression-based BioChips, blood parameter and stress hormone analyses, and MonitorFish GmbH as a project partner performs real-time monitoring of the fish. The company has a self-developed cloud-based technology for comprehensive real-time monitoring of fish in aquaculture for sustainable improvement of fish health. The system is allowing a non-invasive cloud-based real-time fish monitoring. Artificial neural networks (KNNs) are then used to establish relationships between water parameters, fish appearance and movement patterns. KNNs require only a fraction of data sets to generate a diagnostic correlation with recommended action. These data are supportive for functional classification of movement patterns in relation to physical and chemical characteristics of the water. Based on the evaluations of all variables, the software generates recommended actions to maintain optimal water quality and fish health for optimal productivity and yield. Thus, sensor analytics allow early assessment and even prediction of animal welfare in real time.

In the frame of the BioFia-project MonitorFish continuously records the water quality with standard built-in sensors and works on the extension of the software module for camera control to include digital focus, its porting for the new development platform (Raspberry Pi 4, Ubuntu) and the elimination of various driver problems.

In addition, other project partners are monitoring the fish for parasites, fillet quality is being tested, and the microbiology of the aquaculture filtration system during challenge experiments is being verified.

(Continued on next page)

Results

Preliminary results of the image analysis and the interaction of stress management in aquaculture will be presented at the AE2021. BioFia focuses on stress detection and visualisation for salmon. The main focus of the current experiments is:
- appetite/satiation detection
- Stress detection via movement behaviour

For both detections a much higher image rate than currently used will be necessary. A higher image rate also means a larger amount of data. It will also be evaluated whether this data can be processed on site with greater computing power or whether it should be loaded into a cloud in a correspondingly compressed form. At AE2021, the obstacles that are encountered in such complex experiments will be presented.

Discussion and conclusion

Preliminary results on automated feeding and how fish are stressed by the feeding procedure as well as by manual fish biomass determination (as key data for successful software development) will be presented at EAS 2021. The reliability of the baseline data (number of digital counts vs. manual counts and vs. starvation status) will be discussed and approaches for detection algorithms comparing metadata with a fish behavior database will be presented.

References

ROBENS S (2018) Fischkamera gewinnt bei Rentenbank. Agrarzeitung
RUHRPOTT START-UPS (2017) MonitorFish ist der Gewinner der Deutschen-Runde der Future Agro Challenge.
RUSCHE E (2018) Can IoT tool help fish farmers sleep better at night. Global Aquaculture Alliance.
THE HUNDERT (2018) The Hundert Startups of Germany. https://nkf.media/category/the-hundert/
BMWI (2018) Gründerwettbewerb - Digitale Innovation.
ROSNER F (2018) MonitorFish – Internet of Things meets Deep Learning. Codecentric Blog.

EFFECTS OF DIFFERENT MICROALGAL DIETS ON *Ostrea edulis* BROODSTOCK CONDITIONING AND REPRODUCTION. DEVELOPMENT OF A NON-DESTRUCTIVE TECHNIQUE FOR THE MONITORING OF OYSTER GAMETOGENESIS AND SEX DIFFERENTIATION

M. Fabra*, T. Bean, G. Watson and J. Preston

*Institute of Marine Sciences, University of Portsmouth, Portsmouth, UK
Email: monica.fabra@port.ac.uk

Reflecting the global decline of oyster reefs, the current distribution of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758), across the UK and Europe represents only a small fraction of the historic distribution. Despite the numerous restoration projects conducted in the past decades, seed oyster supply is currently a key limiting factor for native oyster restoration. Several hatcheries are being established across Europe to assist in restoring *O. edulis* populations, providing oyster seed. However, *O. edulis* hatchery production is still prone to failure. The scarce capacity to produce large quantities of larvae is firstly due to *O. edulis* complex lifecycle which makes it difficult to manipulate spawning and fertilisation in a controlled environment. The knowledge gaps surrounding *O. edulis* biology and reproduction, and the factors responsible for the gametogenesis and sex differentiation, also make it difficult to control the sex ratio, leading to disproportionate gametic contribution. The biochemical composition of diet is important for broodstock reproduction, with fatty acid-enriched diets expected to provide sufficient reserves for the development of ovogonia, whilst low concentrations of fatty acids promote the development of more spermatogonia and male-based populations. The effects of four single microalgal diets (*Isochrysis galbana, Nannochloropsis oculata, Tetraselmis suecica, Thalassiosira pseudonana)* on *O. edulis* broodstock conditioning were assessed for oyster survival, growth, filtration rate, absorption efficiency and gonadal allocation of fatty acids. Techniques used to monitor the effectiveness of broodstock conditioning protocols, usually require destructive sampling of gonad tissue for histological analysis. This may involve the sacrifice of large numbers of oysters, which is undesirable for many restoration projects with limited broodstock. A novel non-sacrificial sampling technique was tested with effects on oyster survival, growth and filtration rate. If successful, this non-destructive approach will allow to monitor the gametogenesis and sex differentiation of individual oysters through the whole reproductive season, without sacrificing broodstock.

GENOMICALLY ASSISTED RECONSTRUCTION OF THE ANCESTRAL *Dicentrarchus labrax* L. MEDITERRANEAN LINEAGE

Faggion, S.[1*], Duranton, M.[2], Gagnaire, P.-A.[2], Vandeputte, M.[1,3], Allal, F.[1]

[1] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France
[2] ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France
[3] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France
E-mail: francois.allal@ifremer.fr

Introduction

Whole-genome sequences studies of sea bass have recently revealed two ancestral lineages that diverged in allopatry for 270,000 years in the Atlantic and the Mediterranean. A postglacial contact between those lineages resulted in an asymmetrical introgression from the Atlantic to the Mediterranean background (Figure 1 - Duranton et al. 2018). In our study the base western population (WM) is introgressed at 49% by Atlantic genome, while our east-Med population (EM) at 23%. We explored different strategies to reconstruct the ancestral Mediterranean lineage by local ancestry deconvolution.

Material and Methods

A candidate population (G1) of 605 individuals was obtained after a backcross between (WM × EM) females and EM sires. Every animal was genotyped on a 57k SNP chip. We used APIS to recover the pedigree, Lep-MAP3 to construct a genetic map, and Beagle 5.1 for the phasing of the genotypes. The local ancestry for each haplotype of admixed individuals was inferred with Loter. Haplotype-resolved Atlantic genomes and Mediterranean desintrogressed haplotype-resolved genomes were used as reference for both ancestral lineages (Duranton et al. 2018). A custom-made R script was used to simulate generations of selection across different breeding strategies (backcross, BC, intercross, IC, or combinations of those). Six selection criteria were tested on each type of crossing: whole genome admixture (further referred as Med-ratio), chromosome-based Med-ratio, rarefaction of homozygous Atlantic tracts or combinations of those. For each scenario, factorial matings of 24♀×24♂ across 8 generations were simulated and repeated 20 times.

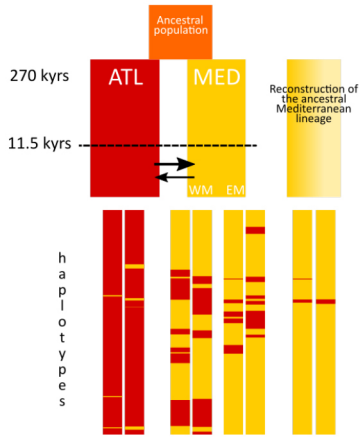


Figure 1. Divergence and introgression history of Atlantic and Mediterranean D. labrax populations (adapted from Duranton et al., 2018). The Atlantic and the Mediterranean populations diverged for 270k years; a postglacial contact between them resulted in asymmetrical introgression (black arrows). The Atlantic genomic background is in red, and the Mediterranean genomic background is in yellow. The aim of the study is to reconstruct the ancestral Mediterranean lineage, with a ~2 % of Atlantic introgression

(Continued on next page)

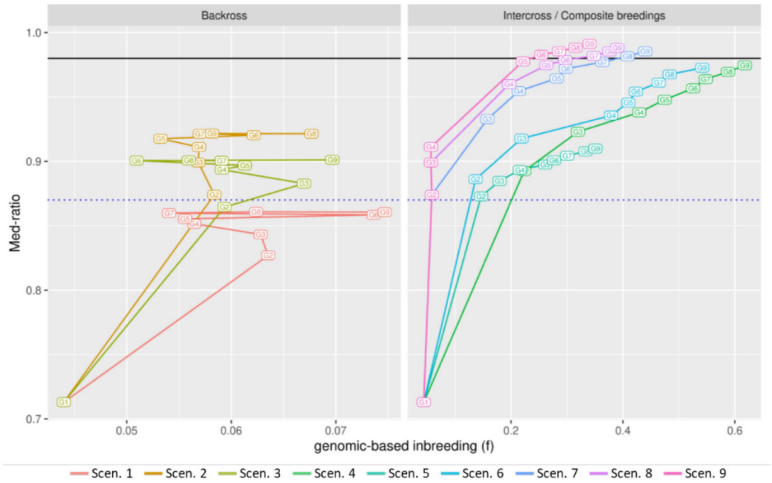


Figure 1. Evolution of mean genomic-based inbreeding and of mean Med-ratio over generations for breeding different scenarios. Solid black line represents the targeted Med-ratio to recover an ancestral Mediterranean lineage, while blue dashed line represents the mean Med-ratio observed in the present Eastern Mediterranean population

Results and Discussion

The Figure 2 display the evolution of the Med-ratio and the genomic-based inbreeding (f) across the generation for the different scenarios tested. We showed that from the candidate generation G1, displaying a Med-ratio level of 71%, a classical BC (Scenario 1) induced a stagnation at a Med-ratio level of 86% from G6. Other BC scenarios with selection of the Med-ratio (Scenarios 2 and 3) allowed to overcome the mean Med-ratio observed in the present Eastern Mediterranean population, maintaining a low level of inbreeding (below 7%), but did not allowed to reach the ancestral Mediterranean target. Pure IC strategies (Scenarios 4, 5 and 6) did not allow to reach the 98% Med-ratio target and induced a high level of inbreeding especially for Scenarios 4 and 6. The best performing strategies were those using the selection criteria on whole genome Med-ratio and including 1, 2 or 3 generations of BC followed by IC (for respectively Scenarios 7, 8 and 9). These strategies allowed to overcome the Med-ratio level of the actual EM population after just one extra generation, and to reach the breeding goal of a 98% pure Mediterranean strain at G6 for Scenario 7.

This recovered ancestral line will be key to understand the dynamics of speciation and secondary admixture in Mediterranean and Atlantic sea bass, and may represent a key resource for sea bass aquaculture in extreme environments.

Acknowledgement

This study was supported by AQUAEXCEL^{2020} project which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 652831, and by the French Ministry of Environment under grant CRECHE2020.

References

Duranton, M., Allal, F., Fraïsse, C., Bierne, N., Bonhomme, F., Gagnaire, P.A. (2018). The origin and remolding of genomic islands of differentiation in the European sea bass. Nat. Commun. 9: 2518.

GENETIC BASIS OF RESISTANCE TO VIRAL NERVOUS NECROSIS IN GILTHEAD SEA BREAM (*Sparus aurata*) AT THE LARVAL STAGE

S. Faggion[1*], R. Franch[1], M. Babbucci[1], F. Pascoli[2], G. Dalla Rovere[1], L. Biasini[2], S. Iori[1], M. Caggiano[3], H. Chavanne[3], A. Toffan[2], P. Carnier[1], L. Bargelloni[1]

[1] Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università, 16, 35020 Legnaro (PD), Italy
[2] Division of Comparative Biomedical Sciences, OIE Reference Centre for viral encephalopathy and retinopathy, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padova, Italy
[3] Panittica Italia Società Agricola S.R.L., Strada del Procaccio 72016 Torre Canne di Fasano, Italy
Email: sara.faggion@unipd.it

Introduction

Nervous Necrosis Virus (NNV) is one of the major viral pathogens in aquaculture, affecting a wide range of fish species and causing high mortality rates. The gilthead sea bream (*Sparus aurata*) has long been considered resistant to NNV, until recently, when significant mortalities caused by a reassortant NNV strain were reported in sea bream hatcheries (Volpe et al. 2020). Since the larval stage is the most susceptible life-stage to NNV, vaccination is not a feasible option due to the immaturity of the immune system. Selective breeding to enhance resistance against the reassortant NNV strain might be a possibility as a disease preventive action. Here, we analysed for the first time the genetic basis of viral nervous necrosis (VNN) mortality in gilthead sea bream larvae and we assessed the accuracy in the genomic prediction of this trait.

Materials and methods

The experimental fish were generated at a commercial hatchery through controlled crosses in three independent full factorial matings (10 sires × 12 dams; 10 sires × 7 dams; 10 sires × 5 dams). At 15 days post-hatching (dph), larvae were transferred to the IZSVe experimental facility. At 19 dph, larvae were infected by immersion adding to the tank the reassortant strain VNNV/S.aurata/Farm1/461-1/Nov2014. The final infectious titre was verified by titration of the water ($10^{5.45}$ $TCID_{50}$/ml). The challenge trial ended at day 9, when no more symptomatic/dying larvae were detected for at least 24 hours. Larvae showing symptoms of VNN infection and surviving individuals were collected for DNA analysis and recorded as 0 (asymptomatic) or 1 (symptomatic). The experimental infection protocol was evaluated by the IZSVe Animal Welfare Body and Ethics Committee (Opinion CE.IZSVE.3/2016 of 24/10/216) and subsequently approved by the Italian Ministry of Health (Law decree 101/2017-PR of 02/02/2017). All the experimental fish and their parents were genotyped using the Med_Fish SNP array, which contains over 27,000 SNPs for the gilthead sea bream (Peñaloza et al. 2021). Variance components for mortality was estimated using Bayesian procedures with a univariate sire-dam threshold model. Heritability was computed using the sire variance only, to avoid potential non-genetic maternal effects. Genome-wide association analysis (GWAS) was performed to test the association between VNN mortality phenotype and SNPs. Genomic prediction of VNN mortality was performed implementing three Bayesian regression models: Bayes B (BB), Bayes C (BC) and Bayesian Ridge Regression (BRR; GBLUP equivalent). Prediction performance was assessed by means of 5 independently-generated 5-fold cross validations (CV). In each CV, 80% of the data were used to train the model and 20% served as a validation set. Three metrics were used to evaluate model performance in classification: Matthews correlation coefficient (MCC), the area under the ROC curve (AUC) and accuracy (ACC). Pedigree indices were estimated using an animal model through 5-fold CV: in each CV, 80% of the data was used to estimate the indices of the remaining 20% of the animals. Performance of the indices in classification of VNN mortality was assessed using the same metrics used for genomic prediction (MCC, AUC, ACC).

Table 1. Average metrics (AUC, ACC, MCC) of classification performance of VNN mortality in 5 independent 5-fold cross-validations; standard deviation (SD) in brackets

Bayesian model	Metric		
	AUC	ACC	MCC
BB	0.5813 (0.0166)	0.5694 (0.0162)	0.1752 (0.0239)
BC	0.5969 (0.0134)	0.5811 (0.0131)	0.1758 (0.0231)
BRR	0.6014 (0.0134)	0.5825 (0.0088)	0.1778 (0.0215)

(Continued on next page)

Results

First symptoms of VNN infection were detected at day 6 post-challenge, followed by a peak of two day; then mortality sharply decreased up to day 8. A total of 1184 individual larvae were collected (513 dying and 671 survivors). Genomic DNA was extracted from the tissue of 1044 larvae and 54 parents and used for individual genotyping. A total of 974 larvae, 47% symptomatic and 53% asymptomatic, were successfully genotyped and parentage assignment to a unique parental pair was achieved for all the 974 fish. Individuals were allocated to 160 families, with a number of offspring per family ranging from 1 to 55. After removing one sire and one dam that generated only one offspring, 972 individuals from 28 sires and 22 dams were retained. Overall, 26,591 SNPs with MAF > 0.05 were scored. The estimate of heritability for VNN mortality was moderate (h^2 = 0.1921; 95% highest posterior density intervals: 0.0006, 0.5790), with a probability being greater than 0.2 equal to 0.49. Classification of the observed VNN mortality using the genomic prediction of the phenotype of mortality as classifier was significantly more accurate than random guessing of the classes, with consistent results across Bayesian models (Table 1); using the pedigree indices to classify the mortality phenotype resulted in similar performances (AUC = 0.5875, ACC = 0.5798, MCC = 0.2550). The GWAS failed to identify any genome-wide QTL for VNN mortality overcoming the significance threshold.

Discussion

VNN is an emerging threat for gilthead sea bream hatcheries and this is the first study that explored the genetic basis of resistance to VNN in this species. Experimental infections in early developmental stages are scarcely reported, especially with adequate sample size to estimate variance components and genetic parameters. The estimate of heritability for VNN mortality suggests the feasibility of selective breeding programmes for increased resistance to VNN of fish larvae/juveniles, overcoming the problem of vaccination. The practical exploitation of genomic information due to the availability of genome-wide dense marker panels might offer the opportunity of developing prediction tools for the studied trait.

References

Peñaloza C, Manousaki T, Franch R, Tsakogiannis A, Sonesson A, Aslam ML, Allal F, Bargelloni L, Houston RD, Tsigenopoulos CS. (2021). Development and testing of a combined species SNP array for the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). Genomics, in press.

Volpe E, Gustinelli A, Caffara M, Errani F, Quaglio F, Fioravanti ML, Ciulli S. (2020). Viral nervous necrosis outbreaks caused by the RGNNV/SJNNV reassortant betanodavirus in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Aquaculture, 523: 735155.

IMMUNE RESPONSES OF RAINBOW TROUT (*Onchorhynchus mykiss*) AFTER CHALLENGE WITH *Yersinia ruckeri*

C. Fajardo[1,2], P. Santos[1,2,3], I.A. Ferreira[2,3,4], L. Ramos-Pinto[2], A. Cunha[2,3], M. Hinzmann[2], T. Baptista[1], B. Costas[2,3*]

[1]MARE - Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, 2520-620 Peniche, Portugal
[2]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto, Portugal
[3]Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[4]Fish Immunology and Vaccinology Group, IBMC-Instituto de Biologia Molecular e Celular, University of Porto, 4200-135 Porto, Portugal
*E-mail: bcostas@ciimar.up.pt

Introduction

Aquaculture production of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) has become a modern multibillion dollar industry. However, the development of this sector has not been exempt from pitfalls related to the presence of pathogens. Among these, the recurrent presence of pathogens of bacterial origin can be highlighted, as is the case of *Yersinia ruckeri*, a Gram negative rod with rounded ends, not forming spores or capsules, but often with the presence of flagella that provides it variable mobility. *Y. ruckeri* is the causative agent of the infectious pathology known as Enteric Red Mouth Disease (ERM). ERM is a systemic infection that can affect *O. mykiss* for its entire life cycle, being especially susceptible the juvenile stages cultivated in fresh water. Although *Y. ruckeri* can affect different types of salmonid and non-salmonid fish, both in fresh and salt water, *O. mykiss* is the species that has been reported as the most sensitive and vulnerable to ERM, causing serious economic losses that can be as high as 30–70% of the stock in some cases. ERM has also been referred to as yersiniosis since affected fish do not always present the characteristic reddened areas of the mouth. Likewise, this term is used to distinguish a chronic infection compared to ERM that appears acutely. *Y. ruckeri* infection generally results in the development of acute or chronic septicaemia characterized by the presence of haemorrhages around the mouth and anus, at the base of the fins, and on the surface of internal organs. Although several studies have yet been performed regarding the pathogen features and virulence factors, few information about the host defence mechanisms activated after infection is available. Given this perspective, this study aimed to evaluate rainbow trout innate immune response against the infection with *Y. ruckeri*.

Materials and Methods

A time-course study was performed at CIIMAR (Matosinhos, Portugal) facilities with 72 rainbow trout (*Oncorhynchus mykiss*) juveniles (16.7 ± 4.4 g). After 2 weeks of acclimation, 12 fish were sampled before infection (time 0). The remaining animals were randomly selected and intraperitoneally (i.p.) injected with 100 μl PBS (placebo group) or 100 μl of exponentially growing *Y. ruckeri* ($2 * 10^8$ CFU mL^{-1}; infected group) and distributed as a complete randomized design in 6 recirculating seawater systems (i.e. triplicates *per* experimental condition). Two animals per tank (n = 6 *per* treatment) were sampled at 3, 6, 9, 24 and 48 h after i.p. injection. Fish were euthanized with 2-phenoxyethanol (0.5 mL L^{-1}) and blood samples were collected for haematological procedures. The remaining blood was centrifuged for plasma collection and innate humoral parameters (i.e. peroxidase, lysozyme, anti-protease, and nitric oxide activities) were evaluated.

Results

In general, the infection induced a host anemic state related with the hemolytic effect caused by the infection with *Y. ruckeri* since red blood cells and haemoglobin dropped their levels in infected animals compared to placebo individuals. In particular, red blood cells count showed significant differences between the placebo and the infected groups after 24 and 48 h. In relation to the haemoglobin parameter, differences were only recorded between the placebo group and the infected group at 48 h, being the value of this parameter lower in the infected group. Regarding haematocrit, significant differences were recorded between the control group (0 h) and the infected group after 48 h. Similarly to that observed for red blood cells and haemoglobin concentration , haematocrit also decreased significantly in infected animals compared to placebo

(Continued on next page)

individuals at times 24 and 48 h. Regarding white blood cells numbers, differences were verified between the control group (0 h) and the infected animals at times 3, 6, 9, 24, and 48 h. In all the cases, there was a sustained decrease in the white blood cells in infected specimens. Moreover, white blood cells also dropped in placebo trout at 6 and 9 h compared to the control group (0 h), a decreased most likely explained as a reaction to the injury produced by the injection of PBS; however, after 48 h the number of white blood cells returns to its basal state. On the other hand, there were no differences in the parameters of peroxidase, anti-protease, lysozyme, and the nitric oxide activity.

Discussion and Conclusion

The results of this study described some mechanisms that contribute to the immune response of *O. mykiss* against the infection with *Y. ruckeri*. The activation of defense mechanisms linked to haematological cellular parameters was verified, such as the amount of white blood cells, which decreased in number in the blood as the infection developed at times 3, 6, 9, 24, and 48 h respectively, presumably as a response of migration of white blood cells to the focus of the infection to combat it. On the other hand, there was a sustained decrease over time in both the number of red blood cells and hematocrit (being significant after 24 and 48 h, respectively), and also in haemoglobin (being significant after 48 h). These results show a preliminary vision of the effects caused in *O. mykiss* by infection with *Y. ruckeri*, which may be useful for the establishment of biomarkers that may be used for the early detection of ERM.

Acknowledgements

This work was supported by project BE4AQUAHEALTH: RASTREIO NACIONAL DE PATOLOGIAS DE PEIXES DE AQUACULTURA: UMA APOSTA NA PREVENÇÃO (16- 02-05-FMP-0013), funded by Mar2020 Operational Programme and the European Union through FEDER, and by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. IAF and BC were supported by FCT (SFRH/BD/147750/2019 and IF/00197/2015, respectively).

SPERMIATION ENHANCEMENT OF GREATER AMBERJACK *Seriola dumerili* IN RESPONSE TO GnRHa IMPLANTS OR hCG

Ioannis Fakriadis[1], Maria Papadaki[1] and Constantinos C. Mylonas[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, P.O. Box 2214, Heraklion, Crete 71003, Greece
E-mail: fakriadis@hcmr.gr

Introduction

The greater amberjack (*Seriola dumerili*) is a species with high potential for the diversification of the aquaculture production, due to its excellent flesh quality and high worldwide consumer acceptability. However, reproductive dysfunctions in captivity have been observed in males, even more pronounced than in females (Zupa, et al., 2017). Additionally, sperm quality and quantity were not different after rearing in sea cages or in land-based tanks, but showing remarkable decreased milt production compared to the wild (Fakriadis, Mylonas, 2021). Agonists of gonadotropin-releasing hormone (GnRHa) and human chorionic gonadotropin (hCG) have been used to overcome these problems in many fishes, and the present study compared these hormonal spermiation enhancement methods in terms of plasma sex steroid production and sperm quality.

Materials and methods

Wild-captured breeders (11.6-20.0 kg) were kept in Aqualabs, Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion, Crete, Greece, in a 40 m^3 tank under simulated photoperiod and temperature profile, and were fed with a broodstock diet (Skretting, Vitalis Cal, 22mm). Males, after blood and sperm collection, were treated either with a GnRHa implant (n=3) with an effective dose of 59±2 μg GnRHa kg^{-1} or hCG injection (n=4) with an effective dose of 617±46 IU kg^{-1} (day 0). Fish were sampled again for blood and sperm on day 3, 7 and 14 after the hormonal treatments. Plasma testosterone (T), 11-ketotestosterone, 17-β estradiol (E_2) and 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) were quantified with the use of enzyme-linked immunosorbent assays (ELISAs). Sperm quantity was evaluated according to their spermiation condition –which is a measure of the available milt in the testes- after gentle abdominal pressure was applied, determined by a subjective scale from 0 to 3, as follows: Spermiation index S0 = no milt released, S1= only a drop of milt released after multiple stripping attempts, S2= milt was released easily after the first stripping attempt and S3= copious amounts of milt released with very little pressure. The sperm quality parameters that were evaluated included: (a) sperm density (number of spermatozoa ml^{-1} of milt), (b) survival of spermatozoa under cold storage at 4°C (spermatozoa survival, days), (c) initial percentage of spermatozoa showing forward motility immediately after activation (spermatozoa motility, %) and (d) duration of forward spermatozoa motility of ≥5% of the spermatozoa in the field of view (motility duration, min).

Results and discussion

Males did not release sperm after abdominal pressure on day 0. Half of the fish (n = 2) after hCG treatment were in S2 on day 3 and S3 on day 14 after treatment, while one fish was found to be in S2 3 days after treatment with GnRHa implants. Plasma T concentration increased significantly 7 days after treatment with GnRHa implants while it did not differ significantly after treatment with hCG (Figure 1), while 11-KT also increased significantly, but there was no difference between the different treatments. Both E2 and 17.20β-P did not differ significantly after treatments. Hormonal therapies did not affect significantly sperm motility and motility duration. Sperm density decreased significantly 3 days after hCG treatment, and remained decreased on day 7, but recovered to baseline on day 14 after treatment. Treatment with GnRHa implants caused a significant reduction in sperm density 14 days after treatment. Regarding the survival of sperm under cold storage, a significant reduction was observed 7 days after hCG treatment, while no changes were observed after treatment with GnRHa implants.

(Continued on next page)

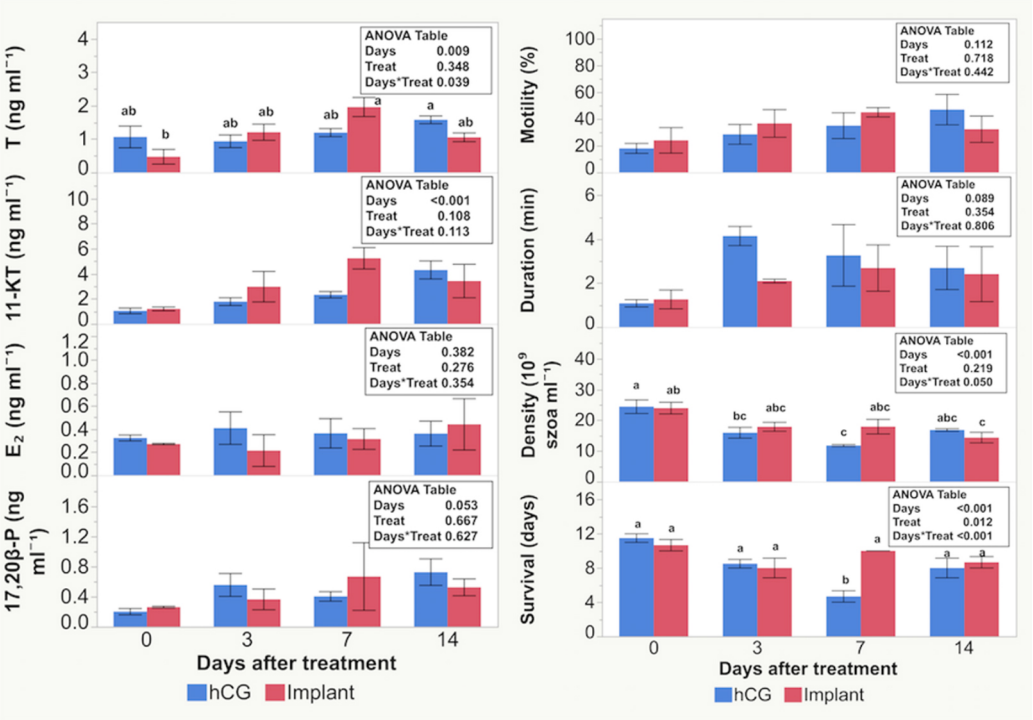


Fig.1. Mean (± SEM) concentration of blood plasma testosterone (T), 11-ketotestosterone (11-KT), 17-β estradiol (E_2), 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P), sperm motility, sperm motility duration, sperm density and survival under cold storage of greater amberjack males treated on day 0 either with hCG injection or GnRHa implant. Lowercase letters indicate significant statistical differences (2-way ANOVA, Tukey HSD, $P < 0.05$).

References

Fakriadis, I., Mylonas, C.C., 2021. Sperm quality of greater amberjack *Seriola dumerili* throughout the reproductive season and in response to GnRHa treatment with controlled release implants. Fish Physiol Biochem. 47, 281-292.

Zupa, P., Fauvel, C., Mylonas, C.C., Pousis, C., Santamaría, C.A., Papadaki, M., Fakriadis, I., V., C., 2017. Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, *Seriola dumerili* (Risso, 1810). J. Anim. Sci. 95, 4085-4100.

Acknowledgments

This project has received funding from the EPAnEK Operational Programme, Special Actions: Aquaculture (T6ΥΒΠ-00068).

European Union
European Structural and Investment Fund
HELLENIC REPUBLIC
MINISTRY OF ECONOMY & DEVELOPMENT
SPECIAL SECRETARY FOR ERDF & CF
MANAGING AUTHORITY OF EPAnEK
EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS
ENTREPRENEURSHIP
INNOVATION
ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement 2014 - 2020
Co-financed by Greece and the European Union

THE USE OF AN ENVIRONMENTAL ENRICHMENT MODULATES STRESS RESPONSE AND CHEMICAL COMMUNICATION IN SENEGALESE SOLE (*Solea senegalensis*)

Elvira Fatsini*, Zélia Vélez, Peter C. Hubbard, Catarina C.V. Oliveira, Catarina Marques, Florbela Soares, Pedro Pousão, Tomás Santos, Bernardete Rodrigues and Elsa Cabrita

Centre of Marine Sciences (CCMAR), Universidade do Algarve (Campus Gambelas), 8005-139, Faro (Portugal)
E-mail: effernandez@ualg.pt

Introduction

Environmental enrichment is used to promote fish welfare and, in some cases, to reduce detrimental characteristics that fish develop in captive conditions. Senegalese sole is a promising species for European aquaculture, above all in the South of Europe, for its good growth rate and high market price (Morais et al. 2016). However, Senegalese sole shows a reproductive dysfunction related to the lack of reproductive behaviour which does not allow to close the cycle in captivity. Several approaches have been studied to increase knowledge and try to solve this disorder. The problem remains unsolved, and the production is based on breeders caught in nature. Recently, it has been observed that urine is the communication vehicle for reproduction in Senegalese sole and its potency variates depending on fish maturity and sex (Fatsini et al. 2017). Our hypothesis was that conditioning the sole to natural environmental factors in early life stages may help to develop healthier fish and future potential breeders. Therefore, the present study aimed to assess the effect of using a substrate (sand) as an environmental enrichment in the stress response and chemical communication of pre-pubertal Senegalese Sole (*Solea senegalensis*).

Material and methods

The present study was divided in two parts. The first part was carried out to monitor the evaluation of the stress response in sole established in different environmental conditions. For this purpose, a total of 1500 sole (~10g; 8 months) were established in 6 outdoor tanks in the experimental station of IPMA (Olhão, Portugal) maintaining natural temperature and photoperiod according to each season. The bottom of 3 tanks were covered by 2 cm of sand and the other 3 remained without sand (fiberglass). These fish were maintained in the same conditions for two years and 4 samplings (every 6 months) were conducted to collect blood samples (n=20 fish per treatment and sampling) to evaluate cortisol in plasma by ELISA. Biometric parameters were also registered. In the last sampling a total of 72 pre-pubertal fish (n=36 per treatment) were transported to Ramalhete station (Faro, Portugal) to conduct the second part of this study. This part was performed to observe the influence of the environment in chemical communication in sole using urine from sole reared with and without sand. The fish were divided in 6 tanks and 3 tanks had the same origin sand in the bottom to mimic the original conditions. Urine samples were collected from 24 fish (n=12 fish per treatment) and 4 different pools were made per sex and environment. A total of 22 sole (7 females from sand and 5 from fiberglass; 5 males from sand and 5 from fiberglass) were used as receivers to perform electro-olfactogram (EOG) to evaluate the olfactory sensitivity and potency for smelling the same urine pools. The EOG were performed in different months to avoid seasonal factor.

Results and discussion

Significant differences ($P < 0.05$) were observed in cortisol plasma levels of Senegalese sole reared with and without sand during the 1st, 2nd and 3rd samplings where the fish maintained in sand obtained higher levels of cortisol in plasma than sole without sand. This could be associated with the stress response to mechanical agitation in the moment of catching the fish (Bates et al. 2014). However, all blood samples were collected in the first 4 minutes after catching the fish to try to avoid the rising of basal levels, suggesting that fish reared with sand were more sensitive to stress disturbance. It was noted that sole reared in sand presented less locomotor activity than fish reared without sand, which might decrease the baseline levels of several physiological parameters including cortisol. In terms of biometric data, sole reared with sand grew faster than sole reared without sand. These results coincided with other species reared using substrates as environmental enrichment (Bates et al. 2014).

In relation with chemical communication, no differences were observed in the potency of urine from females and males reared under different conditions; however, differences were observed in the olfactory sensitivity between females and males reared with and without sand smelling males' urine. Intriguingly, the olfactory sensitivity from females reared with sand to urine was higher than females reared in fiberglass. However, this situation might not be conclusive because females presented high variability in olfactory responses including within the same condition. In the case of males, it was observed

(Continued on next page)

the opposite, males reared without sand had higher olfactory sensitivity than males reared with sand. Besides, the olfactory responses from males reared without sand are described by a three-parameter Hill curve, while responses from males reared with sand are better described by a linear regression. These results might be explained by the fact that males are smelling different compounds or the olfactory epithelium receptors have a different location changing the perception of the stimuli. Nonetheless, more studies are needed to understand the implications of these results in chemical communication and the use of sand as environmental enrichment for the correct development and maturation of F1 breeders.

References

Bates, LC., Boucher, MA., Shrimpton, JM. (2014) Effect on temperature and substrate on whole body cortisol and size larval white sturgeon (*Acipenser transmontanus*). J. App. Ichthyol. 30, 1259-1263.

Fatsini, E., Carazo, I., Chauvigné, F., Manchado, M., Cerdà, J., Hubbard, P., Duncan, NJ. (2017). Olfactory sensitivity of the marine flatfish *Solea senegalensis* to conspecific body fluids. JEB, 220, 2057-2065.

Morais, S., Aragão, C., Cabrita, E., Conceição, L., Constenla, M., Costas, B., et al. (2016). New developments and biological insights into the farming of *Solea senegalensis* reinforcing its aquaculture potential. Rev. Aquaculture, 8, 227-263.

Acknowledgements

This study was funded by ReproF1 Project (Programa Operacional Mar2020, MAR-16-02-01-FMP-0059), CONDISOLE (CeiMar funds, CEIJ-005 awarded EF), ODORACID Project (PTDC/BIA-BMA/30262/2017), and Portuguese national funds (FCT-Foundation for Science and Technology) through project UIDB/04326/2020.

APPROACHES TO ENHANCE SPERM PRODUCTION AND QUALITY IN SENEGALESE SOLE

E. Fatsini[1*], P. Gayo[2], M. Manchado[2], C. Berbel[2], C. Oliveira[1], C. Anjos[1], F. Félix[1], R. Zerolo[3], E. Cabrita[1]

[1]Center for Marine Sciences-CCMAR, University of Algarve, 8005-139 Faro, Portugal. effernandez@ualg.pt
[2]IFAPA Centro El Toruño, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
[3]CUPIMAR, Ctra. Carraca, s/n, Salina San Juan Bautista, San Fernando, Spain

Introduction

Senegalese sole is a promising species for European aquaculture. However, its production is not yet sustainable since breeders from aquaculture (F1 broodstock), unlike wild breeders, do not spawn naturally. Artificial fertilization and cryopreservation have been identified as potential solutions to counteract reproductive problems, however, the low volume of sperm produced by Senegalese sole males, specially F1 individuals, and the high male variability, make difficult the improvement of this technique (Cabrita et al., 2014). In the present study, several approaches were used to improve sperm quality parameters in F1 individuals trying to answer the following questions: (i) Could the cohabiting between wild and F1 males influence sperm quality?; (ii) Could the type of food affect the sperm quality of F1 males? (iii); Could diet supplementation with Vitamin C and β-Glucan influence sperm quality? Cohabiting has been previously demonstrated to positively influence F1 male reproduction (Fatsini et al., 2020). Related to nutrition, it has been observed that F1 fish fed with fresh food during a long period of time improved reproductive fitness including sperm quality (Martin et al., 2021). Diet supplementation with antioxidants and β-Glucan have positive effects in sperm quality in several fish species (Félix et al., 2021), including Senegalese sole. This study presents valuable results to keep investigating natural sources to improve sperm quality to ameliorate male reproductive performance in Senegalese sole.

Material and methods

A total of 157 male breeders (17 wild = 1657.4 ± 445.7 g; 140 F1 = 1020.6 ± 310.1 g) were randomly distributed in 4 different experimental groups in 8 tanks. These individuals were maintained in an open flow system with natural temperature according to season (11 - 22 ºC) in IFAPA-El Toruño facilities in circular tanks of 12 m^3. A total of 61 F1 females (1336.2 ± 286.6 g) were also added to the different experimental groups to simulate a functional broodstock unit. The fish distribution per group (in duplicate tanks) was as follow: 41 males (17 wild and 24 F1(F1cohab)) for cohabiting experiment fed with fresh feed, 36 F1 males for the fresh feeding group (F1f), 42 F1 males for the dry feeding group (F1d), 38 F1 males fed with dry feed supplemented with VitC (n=18) or with β-Glucan (n=20) (SPAROS lda.). Fish were maintained under these conditions for 6 months before the experiment started. Samplings to collect sperm were performed once a month from March to May. Before handling, fish were anesthetized with 2-phenoxyethanol (300 ppm), sperm was collected, and production and quality evaluated. Sperm was centrifuged to eliminate seminal plasma and possible urine contamination and resuspended with the same volume of Ringer. Sperm motility was evaluated using CASA system (ISAS Software, Proiser), viability was evaluated using PI probe by flow cytometry and DNA fragmentation was assessed by Komet assay technique developed by our group for Senegalese sole. Statistical differences were examined using a Student's t-test and Repeated-Measures ANOVA in SPSS v25.

Results and Discussion

Could the cohabiting between wild and F1 males influence sperm quality?

To respond to this question, sperm characteristics were compared between F1 males (F1f) and F1 cohabiting males (F1cohab) fed with fresh food and wild fish. No differences were observed in the percentage of motile spermatozoa (TM) and in curvilinear velocity (VCL). However, significant differences were found in straight-line velocity (VSL) = Wild (50.9 ± 18.5), F1cohab (48.8 ± 11.9); F1f (42.0 ± 12.0)), where wild fish sperm had significant higher VSL than F1f sperm ($P < 0.0001$) and also F1cohab sperm had significant higher VSL than F1f. No differences were observed in viability nor in DNA fragmentation. These preliminary results suggest that cohabiting might enhance sperm quality in F1 individuals. However, the differences among groups will be higher if the period of cohabitation would be longer, since these individuals were cohabiting for just 6 months before the experiment started. This situation was also observed by Fatsini et al. (2020), where in the first spawning season the fish did not react to the cohabitation influence, showing the importance of the establishment of the proper hierarchy and chemical effect in a specific population.

(Continued on next page)

Could the type of food affect the sperm quality of F1 males?

Comparisons were evaluated between F1 males fed on fresh feed (F1f) *and* F1 males fed with dry feed (F1d). No significant differences were observed in spermatozoa TM, VCL or VSL. No differences were observed in viability and DNA fragmentation, showing a tendency similar to motility parameters. These preliminary results propose that dry food might substitute fresh food in terms of sperm quality parameters. However, Martin et al. (2021) observed that feeding Senegalese sole juveniles with just fresh food significantly improved the reproductive performance of these animals at breeder stage.

Could diet supplementation with Vitamin C and β-Glucan influence sperm quality?

Differences were evaluated between F1 males fed on dry food and F1 males fed with diet supplemented with vitamin C (F1vitC) or β-Glucan (F1bgluc). No significant differences were observed in spermatozoa TM, VCL or VSL, although a slight tendency was observed for higher motility parameters in supplemented trials. No differences were observed in viability, showing the same tendency than motility parameters. The results suggest that these diets can be promising if provided periodically to potentially improve sperm quality in F1 males. This was previously observed with the administration of Vitamin E in Senegalese sole breeder's feeds (Beirão et al., 2015). More studies need to be conducted to improve supplementation efficiency in male reproductive performance.

Acknowledgments

This study was funded by projects EBB "EAPA-501/2016" and ERANET-BLUEBIO COFUND "BestBrood-CI2020-111994/AEI/10.13039/501100011033".

References

Cabrita, E., Martínez-Páramo, S., Gavaia, P. Riesco, M., Valcarce, et al. (2014). Factors enhancing fish sperm quality and emerging tolos for sperm analysis. Aquaculture 432, 389-401.

Fatsini, E., González, W., Ibarra-Zatarain, Z., Napuchi, J., Duncan, N. (2020). The presence of wild Senegalese sole breeders improves courtship and reproductive success in cultured conspecifics. Aquaculture 519, 734922.

Martín, I., Riesco, M., Chaves-Pozo, E., Rodríguez, C., Martínez-Vázquez, J.M., et al. (2021). Natural feed after weaning improves the reproductive status of Solea senegalensis breeders. Aquaculture 530, 735740.

Félix, F., Oliveira, C., Cabrita, E. (2021). Antioxidants in fish sperm and the potential role of melatonin. Antioxidants 10(1), 36.

Beirão, J., Soares, F., Pousão-Ferreira, P., Diogo, P., Dias, J., Dinis, M.T., Herráez, P., Cabrita, E. (2015). The Effect of enriched diets on Solea senegalensis sperm quality. Aquaculture 435, 187-194.

APPROACHES TO ENHANCE SPERM PRODUCTION AND QUALITY IN SENEGALESE SOLE

E. Fatsini[1*], P. Gayo[2], M. Manchado[2], C. Berbel[2], C. Oliveira[1], C. Anjos[1], F. Félix[1], E. Cabrita[1]

[1]Center for Marine Sciences-CCMAR, University of Algarve, 8005-139 Faro, Portugal. effernandez@ualg.pt
[2]IFAPA Centro El Toruño, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain

Introduction

Senegalese sole is a promising species for European aquaculture. However, its production is not yet sustainable since F1 broodstocks, unlike wild breeders, do not spawn naturally. Artificial fertilization and cryopreservation have been observed to be prospective solutions to counteract reproductive problems, however, the low volume of sperm produced by Senegalese sole males, specially F1 individuals, and the high male variability, difficult the improvement of this technique (Cabrita et al., 2014). In the present study, several approaches were used to improve sperm quality parameters in F1 individuals trying to answer the following questions: (i) Could the cohabitation among wild and F1 males influence sperm quality?; (ii) Could the type of food affect the sperm quality of F1 males? (iii); Could diet supplementation with Vitamin C and β-Glucan influence sperm quality? Cohabitation has been previously demonstrated to influence positively F1 male reproduction (Fatsini et al., 2020). Related to nutrition, it has been observed that F1 fish fed with fresh food during a long period of time improved reproductive fitness including sperm quality (Martin et al., 2021). Diet supplementation with antioxidants and β-Glucan have positive effects in sperm quality in several fish species (Félix et al., 2021), including Senegalese sole. This study presents valuable results to keep investigating natural sources to improve sperm quality to ameliorate male reproductive performance in Senegalese sole.

Material and methods

A total of 157 male breeders (17 wild = 1657.4 ± 445.7 g; 140 F1 = 1020.6 ± 310.1 g) were randomly distributed in 4 different experimental groups in 8 tanks. These individuals were maintained in an open flow system with natural temperature according to season (11 - 22 ºC) in IFAPA-El Toruño facilities in circular tanks of 12 m^3. A total of 61 F1 females (1336.2 ± 286.6 g) were also added to the different experimental groups to simulate a functional broodstock unit. The fish distribution per group was as follow: 41 males (17 wild and 24 F1) for cohabitation experiment fed with fresh food, 36 F1 males for the fresh food feeding group, 42 F1 males for the dry food feeding group, 38 F1 males fed with dry food supplemented with Vit. C (n=18) or with β-Glucan (n=20) (SPAROS lda.). Fish were maintained under these conditions 6 months before the experiment started. Samplings to collect sperm were performed once a month from March to May. Before handling, fish were anesthetized with 2-phenoxyethanol (300 ppm), sperm was collected, and production and quality evaluated. Sperm was centrifuged to eliminate seminal plasma and possible urine contamination and resuspended with the same volume of Ringer. Sperm motility was evaluated using CASA system (ISAS Software, Proiser), viability was evaluated using PI probe by flow cytometry and DNA fragmentation was assessed by Komet assay technique developed by our group for Senegalese sole. Statistical differences were examined using a Student's t-test and Repeated-Measures ANOVA in SPSS v25.

Results and Discussion

Could the cohabitation among wild and F1 males influence sperm quality?

For this purpose, comparisons were made among F1 males (F1f) and F1 males (F1cohab) cohabiting with wild fish, all being fed with fresh food. No differences were observed in the percentage of motile spermatozoa (TM) and in curvilinear velocity (VCL). However, significant differences were found in straight-line velocity (VSL) = Wild (50.9 ± 18.5), F1cohab (48.8 ± 11.9); F1f (42.0 ± 12.0)), where wild fish sperm had significant higher VSL than F1f sperm ($P < 0.0001$) and F1cohab sperm. No differences were observed in viability nor in DNA fragmentation. These preliminary results suggest that cohabiting might enhance sperm quality in F1 individuals. However, the differences among groups might be higher if the period of cohabitation would be longer, since these individuals were cohabiting for just 6 months before the experiment started. This situation was also observed by Fatsini et al. (2020), where in the first spawning season the fish did not react to the cohabitation influence, showing the importance of the establishment of the proper hierarchy and chemical effect in a specific population.

(Continued on next page)

Could the type of food affect the sperm quality of F1 males?
Comparisons were evaluated between F1 males fed on fresh food *vs* F1 males fed with dry food. No significant differences were observed in spermatozoa TM, VCL or VSL. No differences were observed in viability and DNA fragmentation, showing the same tendency than motility parameters. These preliminary results propose that dry food might substitute fresh food in terms of sperm quality parameters. However, Martin et al. (2021) observed that feeding Senegalese sole juveniles with just fresh food significantly improved the reproductive performance of these animals at breeder stage.

Could diet supplementation with Vitamin C and β-Glucan influence sperm quality?
Differences were evaluated between F1 males fed on dry food and F1 males fed with diet supplemented with vitamin C (F1vitC) or β-Glucan (F1bgluc). No significant differences were observed in spermatozoa TM, VCL or VSL, although a slight tendency was observed for higher motility parameters in supplemented trials. No differences were observed in viability, showing the same tendency than motility parameters. The results suggest that these diets can be promising if provided periodically to potentially improve sperm quality in F1 males. This was previously observed with the administration of Vitamin E in Senegalese sole breeder's feeds (Beirão et al., 2015). More studies need to be conducted to improve supplementation efficiency in male reproductive performance.

Acknowledgments

This study was funded by projects EBB "EAPA-501/2016" and ERANET-BLUEBIO COFUND "BestBrood-CI2020-111994/AEI/10.13039/501100011033".

References

Cabrita, E., Martínez-Páramo, S., Gavaia, P. Riesco, M., Valcarce, et al. (2014). Factors enhancing fish sperm quality and emerging tolos for sperm analysis. Aquaculture 432, 389-401.
Fatsini, E., González, W., Ibarra-Zatarain, Z., Napuchi, J., Duncan, N. (2020). The presence of wild Senegalese sole breeders improves courtship and reproductive success in cultured conspecifics. Aquaculture 519, 734922.
Martín, I., Riesco, M., Chaves-Pozo, E., Rodríguez, C., Martínez-Vázquez, J.M., et al. (2021). Natural feed after weaning improves the reproductive status of Solea senegalensis breeders. Aquaculture 530, 735740.
Félix, F., Oliveira, C., Cabrita, E. (2021). Antioxidants in fish sperm and the potential role of melatonin. Antioxidants 10(1), 36.
Beirão, J., Soares, F., Pousão-Ferreira, P., Diogo, P., Dias, J., Dinis, M.T., Herráez, P., Cabrita, E. (2015). The Effect of enriched diets on Solea senegalensis sperm quality. Aquaculture 435, 187-194.

SHORT-TERM EFFECT OF A LOW PROTEIN HIGH CARBOHYDRATE DIET ON GLUCOSE AND LIPID METABOLISM IN THE LIVER OF MATURE FEMALE AND MALE, AND NEOMALE RAINBOW TROUT

N. Favalier*, H. Li, M. Marchand, A. Surget, P. Maunas, N. Turonnet, S. Panserat and L. Marandel

University of Pau and Pays de l'Adour, INRAE, E2S UPPA, NUMEA, Saint-Pée-sur-Nivelle, France
E-mail : lucie.marandel@inrae.fr

Introduction

Aquaculture and more specifically carnivorous fish farming, such as trout production, is highly dependent on fish derived products such as fish meal (FM) and fish oil (FO) as sources of proteins and lipids, respectively, in the aquafeed formula. However, FM and FO rely on fishing from wild stocks, which can be seen as a hindrance to the sustainable development of salmonid farming [1]. This applies in particular to broodstocks, which are large animals that consume large amounts of feed. Carbohydrates represent good candidates for the replacement of FM [2]low polluted and nutrient rich high quality artificial feeds. Like terrestrial animals around 40 essential nutrients are required by the aquatic organisms which includes protein, carbohydrate, fatty acids, vitamins, minerals, growth factors and other energy sources essentially for maintaining growth, reproduction and other normal physiological functions. The variation in the nutritional requirements can be identified with warm water or cold water, finfish or shell fish and marine water or freshwater species. Successful production of good quality fishes can be achieved by feeding the fishes with nutritionally balanced feeds. The nutritional requirements of various fish species are fulfilled by a different animal and plant based artificial feeds. Standardization of feeding method is another innovative way for preserving sustainable production of aquatic organisms in cages, ponds and short seasonal tanks. Ideal fish protein concept is also the superlative advance towards maximizing the effective utilization of protein by the fishes through the production of cost efficient, nutritionally high and low polluted feeds.","language":"en","page":"7","source":"Zotero","title":"An overview on significance of fish nutrition in aquaculture industry","author":[{"family":"Prabu","given":"E"},{"family":"Felix","given":"S"},{"family":"Felix","given":"N"},{"family":"Ahilan","given":"B"},{"family":"Ruby","given":"P"}]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} . However, rainbow trout is considered as a poor user of digestible carbohydrates [3]. This is linked to several hypothesis such as the non-inhibition of the production of endogenous glucose through gluconeogenesis or the poor induction of *de novo* lipogenesis [4,5]if ever, regulated by carbohydrates, suggesting that this metabolic pathway is involved in this specific phenotype. In this study, we hypothesized that the fate of duplicated genes after the salmonid-specific 4th whole genome duplication (Ss4R when fed a high carbohydrate diet. If they have been deeply explored in juveniles, data concerning broodstock remained scarce. In this context, this study aimed to evaluate the consequences of feeding rainbow trout broodstocks with a high carbohydrate diet for two days on glucose and lipid metabolism.

Results

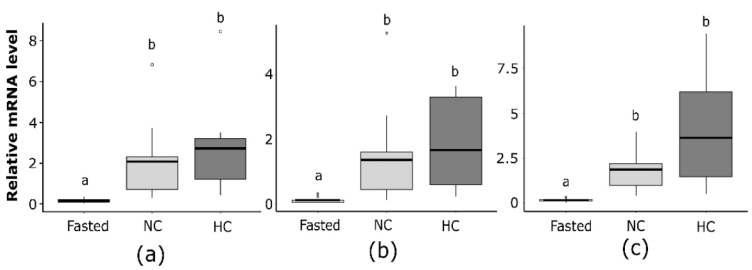


Figure 1 : Illustration of the up-regulation of the *de novo* lipogenesis with *aca alpha a* mRNA levels in (a) mature female, (b) mature male and (c) neomale 15 days fasted (Fasted, white) and then refed with either the NC (non-carbohydrate diet, grey) orthe HC diet (High-carbohydrate diet, dark grey). Data (n=9 fish per condition) were analysed with a Kruskal Wallis test, followed by a post hoc Tukey test in case of significant difference ($p<0,05$, indicated with different letters).

(Continued on next page)

Materials and methods

In the present study, we analysed the three sex commonly used in aquaculture as broodstocks, female, male and neomale that are for the latter masculinized females used to produce all-female lines. Mature female and male, and neomale rainbow trout were fed for two days with a low protein high carbohydrate diet (32% carbohydrates, 42% proteins - HC) or a diet containing no carbohydrates (0% carbohydrates, 66% proteins - NC).Six hours after the last meal, we analysed plasma metabolites, mRNA levels and enzymatic activities of glucose and lipid metabolism-related actors. All these parameters were studied in the liver that is the key organ for the regulation of intermediary metabolism.

Results

Results demonstrated that the glucose metabolism was regulated at the molecular level by the nutritional status in all sex irrespective of the diet composition. Glycolysis was up-regulated in fed neomales and females with an increase of mRNA levels of glucokinase and phosphofructokinase encoding genes as well as an increase in the enzymatic activity of the pyruvate kinase. Concerning gluconeogenesis only glucose-6-phosphatase coding genes were regulated by the nutritional status irrespective of diet composition with the repression of *g6pcb1b* and the induction of *g6pcb2a*. However, no differences in enzymatic activities were highlighted for this pathway. Finally, concerning the lipid metabolism, our results clearly demonstrated for neomales and males the up-regulation of the *de novo* lipogenesis and the down-regulation (example of *aca alpha-a*, figure 1) of the beta-oxidation,while only an up-regulation of the *de novo* lipogenesis occurred in females.

Discussion and conclusion

The present study investigated the glucose and lipid metabolism at the molecular level in mature female and male, and neomale rainbow trout fed for two days with a NC diet or a HC diet. The results obtained for the glucose metabolism highlighted an activation of the glycolysis pathway by the nutritional status in all sex irrespectively of the diet. The up-regulation of the *de novo* lipogenesis was also demonstrated in fed animals. These data point out that carbohydrate intake during a short period (5 meals) does not induce specific metabolic changes after two days of feeding in mature female and male, and neomale. Moreover, we demonstrated for the first time sex differences regarding the effect of two days feeding on metabolism, especially with neomales displaying more differences in the regulation of the glucose and lipid metabolism than male and female broodstock. Such differences may be explained by the fact that neomales are still immature fish. In conclusion, there is no negative effect of feeding mature female and male, and neomale rainbow trout with a high carbohydrate diet for a short period of time.

References

1. Naylor, R.L.; Hardy, R.W.; Buschmann, A.H.; Bush, S.R.; Cao, L.; Klinger, D.H.; Little, D.C.; Lubchenco, J.; Shumway, S.E.; Troell, M. A 20-Year Retrospective Review of Global Aquaculture. *Nature* **2021**, *591*, 551–563, doi:10.1038/s41586-021-03308-6.
2. Prabu, E.; Felix, S.; Felix, N.; Ahilan, B.; Ruby, P. An Overview on Significance of Fish Nutrition in Aquaculture Industry. 7.
3. Polakof, S.; Panserat, S.; Soengas, J.L.; Moon, T.W. Glucose Metabolism in Fish: A Review. *J Comp Physiol B* **2012**, *182*, 1015–1045, doi:10.1007/s00360-012-0658-7.
4. Marandel, L.; Seiliez, I.; Véron, V.; Skiba-Cassy, S.; Panserat, S. New Insights into the Nutritional Regulation of Gluconeogenesis in Carnivorous Rainbow Trout (*Oncorhynchus Mykiss*): A Gene Duplication Trail. *Physiological Genomics* **2015**, *47*, 253–263, doi:10.1152/physiolgenomics.00026.2015.
5. Panserat, S.; Skiba-Cassy, S.; Seiliez, I.; Lansard, M.; Plagnes-Juan, E.; Vachot, C.; Aguirre, P.; Larroquet, L.; Chavernac, G.; Medale, F.; et al. Metformin Improves Postprandial Glucose Homeostasis in Rainbow Trout Fed Dietary Carbohydrates: A Link with the Induction of Hepatic Lipogenic Capacities? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2009**, *297*, R707–R715, doi:10.1152/ajpregu.00120.2009.

IS THERE A MELATONIN PROTECTIVE EFFECT DURING GILTHEAD SEABREAM SPERM CRYOPRESERVATION?

F. Félix*, R. Antunes, L. Leoni, C.C.V. Oliveira, E. Cabrita

CCMAR, University of Algarve, Campus de Gambelas, ed 7, 8005-139 Faro, Portugal
* Corresponding author ffmelo@ualg.pt

Introduction

Melatonin is widely present in nature and is commonly known for the regulation of circadian rhythms (Falcón et al., 2010). For many years, it was thought to be produced only by the pineal organ, but both autocrine and paracrine mechanisms of action are already described in different organs (Félix et al., 2021). Its important role in fish gonad maturation have been studied in fish, providing evidences of antioxidant capacity (Maitra and Hasan, 2016). Therefore, our hypothesis is that melatonin antioxidant properties may provide additional protection to sperm during cryopreservation, reducing oxidative stress. Cryopreservation has been used as a tool to help in artificial reproduction in many aquaculture species. Since these protocols for gilthead seabream (*Sparus aurata*) are well stablished (Cabrita et al., 2005), we aim to understand the effect of endogenously produced melatonin by night at the spermatozoa quality level and its effect as a supplemented antioxidant on a cryopreservation medium.

Methodology

For this experiment, gilthead seabream broodstock was acquired from a semi-intensive aquaculture, Aqualvor (Portugal) and maintained at the Ramalhete station at the University of Algarve. Fish had a mean body weight of 520 ± 14g and were kept under a controlled short photoperiod of 8h light: 16h darkness, in order to simulate the normal environmental conditions of the reproductive season (Bromage, 2001). Sperm samples were collected by abdominal massage at two different points, mid light (ML) and mid dark (MD) (6 males at each point), avoiding any contamination, and kept at 4ºC until analysis. Afterwards, a cryopreservation protocol developed by our group was used (Cabrita et al., 2005), supplemented with melatonin (MEL) to evaluate its protection role. According to the available literature, three different MEL concentrations were chosen: 0.001 mM, 0.01 mM and 0.1mM melatonin, together with a control group without MEL. Sperm motility and concentration were assessed using a Computer Assisted Sperm Analysis software (CASA) to check potential differences in fresh and cryopreserved sperm quality between day and night. On a second experiment, cell viability and DNA fragmentation, by Comet assay, were tested (N=8) as described by Cabrita et al. (2005) for gilthead seabream sperm. For cell viability, samples were diluted 1:100 in 1% NaCl and a mix of Propidium Iodide (PI) and SYBR-green dyes were used to stain non-viable and viable cells, respectively. Three photos of different fields were taken per sample, and at least 100 cells per field were counted under a fluorescent microscope (Nikon E200, Tokyo, Japan) using the "cell counter" feature from ImageJ (Java) software. Results were expressed as percentage of viable cells. Statistical analysis was performed using SPSS software (IBM). Data that assumed normality and homogeneity of variance was analyzed with a T-test or One-way ANOVA, followed by a Student–Newman–Keuls (SNK) *post-hoc* test to identify statistical differences between groups. Mann-Whytney or Kruskal-Wallis nonparametric tests were respectively applied to data that did not assume the above mentioned principles.

Results

Results from day and night cryopreservation experiment revealed that cell concentration at MD was higher than ML, and all motility parameters analyzed, total motility (TM), progressive motility (PM), curvilinear velocity (VCL), straight line velocity (VSL) and linearity (LIN), revealed to be influenced by melatonin. Although TM in fresh samples from ML and MD points did not differ, all cryopreserved treatments, including the control only with DMSO, had higher TM and VCL values at nighttime. Regarding DNA fragmentation, no differences were observed between treatments in Tail DNA (%), but Olive Tail Moment (OTM) was consistently higher at ML.

Discussion

The present results can be explained by the potential antioxidant activity of melatonin (Mironczuk-Chodakowska et al., 2018) when supplemented in the cryoprotectant medium, as described by Gao et al. (2019) for paddlefish (*Polyodon spathula*) sperm. However, the fact that control group also revealed an increase in TM and VCL at MD may suggest that the higher levels of melatonin endogenous production at night (Reiter, 1991) can have an important role in spermatozoa protection, especially during cryopreservation (Len et al., 2019). Further research is needed in order to understand this melatonin mechanism of action (Zhao et al., 2019) and either if it is produced locally in the testis or absorbed by the blood stream (Acuna-Castroviejo et al., 2007; Falcón et al., 2010).

(Continued on next page)

Acknowledgments

This work is part of a PhD programme funded by Portuguese national funds from FCT through the grant SFRH/BD/148280/2019 to F.F and contract DL 57/2016/CP1361/CT0007 to C.C.V.O. The work was funded through projects UIDB/04326/2020 and PTDC/CVT-CVT/4109/2020 (FCT, SpermAntiOx), ASSEMBLE+ JRA2-H2020-INFRAIA-2016-2017 (No 730984), EBB-EAPA_501/2016 (Interreg Atlantic Area) and CCMAR/Multi/04326/2021 (FCT).

References

Acuna-Castroviejo, D., Escames, G., Rodriguez, M.I., Lopez, L.C., 2007. Melatonin role in the mitochondrial function. Front. Biosci. 12, 947-963.

Bromage, N., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture. 197, 63-98.

Cabrita, E., Robles, V., Rebordinos, L., Sarasquete, C., Herraez, M.P., 2005. Evaluation of DNA damage in rainbow trout (Oncorhynchus mykiss) and gilthead sea bream (Sparus aurata) cryopreserved sperm. Cryobiology. 50, 144-153.

Falcón, J., Migaud, H., Munoz-Cueto, J.A., Carrillo, M., 2010. Current knowledge on the melatonin system in teleost fish. Gen. Comp. Endocrinol. 165, 469-482.

Félix, F., Oliveira, C.C.V., Cabrita, E., 2021. Antioxidants in Fish Sperm and the Potential Role of Melatonin. Antioxidants. 10, 36.

Gao, Y., Yang, C., Huang, C., Zhang, G., Ji, H., Dong, W., 2019. The effects of melatonin supplement on paddlefish (*Polyodon spathula*) sperm quality and ATP content in sperm during *in vitro* storage. Aquaculture. 503, 475-482.

Maitra, S.K., Hasan, K.N., 2016. The Role of Melatonin as a Hormone and an Antioxidant in the Control of Fish Reproduction. Front. Endocrinol. 7, 38.

Mironczuk-Chodakowska, I., Witkowska, A.M., Zujko, M.E., 2018. Endogenous non-enzymatic antioxidants in the human body. Adv. Med. Sci. 63, 68-78.

Reiter, R.J., 1991. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr. Rev. 12, 151-180.

Zhao, D., Yu, Y., Shen, Y., Liu, Q., Zhao, Z., Sharma, R., Reiter, R.J., 2019. Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. Front. Endocrinol. 10, 249.

SULFATE REMOVAL BY NANOMEMBRANE FILTRATION REDUCES THE RISK OF BIOFILTER-RELATED HYDROGEN SULFIDE INCIDENTS IN RAS

P.M. Fernandes *[1], S.L. Aalto [2], H.Ø. Åsnes [1], P. Rojas-Tirado [1], Å. Åtland [1], and C. Letelier-Gordo[2]

* corresponding author (paulo.fernandes@niva.no)
[1] NIVA, Aquaculture section, Thormøhlensgate 53D, NO-5006 Bergen, Norway
[2] DTU Aqua, Technical University of Denmark, Section for Aquaculture, The North Sea Research Center, P.O.Box 101, DK-9850 Hirtshals, Denmark

Introduction

In recent years, hydrogen sulfide (H_2S) incidents leading to acute and severe fish mortality has been identified as one of the most important challenges for marine, land-based recirculating aquaculture systems (RAS). It has been demonstrated that the highest potential for H_2S production in RAS is in saline environments with high sulfate (SO_4^{2-}) content (Letelier-Gordo et al., 2020) and in RAS biofilters with the higher sulfate-reducing microbial activity (Rojas-Tirado et al., 2021). Other factors can also play an important role in H_2S incidents, such as organic matter and nitrate availability. Therefore, it is important to find solutions that reduce the risk of H_2S -related incidents in RAS. The presence, level and availability of sulfate is still one of the key drivers of H_2S-related incidents. One of the options to prevent H_2S formation is to remove sulfate from the seawater intake via nanomembrane filtration to use in saline-environment RAS.

Material & Methods

A nanofiltration membrane setup (Hydronautics, Nitto-Group Company, USA) was installed at a commercial salmon smolt farm in Vestland county (Norway) to remove the SO_4^{2-} ions of the seawater intake. The farm was operated at 15-17 ppt and with a feed loading of 1.3-2.2 kg feed/m^3 of make-up water. At continuous and constant operation, the membrane maintained the SO_4^{2-} levels in the farm water at 102 mg SO_4^{2-}/L. The sulfate content of the local seawater varies between 2181-2830 mg SO_4^{2-}/L. This means that, before mixing with freshwater to a salinity of 17 ppt, the constant operation of the membrane had the capacity to decrease sulfate by 10 to 14 times.

Mature RAS nanofiltered water, freshwater and seawater, and mature moving bed biofilter elements (BTW 15, Biowater Technologies, USA) were obtained from the farm only after two consecutive weeks of membrane operation without significant downtime. 400 mL of biofilter elements (ca. 278 biomedia elements) were incubated in closed glass bottles with 2 L of nanomembrane filtered water (n =3) or 2 L of unfiltered water (n = 3) to a salinity of 15 ppt. Initial sulfate levels in the filtered water (102 mg SO_4^{2-}/L) were 10-fold lower than in the unfiltered water (1060 mg SO_4^{2-}/L).

Water samples were collected from each reactor every second day and analyzed for O_2, pH, temperature, ORP (oxy-redox potential), total sulfide and H_2S, sCOD (dissolved chemical oxygen demand), NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}. All reactors were spiked with 180 mg acetate/L on day 27 to maximize H_2S production. The trial lasted 42 days. TCOD (total chemical oxygen demand) from water and biomedia, and microbial samples from biomedia and water of each reactor were collected at the start (day 0) and end of the trial (day 42).

Results

Data analysis is ongoing, and we expect to present the results during the conference.

References

Letelier-Gordo, C.O., Aalto, S.L., Suurnäkki, S., Pedersen, P.B. (2020) Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. Aquacultural Engineering, 89: 102062.

Rojas-Tirado, P., Aalto, S.L., Åtland, Å., Letelier-Gordo, C. (2021). Biofilters are potential hotspots for H_2S production in brackish and marine water RAS. Aquaculture, 536: 736490.

ZOOTECHNICAL PERFORMANCE OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FED PLANT-FEEDSTUFFS-BASED DIETS PRE-TREATED WITH CARBOHYDRASES PRODUCED BY SOLID-STATE FERMENTATION OF BREWER'S SPENT GRAIN

Fernandes, H[1,2*]; Castro, C[1,2]; Quinzico, I.[1]; Salgado, J.[3]; Moyano, F.[4]; Ferreira, P.[1]; Oliva-Teles, A.[1,2]; Belo, I.[3]; Peres, H.[1,2]

[1*]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre Ed. FC4, 4169-007 Porto, Portugal
[2]CIMAR/CIIMAR-Centro Interdisciplinar Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos 4450-208 Matosinhos, Portugal
[3] CEB – Centre of Biological Engineering of University of Minho, Campus de Gualtar 4710-057 Braga, Portugal
[4] Dpto. Biología y Geología, CEIMAR, University of Almería, Almería

Corresponding author: helenafernandes012@gmail.com

Introduction

Plant feedstuffs (PF) have non-starch polysaccharides (NSP) that may negatively impact fish growth, feed utilization and nutrients digestion (Kokou & Fountoulaki, 2018). Given the increasing utilization of PF in fish diets as replacers of fishmeal, innovative strategies need to be considered to increase PF nutritional value. One of such strategies is the use of carbohydrases to digest NSP (Castillo & Gatlin, 2015). This work aims to assess the potential of using carbohydrases produced by solid-state fermentation (SSF) of brewer's spent grain (BSG), either directly incorporated in the diets or used to pre-treat the diet PF mixture, on growth, feed utilization, body composition and digestive enzymes activities of European seabass (*Dicentrarchus labrax*) juveniles.

Materials and Methods

BSG was fermented (SSF) with *Aspergillus ibericus* (MUM 03.49) for 7 days, with 75% moisture and at 25 °C. Then, an aqueous extraction was carried out in the fermented product (5 ml distilled water/g fermented BSG) and the enriched-carbohydrases extract was lyophilized. Five isonitrogenous (50% crude protein) and isolipidic (18% crude lipids) diets were formulated to contain 25% fishery products and 55.4% PF: a control diet (with no carbohydrases); two diets similar to the control but inclusing 0.4% (BSG4) and 0.8% (BSG8) lyophilized SSF-carbohydrases, corresponding to 4000 and 8000 U cellulase/kg diet, respectively (28230 and 56460 U xylanase/kg, respectively); two diets similar to the control but with the PF-mixture pre-treated (4 h, 45 °C, 40% moisture) with the lyophilized SSF-carbohydrases in sodium citrate buffer (0.05 N, pH 4.8) at the concentration of 4000 (PreBSG4) and 8000 U cellulase/kg PF (PreBSG8). European seabass (*Dicentrarchus labrax*) juveniles (IBW 21.5±1 g) were randomly distributed in 18 groups (18 fish/group). The trial lasted 66 days and fish were fed twice a day, 6 days a week. At the end of the trial, fish were bulked weighed and body composition (n=3) and activities of intestinal digestive enzymes (n=9) measured. All experimental diets were compared to the control diet (Dunnett's test). Two-way-Anova was carried out with BSG levels (0.4% and 0.8%) and incorporation method (IM) (direct or as pre-treatment) as factors.

Results

Compared to the control, fish fed the BSG8 diet showed higher growth, although the PreBSG4 diet also showed similar values but with no significant differences. Further, FI was lower while PER was higher in fish fed both pre-treated diets, and FE was higher in the PreBSG4 group. BSG levels did not affect growth performance and feed utilization of the fish, while pre-treatment of the PF improved PER and NR (% NI) compared to groups fed the diets directly supplemented with the extract. Whole-body dry matter and lipid content were also affected by the inclusion method of SSF-carbohydrases at 0.8%, as BSG8 group presented higher values than PreBSG8 group. Energy body content was similar among groups. Amylase, lipase, and trypsin activities were not affected by diet composition. Total alkaline proteases (TAP) from the 0.4% groups exhibited higher activities when fish were fed the preBSG4 diet.

(Continued on next page)

Table 1: Growth performance and specific digestive enzymes activities (mU mg^{-1} protein) of European seabass fed the experimental diets.

	Control	BSG4	BSG8	PreBSG4	PreBSG8	SEM
IBW (g)	23.0	23.0	22.9	22.9	23.0	0.02
FBW (g)	61.7	61.6	62.8	63.3	60.8	0.61
WG (g kg ABW^{-1} day^{-1})	13.8	13.8	14.1*	14.2	13.7	0.12
DGI[1]	1.68	1.67	1.72*	1.73	1.65	0.02
FI (g kg ABW^{-1} day^{-1})	19.9	18.2	18.9	17.6*	18.0*	0.28
FE[2]	0.70	0.76	0.75	0.80*	0.76	0.01
PER[3]	1.38	1.54	1.53	1.77*	1.64*	0.04
Mortality (%)	0.0	0.0	1.9	0.0	0.0	0.37
TAP	81.4	52.5^{A}	82.4	80.3^{B}	67.2	4.33
Amylase	37.2	44.6	48.8	41.4	43.9	2.49
Lipase	4.11	4.69	5.14	3.36	4.91	0.27
Trypsin	42.1	45.8	47.8	38.8	46.3	2.43

Two-way ANOVA**

Factors	BSG level	IM	BSG level x IM
IBW	0.616	0.773	0.521
FBW	0.934	0.698	0.302
WG	0.998	0.759	0.253
DGI	0.997	0.766	0.251
FI	0.160	0.328	0.696
FE	0.264	0.351	0.592
PER	0.248	**0.015**	0.338
Mortality	0.347	0.347	0.347
TAP	0.399	0.524	**0.035**
Amylase	0.570	0.493	0.884
Lipase	0.109	0.211	0.371
Trypsin	0.479	0.527	0.676

Mean ± standard error mean (SEM), n=3. Two-way-Anova (Fixed factors): BSG levels (0.4% and 0.8%); incorporation method (IM) (direct incorporation or pretreating PF prior inclusion in diets). *Denotes significant differences $p<0.05$ between control diet and each test diet (Dunnett test).

**Two-way ANOVA-excluding the control diet (Sig. differences at $p<0.05$). If interaction was significant, one-way ANOVA was performed for each factor, and means in the same line with different small or capital letters indicate significant differences ($p<0.05$) between both supplementation levels (0.4% or 0.8%) or incorporation method (IM: BSG vs PreBSG), respectively; means with no letters are not significantly different ($p\geq0.05$).

IBW, initial body weight; FBW, final body weight; ABW, average body weight; DGI, Daily growth index; FI, feed intake; FE, feed efficiency; PER, protein efficiency ratio. TAP, total alkaline proteases.

ABW: (IBW + FBW)/2 ; [1]DGI: ((FBW1/3 - IBW1/3)/time (days)) × 100 ; [2]FE: wet weight gain/dry feed intake ; [3]PER: wet weight gain/crude protein intake ; [4]NR = ((FBW × carcass N content) - (IBW × carcass N content))/(ABW × the number of days) ; [5]Energy retention = ((FBW × carcass energy content) - (IBW × carcass energy content))/(ABW × the number of days).

Conclusions

The use of SSF-carbohydrases promoted the growth and feed efficiency of European seabass juveniles compared to fish fed the control diet. Direct supplementation of 0.8% SSF-carbohydrases in the diet promoted growth performance but did not affect feed efficiency, while pre-treatment of PF with 0.4% SSF-carbohydrases promoted growth performance and decreased feed intake, thus improving feed efficiency.

Acknowledgements: supported by PhD grant by FCT ref. SFRH/BD/131219/2017, IJFCT-POCI-01-0145-FEDER-030377 and MAR-02.01.01-FEAMP-0111

References

Castillo, S., & Gatlin, D. M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. *Aquaculture*, *435*, 286–292. https://doi.org/10.1016/j.aquaculture.2014.10.011

Kokou, F., & Fountoulaki, E. (2018). Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. *Aquaculture*, *495*(October), 295–310. https://doi.org/10.1016/j.aquaculture.2018.06.003

HOW DO NOVEL FEED FORMULATIONS AFFECT GROWTH PERFORMANCE, OXIDATIVE STRESS AND IMMUNE RESPONSE OF ATLANTIC SALMON?

A.M. Fernandes[1,2], L.E.C. Conceição[1], J.A. Calduch-Giner[3], G.V. Pereira[1], G. Micallef[4], P. Siriyappagouder[2], B.D. Glencross[5], F. Naya-Català[3], M.C. Piazzon[3], A. Sitjà-Bobadilla[3], J. Johansen[6], J. Pérez-Sánchez[3] and J.M.O. Fernandes[2]

[1]Sparos Lda, Olhão, Portugal
[2]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
[3]Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain
[4]Gildeskål Research station AS, Inndyr, Norway
[5]Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK
[6]Norwegian Institute of Bioeconomy Research, Bodø, Norway
E-mail: anafernandes@sparos.pt

Introduction

The aquaculture industry continues to grow faster than any other sector of food production. The need to make aquaculture as sustainable and more environmentally conscious as possible is becoming clearer everyday (FAO, 2020). With this in mind, the replacement of fishmeal and fish oil in aquafeeds has been studied in Atlantic salmon (*Salmo salar*) (e.g., Bendiksen et al., 2011) with many products emerging as potential alternatives to conventional ones (e.g., Hodar et al., 2020). One of the main objectives of the EU project GAIN is to evaluate new ingredients that are already commercially available using different formulation concepts that consider all the fish nutritional needs. GAIN diets are based on circular economy principles and maximize resource efficiency, while contributing to zero waste in the agri-food value chain, being cost-effective feeds, and having good social acceptability. The present study aims to understand the actual effects of these novel feed formulations on growth performance, nutritional status, immunity and oxidative status.

Methods

Quadruplicate groups of Atlantic salmon were fed *ad libitum* with three different diets. Two diets were developed to facilitate the eco-intensification of aquaculture through increased circularity and resource utilization (NOPAP - formula without processed animal protein - and PAP - formula with processed animal protein). The third diet was a commercial-like formulation that was used as a control. After a 96-day feeding trial, plasma samples were collected to evaluate humoral parameters (protease, anti-protease, bactericidal activity, and IgM). Liver and head kidney tissues (collected at day 45 and 96) were used for the simultaneous profiling by PCR array of a panel of 38 or 28 genes, respectively, as markers of growth performance, lipid and energy metabolism, and immune and antioxidant activities. Liver samples were also used to analyse lipid peroxidation. In addition, after 45 and 96 days, the lice count and fish welfare were also assessed by standard methods. The dorsal skin and foregut were collected at days 45 and 96 for mucosal mapping (mucous cell area, density, and barrier status).

Results

Growth performance was adequate and comparable to commercial standards for the novel diets tested. Other parameters analysed, including those related to key performance indicators, intestinal and skin dorsal mucosal mapping, plasma innate immune defences, and lipid peroxidation in the liver did not significantly differ across diets. Regarding head kidney gene expression, at Day 45, 2 out of 28 genes in the array were differentially expressed ($p<0.05$). Gene expression of fish fed with novel feed formulations showed a pro-inflammatory profile evidenced by the up regulation of *il-8,* and a down regulation of *il-10*.At Day 96, the same genes continued to be differentially expressed, but gene *clec1b* (membrane protein) was also up-regulated. However, the rest of the analyses do not support this pro-inflammatory profile. A longer trial may bring light to some of the current results. In turn, the liver had a differential gene expression only at the second sampling point (Day 96), where 4 out of 38 genes in the array were affected, including growth performance (*igf2*), lipid metabolism, elongases (*elovl4*), and energy metabolism (*ucp2l* and *sirt1*). These transcriptomic changes may be attributed to an initial response to the experimental diets. Cross-analysis of gene expression by time points and dietary treatment (two-way ANOVA) yielded only 2 out of 38 genes that had significantly different expression across treatments. The differentially expressed genes were related to growth performance (i*gf2*) and lipid metabolism (*elovl4*).

(Continued on next page)

Conclusions

The novel feed formulations of the GAIN project for Atlantic Salmon seem to be viable options for the near future. In any case, all results are related to the formulation itself and cannot be attributed to a specific ingredient alteration. More studies are necessary to understand the cost-benefit of these new formulations and their market acceptability to optimize sustainability within the current/predictable European regulatory framework.

Acknowledgments:

This project was financed by the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773330 (GAIN), with additional support from Nord University (Norway) and SPAROS Lda (Portugal).

References

Bendiksen, E. Å., Johnsen, C. A., Olsen, H. J., & Jobling, M. (2011). Sustainable aquafeeds: progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar L.*). Aquaculture, 314(1–4), 132–139.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action.

Hodar, A. R., Vasava, R. J., Mahavadiya, D. R., & Joshi, N. H. (2020). Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: A review. J. Exp. Zool. India, 23(1), 13–21.

A MULTIPARAMETRIC TOOL FOR SCREENING AND IMPROVING THE USE OF ALTERNATIVE RAW MATERIALS IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS

Francisco J. Toledo-Solís[1,2*], Andrea G. Hilerio-Ruíz[1], Francisca P. Martínez[1], Abel Barrios[4], María J. Aznar[1], Ana M. Larrán[5], Ignacio Fernández[5], Francisco J. Moyano[1]

[1]Department of Biology and Geology, University of Almeria, 04120 Almeria, Spain
[2]Consejo Nacional de Ciencia y Tecnología (CONACYT), Av. Insurgentes Sur 1582, Alcaldía Benito Juárez, 03940 Mexico City, Mexico
[3]Laboratorio de Acuicultura Tropical, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, Villahermosa, Mexico
[4]Unidad de Cultivos Herbáceos, Agro-Technological Institute of Castilla y León (ITACyL), Ctra. De Burgos Km. 119, Finca Zamadueñas, 47071 Valladolid, Spain
[5]Aquaculture Research Center, Agro-Technological Institute of Castilla y León (ITACyL), Ctra. Arévalo, 40196 Zamarramala, Segovia, Spain
Email: fermonig@itacyl.es

Introduction:
Aquaculture long-term sustainability has been deeply questioned since it requests increasing amounts of fish oil and meal (FO and FM, respectively; limited resources) as main raw materials for aquafeeds. During the last decades, a large effort has been focused to identify alternative raw materials (ARMs; e.g. soybean, insect, algae and single cell's meal, animal by-products meal, plant-derived oils, microalgae and krill oils, etc.) to substitute FO and FM (reviewed in Turchini et al., 2018). Nowadays, soybean meal (SBM) is still one of the main ARMs currently used in commercial fish diets. However, it is imported in some regions (e.g. Europe). Thus, the identification of locally produced crops able to partially or totally substitute SBM is an urgent need to reduce not only the dependency of SBM from third countries, but also to reduce the carbon footprint.

A two-step screening protocol, including a first set of in vitro assays followed by in vivo test, is proposed to evaluate different protein sources of vegetable origin locally produced in Europe as more sustainable sources for rainbow trout (*Oncorhynchus mykiss*) diets.

Materials and Methods:
Experimental design: Different meals obtained from cultivars of Narbone vetch (*Vicia narbonensis*), in addition to red vetchling (*Lathyrus cicera*) and green pea (*Pisum sativum*) meals (NVM, RVM and PM, respectively) were tested as alternatives to the use of soybean (*Glycine max*) meal (SM). In a first approach, this selection was based on nutritional, economical and environmental factors. Afterwards, a complementary characterization of the nutritional aspect of each ARM was done, including: amino acid profile, buffering capacity, soluble protein content, etc. Also, the presence and their stability/activity of some nutritionally limiting factors (e.g. non-starch polysaccharides, phytate) as well as their *in vitro* digestibility after treatment with an exogenous enzyme was evaluated. In a second step, the most promising ARM to partially replace soy protein concentrate (SPC) in rainbow trout diets was evaluated *in vivo*.

Five experimental diets (iso-nitrogenous, -lipidic and -energetic) were formulated: one diet with FM and SPC as main protein sources (Control), and 4 diets were SPC was partially replaced by 33% or 66% of NVM (the selected ARM) previously treated with exogenous enzyme Rovabio® phytase (A33E and A66E) or not (A33 and A66). Thirteen fish (38.04 ± 0.07 g and 15.10 ± 0.07 cm) per 500 L tank were randomly allocated. Diets were tested in triplicate (15 tanks connected to recirculating aquaculture systems (RAS)). Fish were daily hand-fed (3% of daily feed intake) during 63 days. Rearing conditions were: 14.5 ± 0.3 °C, >8.0 mg/L of dissolved oxygen, and 12:12-h light:dark cycle. All procedures were approved by the Bioethical Committee of ITACyL (Nº 2018/31/CEEA).

Fish sampling, growth performance and histopathological assement: Final body wet weight (FBW) and furcal length (FFL), weight gain (WG), condition factor (CF), SGR, FCR, viscerosomatic (VSI) and hepatosomatic indexes (HSI) were calculated.

Statistical analysis: Results are given as mean values ± standard deviations. Data was checked for normality (Kolmogorov–Smirnov test) and homoscedasticity of variance (Bartlett's test). Results were compared by means of one-way ANOVA and Tukey or Dunnett´s multiple-comparison test. The level of significance was set at $p < 0.05$.

(Continued on next page)

Results and Discussion:
ARMs showed a lower buffer capacity (81.63 to 95.09 μmol H+ per (g * ΔpH)) than SM (248.69 μmol H+ per (g * ΔpH); ANOVA, $P < 0.05$). Alkaline proteases inhibition was higher (47 to 69% of inhibition) in all of them when compared to that of SM (24.18%; ANOVA, $P < 0.05$). Protein solubility was variable in all ARMs (from 2.69 to 4.60 mg/g), while in SM it was 3.76 mg/g. Regarding the presence of phytate, while NVM showed similar content to the one reported in SM, RVM and PM had less than the 50% of the phytate present in SM. While NVM and SM had similar content in phenolic compounds (1.3 and 1.2 g/kg) RVM and PM had lower content (0.8 and 0.9 g/kg). Furthermore, total soluble phosphorus was also lower in RVM and PM than in NVM and SM. Based on these results, NVM autoclaved or not was selected for additional *in vitro* characterization.

Enzymatic pre-treatment of NVM autoclaved or not and SM was able to remove all the phytate, and increased the availability of pentoses and reducing sugars. In not autoclaved NVM, the amount of reducing sugars was increased by 5 times with the enzymatic pre-treatment. Autoclaving is a process that already reduce the presence of phytate in NVM, but it is expensive an unpractical for large quantities. Thus, not autoclaved NVM was selected to partially replace SM in the *in vivo* assay to validate this screening tool.

As shown in Table 1, results of the growth performance trial suggested that 33% of SPC can be substituted by NVM when previously treated with exogenous enzyme. Thus, present screening tool might help to identify/optimize the use of ARMs in fish diets.

References: Turchini et al., 2018. *North Am. J. Aquac. 81*, 13–39.

Acknowledgements: Work funded by "Optimización integral de los sistemas productivos en acuicultura: revalorización de materias primas locales en piensos y en cría de especies en potencial desarrollo (OPTI-ACUA)" project - ERDF. F.J. T-S. acknowledges CONACYT post-doc fellowship No. 2019–000012-01EXTV-00292. I.F. acknowledges Ramón y Cajal (Ref. RYC2018-025337-I) contract MICIU-AEI-European Social Fund.

EXPRESSION OF GENES INVOLVED IN FAT-SOLUBLE VITAMINS METABOLISM ARE ADAPTED TO DIETARY CONTENT IN RAINBOW TROUT *Oncorhynchus mykiss* DIETS

Ignacio Fernández[1*], Fátima Vicente[1], Francisco J. Toledo-Solís[2,3], Silvia Beato[1], Francisco J. Alarcón[3], Juan B. Ortiz[4], Carmen Sarasquete[4], Pedro de la Cuesta[5], Ana M. Larrán[1]

[1]ARC-ITACyL, Ctra. Arévalo, 40196 Zamarramala, Spain
Email: fermonig@itacyl.es
[2]Consejo Nacional de Ciencia y Tecnología, Av. Insurgentes Sur 1582, 03940 Mexico City, Mexico
[3]Department of Biology and Geology, University of Almería, 04120 Almería, Spain
[4]ICMAN/CSIC, Campus Universitario Río San Pedro, 11510, Puerto Real, Cádiz, Spain
[5]Lab. físico-química-ITACyL, Ctra. Burgos Km. 119, Finca Zamadueñas, 47071 Valladolid, Spain

Introduction:
Fat-soluble vitamins (FSVs) play essential roles in vertebrate's development and homeostasis. Thus, efficient and sustainable fish farming deeply depends on the optimization of their dietary levels (Hamre et al., 2013). Although their nutritional minimum requirements have been fixed (at least in salmonids; NRC 2011) through unifactorial nutritional-dose-response trials (Fernández et al., 2018), little is known on their metabolism. Furthermore, the different FSVs have genes in common involved on the processes of assimilation and transport from intestine to liver and target tissues (Fernández et al., 2018). Therefore, how these genes are modulated in response to dietary FSVs contents through a multifactorial and integrative manner, might help to further develop precision fish farming and defining fully nutritionally balanced diets.

Here, growth performance, histopathological status of the digestive system, liver FSV's content and the expression of genes involved in the FSVs metabolism were explored in rainbow trout (*O. mykiss*) juveniles fed with diets containing different levels of FSVs.

Materials and Methods:
Experimental design: All procedures were approved by the Bioethical Committee of ITACyL (Nº 2018/35/CEEA). Nine experimental diets (isonitrogenous, isolipidic and isoenergetic) were specifically formulated to contain: the reference content on vitamin A (VA), D (VD), E (VE) and K (VK) (NRC 2011) and/or 10 or 0.2 times the reference content of each vitamin. Twenty-one fish (94.04 ± 0.81 g mean weight and 20.28 ± 0.09 cm) per 500 L tank were randomly allocated. Experimental diets were tested in triplicate (27 tanks connected to recirculating aquaculture systems (RAS), 9 tanks per RAS unit). Fish were daily hand-fed (3% of daily feed intake) during 90 days. Rearing conditions were: 14.5 ± 0.3 °C, >8.0 mg/L of dissolved oxygen, and 12:12-h light:dark cycle.

Fish sampling, growth performance and histopathological assessment: Final body wet weight (FBW) and furcal length (FFL), weight gain (WG), condition factor (CF), SGR, FCR, viscerosomatic (VSI) and hepatosomatic indexes (HSI) were calculated. In addition, 3 fish from each tank were randomly sampled and sacrificed with an overdose of MS222 to dissect liver and intestine to perform histological, HPLC/DAD and gene expression analyses. Samples for histological analyses were fixed in 10% buffered paraformaldehyde (pH 7.4) during 24 h, dehydrated, embedded in paraffin blocks, sectioned and stained with Haematoxylin-Eosin or Alcian blue periodic acid-Schiff (PAS) solutions. Standard histomorphometric analyses were conducted (Gisbert et al., 2008; Silva et al., 2015).

HPLC/DAD analysis: FSVs content in diets and livers (from 6 fish per dietary group) were extracted, concentrated and quantified using an Agilent HPLC/DAD 1200 equipment.

Expression analysis of genes involved in fat-soluble vitamins metabolism: Total RNA was extracted with TRI-Reagent from 6 fish (2 from each tank) from each dietary group. 1 μg of total RNA was reverse-transcribed, and gene expression was quantified using SsoFast EVAgreen Supermix (Bio-Rad) in a StepOnePlus Real-Time PCR system. The studied genes were previously identified in Fernández et al. (2018).

Statistical analysis: Results are given as mean values ± standard deviations. Data was checked for normality (Kolmogorov–Smirnov test) and homoscedasticity of variance (Bartlett's test). Results were compared by means of one-way ANOVA and Tukey multiple-comparison test. The level of significance was set at $P < 0.05$.

(Continued on next page)

Results and Discussion:
Only slight differences in particular growth performance indexes were found after 90 days of feeding (Table 1). Increased dietary VD content induced a higher condition factor, while reduced VE content lead lower SGR and HSI. While lower dietary VK content decreased SGR, HSI and VSI; and increased VK content decreased condition factor, SGR and HSI.

FSVs content in fish livers reflected that of experimental diets. FSVs dietary content has also affected histopathological status of the digestive system, particularly at the proximal intestine. High dietary VA, VE and VK content increased goblet cells density when compared to low VA, VE and VK, respectively. Fish fed high dietary VK content also have thicker muscular and serosa layers compared to that of fish fed on low VK content.

Finally, we are currently studying how is the expression of some genes involved in FSVs metabolism accordingly to the dietary content included in fish diets, and results will be discussed. These results show how dietary FSVs content altered fish growth and physiology, brings new insights on how their metabolism is adapted and key knowledge on biomarkers to design fully nutritionally balanced diets regarding FSVs.

References:
Fernández et al., 2018. In "*Emerging Issues in Fish Larvae Research*" 159–208.
Gisbert et al., 2008. *Histol. Histopathol. 23*, 1525–1539.
Hamre et al., 2013. *Rev. Aquac.* 5(Suppl. 1), S26–S58.
NRC 2011. *Nutrient Requirements of Fish and Shrimp*. The National Academies Press.
Silva et al., 2015. *Aquaculture 445*, 42–56.

Acknowledgements: MET2VI project (RTI2018-099029-A-I00) funded by ERDF-MICIU-AEI and Ramón y Cajal (RYC2018-025337-I) contract from MICIU and European Social Funds "The European Social Fund invests in your future" from Spain; and CONACYT postdoc fellowship (2019–000012-01EXTV-00292) from México.

FISH MEAL SUBSTITUTION BY *Tenebrio molitor* MEAL IN RAINBOW TROUT *Oncorhynchus mykiss* DIETS AFFECTS FAT-SOLUBLE VITAMINS METABOLISM: IMPLICATIONS FOR FUTURE FORMULATIONS

Fátima Vicente[1*], Federico Melenchón[1], Cristina Tomás-Almenar[1], Blanca Martín[1], Francisco J. Alarcón[2], Valentín Pérez[3], Pedro de la Cuesta[4], Ana M. Larrán[1], Ignacio Fernández[1]

[1]Aquaculture Research Center, Agro-Technological Institute of Castilla y León (ITACyL), Ctra. Arévalo, 40196 Zamarramala, Segovia, Spain
Email: fermonig@itacyl.es
[2]Department Biology and Geology, Ceimar-University of Almería 04120 Almería, Spain
[3]Universidad de León, Campus de Vegazana, s/n, 24071 León, Spain
[4]ITACyL, Ctra. de Burgos Km. 119, Finca Zamadueñas, 47071 Valladolid, Spain

Introduction:
Due to the limited availability of fish meal and oil from wild fisheries, aquaculture sustainability largely depends on the implementation of alternative raw materials in aquafeeds. Among the different alternatives considered, insects have recently attracted the attention as suitable alternatives (Nogales-Mérida et al., 2018). Different species of insects have been evaluated, but we recently found mealworm (*Tenebrio molitor*; TM) as the most promising to replace fish meal up to 30% (Melenchón et al., 2021). Different reports have shown how high levels of fish meal substitution with insect meal might impact fish physiology, but the nutritional causes are not fully understood.

Here, we assessed the effects on fish growth, histopathological status on the digestive system, the content of fat-soluble vitamins (FSVs) and the related gene expression when substituting 50% of fish meal with TM meal (either defatted or not) in rainbow trout (*Oncorhynchus mykiss*) diets.

Materials and Methods:
Experimental design: All procedures were approved by the Bioethical Committee of ITACyL (Nº 2018/35/CEEA). Three experimental diets (isoproteic and isolipidic) were specifically formulated with fish meal (Control), fish meal 50% substituted with defatted TM meal (TD50) and fish meal 50% substituted with TM meal (T50), all of them supplemented with an equal amount of mineral and vitamin premix and tested in triplicates. Twenty-one fish (46.2 ± 0.21 g mean weight and 15.9 ± 0.12 cm) per 500 L tank were randomly allocated in 9 tanks connected to a recirculating aquaculture system (RAS). Fish were daily hand-fed to apparent satiation (up to 3% of daily feed intake) during 68 days. Rearing conditions were: 14.5 ± 0.3 °C, >8.0 mg/L of dissolved oxygen, and 12:12-h light:dark cycle.

Fish sampling, growth performance and histopathological assessment: Final body wet weight (BW) and furcal length (FL), WG, CF, SGR, FCR, viscerosomatic (VSI) and hepatosomatic indexes (HSI) were calculated. In addition, 3 fish from each tank were sacrificed to dissect liver and intestine to perform histological, HPLC/DAD and gene expression analyses. Samples for histology were fixed in 10% buffered paraformaldehyde (pH 7.4) during 24 h, dehydrated, embedded in paraffin blocks, sectioned and stained with Haematoxylin-Eosin or Alcian blue periodic acid-Schiff (PAS) solutions. Standard histomorphometric analyses were conducted (Gisbert et al., 2008; Silva et al., 2015).

HPLC/DAD analysis: FSVs content in diets and livers (from 6 fish per dietary group) were extracted, concentrated and quantified using an Agilent HPLC/DAD 1200 equipment.

Expression analysis of genes involved in fat-soluble vitamins metabolism: Total RNA was extracted with TRI-Reagent from 6 fish (2 from each tank) from each dietary group. 1 μg of total RNA was reverse-transcribed, and gene expression was quantified using SsoFast EVAgreen Supermix (Bio-Rad) in a StepOnePlus Real-Time PCR system. The studied genes were previously identified in Fernández et al. (2018).

Statistical analysis: Results are given as mean values ± standard deviations. Data was checked for normality (Kolmogorov–Smirnov test) and homoscedasticity of variance (Bartlett's test). Results were compared by means of one-way ANOVA and Tukey multiple-comparison test. The level of significance was set at $P < 0.05$.

(Continued on next page)

Results and Discussion:
Only fish fed with T50 diet showed significant differences when compared with the Control group, particularly with reduced body weight and weight gain as well as increased feed conversion rate (Table 1; ANOVA, $P < 0.05$).

No major impacts on the histopathological status of the digestive system have been found among the fish fed the different experimental diets. Only a slightly increased width of the serosa layer in fish fed T50 diet when compared with fish fed Control diet was found (ANOVA, $P < 0.05$), with TD50 group showing intermediate values.

Significant differences were found in the FSVs content in fish livers (ANOVA, $P < 0.05$). In particular, total vitamin A was only significantly reduced in fish fed TD50 diet when compared with the one of fish from Control group, with fish fed T50 diet showing intermediate values. In contrast, independently of being defatted or not, a 50% fish meal substitution by TM meal led to reduced vitamin K levels in fish liver.

Finally, the expression of some genes involved in FSVs metabolism is being studied and results will be presented. Altogether, present results show that although TM meal can partially replace fish meal in rainbow trout diets, dietary FSVs content should be corrected in the premix in order to avoid any potential physiological impact of their deficiency.

References:
Fernández et al., 2018. In "*Emerging Issues in Fish Larvae Research*" 159–208.
Gisbert et al., 2008. *Histol. Histopathol. 23*, 1525–1539.
Melenchón et al., 2021. *Aqua. Nut.* 27, 491–505.
Nogales-Mérida et al., 2018. *Rev. Aqua. 11*, 1080–1103.
Silva et al., 2015. *Aquaculture 445*, 42–56.

Acknowledgements: MET2VI project (RTI2018-099029-A-I00) funded by ERDF-MICIU-AEI; Ramón y Cajal (RYC2018-025337-I) contract from MICIU-ESF; INSECTMEAL (RTA2015-00021-C03) from INIA-FEDER; pre-doc fellowship (BES2017-080567) from ESF; and CONACYT postdoc fellowship (2019–000012-01EXTV-00292) from México.

ECONOMIC IMPACT OF KEY OPERATIONAL FACTORS IN THE EUROPEAN INDUSTRY OF SEA BASS AND SEA BREAM FARMING

José L. Fernández Sánchez*, José M. Fernández-Polanco, Ignacio Llorente, Manuel Luna, Elisa Baraibar-Diez, María D. Odriozola-Zamanillo and Ladislao Luna Sotorrío

IDES Research Group, Faculty of Economics and Business Administration, University of Cantabria, Avda. de los Castros 56, 39005 Santander (Spain)
*E-mail: jluis.fernandez@unican.es

Introduction

European sea bass (*dicentrarchus labrax*) and gilthead sea bream (*sparus aurata*) are both an economically important cultured fish species along the Mediterranean coast. In 2018 the two species represented a 38% of the total value of the European aquaculture sector (STECF, 2021) ranking the second and third species respectively after the Atlantic salmon. The EU is one of the largest producers of sea bass and sea bream in the world, being Greece the largest producer within the EU followed by Spain. Aquaculture of sea bass and sea bream is an industry with a high economic competitiveness in which the profitability of operations is very important and sensible to different operational factors and production scenarios. Despite of its importance, there are few empirical studies about this topic so far. Hence, it would be very interesting for this industry to analyze how changes in different key operational factors affect the economic performance of production facilities. The aim of this work, which is part of the MedAID project funded by the European Commission (H2020, GA727315), is to analyze these economic effects or impacts in the European sea bass and sea bream aquaculture industry at the micro (farm) level.

Methodology

To carry out this analysis, we have designed a deterministic static model, which we have named MedAID Model for Economic Simulation (MMES), composed of a production sub-model and an economic sub-model (see Figure 1) and programmed with the spreadsheet Excel to simulate the annual income statement of a standard (average) aquaculture farm. The model can be employed to simulate the annual economic results of a grow-out facility for growing up sea bass and sea bream as well as a hatchery facility to culture sea bass and sea bream fry and larvae in the Mediterranean Sea. To obtain the baseline values of the model parameters, we have used data from representative facilities from six European countries (Croatia, Cyprus, France, Greece, Italy, and Spain) collected in a survey conducted in MedAID's WP1.

With the former model, a sensitivity analysis has been applied to identify the most important, highly sensitive, parameters in the model. Thus, we have examined the sensitivity of the baseline model results to different assumptions about some key model parameters such as the unit sales price, the survival rate, the harvested weight, the tank or cage density, the growth rate, the fingerling unit cost, the feed unit cost, or the feed conversion ratio (FCR). Each of these model parameters has been varied one by one maintaining the rest of parameters constant (*ceteris paribus*) by 10%, 20% and 30% above and below its baseline value, what allows us to analyze the impacts of those variations on the indicators of economic performance employed in this research such as the gross operating profit, the net operating profit, the average operating cost, and the break-even point. Four different production scenarios have been designed to run the sensitivity analysis. These scenarios have been chosen based on actual data from different European facilities producing sea bass and sea bream, which have been surveyed in the MedAID project. In Table 1 we summarize the four production scenarios.

Results

The economic impact of model parameters changes shows that the unit sales price (p) is the model parameter with the largest impact for all scenarios (i.e., hatcheries and grow-out facilities), followed by survival rate (s) and harvested weight (w_1) parameters in hatcheries and by survival rate (s), feed unit cost (p_f) and FCR (r) in grow-out facilities. On the other hand, the feed unit cost (p_f) and feed conversion ratio (r) are the model parameters with the lowest impact on profits of a hatchery facility (scenario 1), whereas the harvested weight (w_1) and fingerling unit cost (p_j) are the model parameters with the lowest impact on profits of a grow-out facility (scenarios 2, 3 and 4). All these effects are briefly summarized in Table 2 for a standard hatchery facility (scenario 1) and Table 3 for a standard grow-out facility (scenarios 2, 3 and 4).

References

Scientific, Technical and Economic Committee for Fisheries (STECF). *The EU Aquaculture Sector – Economic report 2020 (STECF-20-12)*. Publications Office of the European Union, Luxembourg, 2021, doi: 10.2760/441510.

ANALYSIS OF THE MAIN IMMUNE GENE BIOMARKERS IN *Ruditapes decussatus* AFTER EXPERIMENTAL INFECTION BY *Perkinsus olseni*

S. Fernández-Boo[1*], A. García[1,2], J. Estêvão[1,2], B. Costas[1,2], A. Cruz[3]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Matosinhos, Portugal.
[2] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), University of Porto, Portugal.
[3]Oceano Fresco S.A., Porto de Abrigo, 2450-075 Nazaré, Portugal.

Email: sboo@ciimar.up.pt

Introduction

The grooved carpet shell clam (*Ruditapes decussatus*) is a highly desirable species in the European market. Its population has been in a constant decline due to biotic and abiotic factors, namely, habitat degradation by anthropogenic actions in the case of abiotic factors but also due to competition with alien species and pathogens in the case of biotic factors. The introduction of the alien species *Ruditapes philippinarum* in the decade of 60´s in France and England constitutes one of the main problems due to habitat loss and competition against a faster growth clam [1], also, some introductions of the alien species from Asia in the 80´s brings the Apicomplexan parasite *Perkinsus olseni* to European waters [2, 3]. This parasite not only provoke mortalities in the native clams but also it is able to impairs their reproduction by decreasing the quality of gametes and survival of the larvae [4].

Taking all these details into account, our study is focused on the effect of different doses of the parasite in the ability of clams to survive and to fight against the disease. Also, some genes previously identify as potentially involved in the response to infection were studied in gill and hemocytes during the progression of the infection.

Material and methods

A total of 540 *R. decussatus* clams (43.7 ± 2.9 mm length) were purchased from the fisheries association of San Bartolomé de Noia (Galicia, NW Spain) a known spot for being *P. olseni* free bed. Clams were transferred to CIIMAR facilities and they were placed in 10 tanks (54 clams per tank) at 19ºC. After that, clams from 9 tanks were notched in the shell and injected in the adductor muscle with 100μL of two different doses of parasites (3 tanks with 5000 cells/clam – Low Infection (LI); 3 tanks with 500,000 cells/clam – High infection (HI)) and 3 tanks with marine filtered sea water (control), also one tank was untouched to check it out the effect of the injection into the clams (C-).

At 24 hours, 48 hours, 1, 2 and 4 weeks after injection, 5 clams per tank (15 per treatment) were sampled. Hemolymph was extracted by a 1mL insulin syringe, centrifuged at 2500 rpm, 10 min and 4ºC to separate hemocytes from plasma. Hemocytes were immediately stored at -80ºC. One piece of gill was collected and placed in 0.5 ml of RNA later (Invitrogen, US) and stored at -80ºC. Also, one hemigill was placed in 10 ml of RFTM medium (Casas & Villalba 2012) for *P. olseni* diagnosis and quantification and other was placed in ethanol 96% for DNA extraction and qPCR diagnosis of the parasite according to Rios, Aranguren [5].

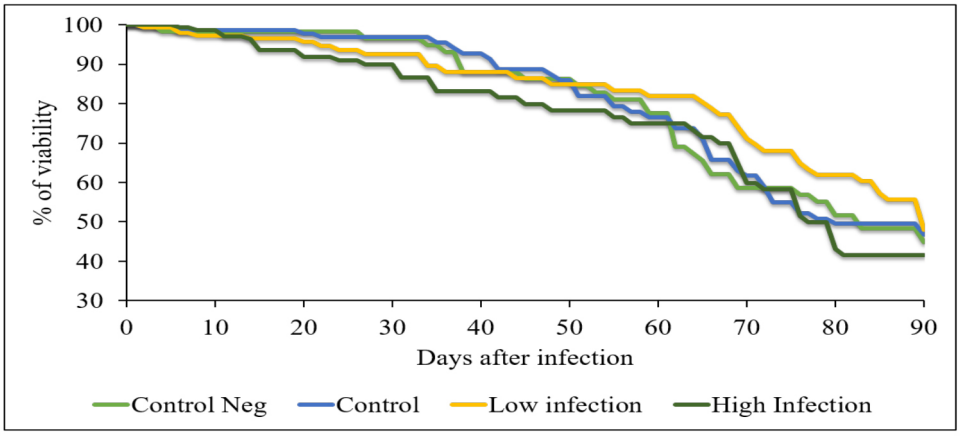


Fig. 1. Mortality curve of clams after infection by two doses of parasites. Y axis represents percentage of survival while x axis represents days after infection.

(Continued on next page)

Results

No differences in mortality was seen among the different treatments during the 3 months of trial (Fig.1). Infection trial was successful and a high correspondence parasite load was obtained by two diagnosis methods: RFTM and qPCR.

Regarding the gene expression results, a high expression was seen in adiponectin in both tissues at all times and infection load, while most of the other genes seem to be down-regulated at first 48 hours to recover after that in gills while no differences were seen in hemocytes.

P. olseni was not able to provoke a high mortality in a small period of time in adult clams although some studies reported high mortalities in juveniles [6]. Regarding the experimental infection, all parasites migrate quickly from adductor muscle to gills and a high parasite load was seen 24 hours post-infection in HI clams suggesting an active infection and confirming the effectiveness of the infection protocol. Also, no differences in mortality were recorded between both control tanks suggesting that notched and injection have not any harmful effect on clams.

Finally, gene expression study shows that adiponectin seems to be the most relevant marker of infection for *Perkinsus* infection in clams.

Acknowledgements

This research was supported by the project Tools4Breed – Challenge test and genetic markers for *Perkinsus* as a tool for *Ruditapes decussatus´* selective breeding with reference FA_05_2017_025 financed by Fundo Azul and República Portuguesa.

Bibliography

1. Cordero, D., et al., *Population genetics of the Manila clam (Ruditapes philippinarum) introduced in North America and Europe*. Sci Rep, 2017. **7**: p. 39745.
2. Vilas, R., Cao, A., Pardo, B. G., Fernández, S., Villalba, A., Martínez, P., *Very low microsatellite polymorphism and large heterozygote deficits suggest founder effects and cryptic structure in the parasite Perkinsus olseni*. Infection, Genetics and Evolution, 2011. **11**: p. 904-911.
3. Azevedo, C., *Fine structure of Perkinsus atlanticus n. sp. (Aplicomplexa, Perkinsea) parasite of the clam Ruditapes decussatus from Portugal*. Journal of Parasitology, 1989. **75**: p. 627-635.
4. Casas, S.M. and A. Villalba, *Study of perkinsosis in the grooved carpet shell clam Ruditapes decussatus in Galicia (NW Spain). III. The effects of Perkinsus olseni infection on clam reproduction*. Aquaculture, 2012. **356-357**: p. 40-47.
5. Rios, R., et al., *Development and validation of a specific real-time PCR assay for the detection of the parasite Perkinsus olseni*. J Invertebr Pathol, 2020. **169**: p. 107301.
6. Shimokawa, J., T. Yoshinaga, and K. Ogawa, *Experimental evaluation of the pathogenicity of Perkinsus olseni in juvenile Manila clams Ruditapes philippinarum*. J Invertebr Pathol, 2010. **105**(3): p. 347-51.

COLD SEAWATER PRE-TREATMENT AFFECTS THE SPERMATOGENESIS AND THE REPRODUCTIVE PERFORMANCE OF MALE EUROPEAN EELS

L. Ferrão[a*], M. Morini[a], V. Gallego[a], A. Felip[b], A. Gómez[b], L. Pérez[a] and J.F. Asturiano[a]

[a] Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain
Email: jfastu@dca.upv.es
[b] Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (IATS), CSIC, Ribera de Cabanes, 12595 Castellón, Spain

Introduction

The European eel (*Anguilla anguilla*) is a highly valued species targeted for aquaculture production. However, it is considered a critically endangered species by CITES, and reproduction in captivity seems to be the only realistic alternative to solve both economic and ecological problems (Asturiano, 2020). It is possible to obtain gametes for artificial fertilization but it requires hormonal treatment, which is a time-consuming and expensive process (Gallego et al., 2012). Moreover, the variable response to these treatments results in high mortality rates during the early stages of development, which could be caused (at least in part) by low sperm quality and uncontrolled epigenetic factors (Herráez et al., 2017). Considering the environmental conditions experienced during eels oceanic migration, the effects of low temperature combined with hormonal treatment have been used to better understand the onset of eel maturation (Peñaranda et al., 2016). Thus, the present study considers the use of low temperature as a pre-treatment before starting a standard hormonal treatment and evaluates its impact on eel maturation and male reproductive performance.

Material and Methods

Eighty-eight male European eels were maintained for 3-4 days in freshwater at 20 ºC. Then, 6 males were sacrificed, as initial control, and the rest were changed to seawater at 10 ºC, except the control group, which was changed to seawater but maintained at 20 ºC. Low water temperature pre-treatments were applied during 1, 2 or 4 weeks to groups of 20 males. Once finished each pre-treatment, 8 fish per group were sacrificed for sampling. The temperature of the water of the remaining males was increased to 20 ºC in 3-4 days and they started receiving standard hormonal treatment (weekly administration of recombinant chorionic human gonadotropin, rhCG; Ovitrelle, Spain, 1.5 IU/g fish). From sacrificed fish (controls and pre-treated) biometric parameters were measured. Blood and testis samples were used to perform steroid (T, 11-KT and E2) analyses and histology, respectively. Once eels were spermiating, sperm volume, motility and kinetic parameters were evaluated weekly using a CASA system.

Results

The eye, fin and hepatosomatic indexes from 1-week, 2- and 4-weeks pre-treated fish were significantly higher than those of the control group. The gonadosomatic indexes registered in the control group were unusually high and significantly differed with 1-week pre-treatment group but not with the 2- and 4-weeks groups. Androgen levels (T, 11-KT) were significantly higher after 4-weeks of pre-treatment in comparison with the control. The E2 levels did not show the same increasing profile. The control and 1-week pre-treated males showed significantly higher percentages of undifferentiated and differentiated type A spermatogonia in comparison with early spermatogonia type B. The males after 2- and 4-weeks of pre-treatment revealed a significantly increased proportion of differentiated spermatogonia type A. After the rhCG treatment, males from the 4-weeks pre-treatment group started to produce sperm earlier than males from the other groups. The sperm volume in the 4-weeks pre-treatment group increased during the first weeks of spermiation and in the control group at the end of the experiment, but no significant differences were found. Sperm motility and kinetics parameters were low in pre-treated groups throughout the whole experiment while in the control group increased significantly at the end of the hormonal treatment.

(Continued on next page)

Discussion

The onset of maturation in the European eel is controlled by environmental conditions found during their migration. The application of pre-treatments can be important to induce maturation in aquaculture eels, which do not encounter these conditions (Palstra and van den Thillart, 2009). Several factors such as temperature, salinity, forced swimming and pressure have been used as part of pre-treatments applied to eels (reviewed by Asturiano et al., 2020). Rozenfeld et al. (2019) evaluated the effects of cold seawater in European eel maturation without hormonal treatment. In eels treated at 10 ºC, the T and 11-KT levels were higher and there was an increase in the proliferation of differentiated type A spermatogonia. Our results also corroborate that low-temperature pre-treatment (4-weeks at 10 °C) induces an early sexual development, thus leading to the release of androgens which promote the proliferation of type A spermatogonia. However, the low sperm quality found in the pre-treated groups revealed that thermal pre-treatment followed by rhCG treatment at high temperatures impairs spermatogenesis. Low-temperature pre-treatments induce testis cell proliferation and synchronization but inhibit further maturation. Peñaranda et al. (2016) suggested that low seawater temperature inhibits the gene expression of enzymes responsible for the synthesis of maturation-inducing steroids (MIS) during eels oceanic migration. Thus, the premature maturation of the testis is prevented before eels arrive to the spawning areas and a temperature threshold is reached allowing the change of the steroidogenic pathway to change from androgens to MIS. In our experiment, low-temperature pre-treated males had a time-dependent and fast response to rhCG treatment (in comparison with control males maintained at 20 °C), but their spermiation period was shorter and their sperm motility parameters were significantly lower. If this is evidencing a lower rate of gene expression in the testis, and thus limiting the final sperm maturation process, is an open question that requires further research.

Bibliography

Asturiano, J.F., 2020. Improvements on the reproductive control of the European eel, in: Yoshida, M., Asturiano, J.F. (Eds.), in: Reproduction in Aquatic Animals: From Basic Biology to Aquaculture Technology. Springer Nature, Singapore, pp. 293-320.

Gallego, V., Mazzeo, I., Vílchez, M.C., Peñaranda, D.S., Carneiro, P.C.F., Pérez, L., Asturiano, J.F., 2012. Study of the effects of thermal regime and alternative hormonal treatments on the reproductive performance of European eel males (*Anguilla anguilla*) during induced sexual maturation. Aquaculture 354-355, 7-16.

Herráez, M.P., Ausió, J., Devaux, A., González-Rojo, S., Fernández-Díez, C., Bony, S., Saperas, N., Robles, V., 2017. Paternal contribution to development: Sperm genetic damage and repair in fish. Aquaculture 472, 45-59.

Palstra, A., van den Thillart, G., 2009. Maturation and Reproduction, in: van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), Spawning Migration of the European Eel - Reproduction Index, a Useful Tool for Conservation Management. Springer, pp. 253-278.

Peñaranda, D.S., Morini, M., Tveiten, H., Vílchez, M.C., Gallego, V., Dirks, R.P., van den Thillart, G.E.E.J.M., Pérez, L., Asturiano, J.F., 2016. Temperature modulates the testis steroidogenesis in European eel. Comp. Biochem. Physiol. A 197, 58–67.

Rozenfeld, C., García-Carpintero, V., Pérez, L., Gallego, V., Herranz-Jusdado, J.G., Tveiten, H., Johnsen, H.K., Fontaine, R., Weltzien, F.A., Cañizares, J., Asturiano, J.F., Peñaranda, D.S., 2019. Cold seawater induces early sexual developmental stages in the BPG axis of European eel males. BMC Genomics 20: 597.

SEAWEED *Gracilaria gracilis* AND MICROALGAE *Nannochloropsis oceanica*, SINGLE OR IN COMBINATION, MODULATE EUROPEAN SEABASS (*Dicentrarchus labrax*) INTESTINAL MICROBIOTA

Mariana Ferreira[1, 2*], Yousri Abdelhafiz[3], Luisa M.P. Valente[1, 2], Viswanath Kiron[3]

[1] CIIMAR, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal
[2] ICBAS, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
*Presenter author: marianaipf@gmail.com

Introduction

The continuous increase in demand for fish puts pressure on the aquaculture industry to find alternatives to the limited fisheries-based dietary protein sources to avoid overexploitation of marine resources. The traditional fish meal is now being successfully replaced with more economically and environmentally sustainable ingredients. In this context, seaweeds and microalgae, the natural sources of nutrients and bioactive compounds, can improve fish growth and overall health. Furthermore, nutritional manipulation of the intestinal microbiota can have a positive impact on fish welfare and nutrition.

The macroalga *Gracilaria gracilis* and microalga *Nannochloropsis oceanica* have been recently explored by the feed industry, but the impact of the inclusion of such products on the gut microbiota of European seabass (*Dicentrarchus labrax*) is poorly understood. We evaluated the impact of seaweed *G. gracilis* and microalga *N. oceanica*, single or blended, on the composition of microbial community in the intestine of European seabass.

Materials and Methods

European seabass (30 g) in triplicate tanks were fed four diets for 106 days at CIIMAR facility (Matosinhos, Portugal): a commercial-based diet (CTRL) and three experimental diets with the inclusion of 8 % *G. gracilis* (GRA), 8 % *N. oceanica* (NAN), or a blend of 4 % of each alga (NANGRA), at the expense of fish meal and wheat meal. At the end of the trial, mucus from the posterior intestine was collected under sterile conditions. After DNA extraction, the V3-V4 region of the 16S rRNA was amplified and sequenced employing an Illumina® MiSeq platform. After quality filtering, taxonomic assignment of the representative bacterial ASVs was performed using the RDP classifier. The R packages "iNEXT" and "phyloseq" were used to calculate alpha diversity and then employing the functions in "ggplot2", plots were generated for overall species richness, Shannon diversity and Simpson diversity. Kruskal-Wallis test followed by Dunn's test was employed to detect significant differences. The package "microbiome" was employed to determine relative abundance of core taxa and then "DESeq2" was used to identify the OTUs that were differently abundant in the study groups.

Results and Discussion

Illumina sequencing reads were assigned to 4,371 ASVs. The inclusion of the seaweed *G. gracilis* and the microalga *N. oceanica* on diets for European seabass had a significant impact on the alpha diversity: species richness was significantly lower in fish that consumed the GRA diet compared to CTRL; while Shannon and the Simpson diversity measures were significantly reduced in fish fed both GRA and NAN diets compared to fish fed CTRL. Neverthless, when algae were included in a blend (NANGRA diet), the alpha diversity measures remained similar to those observed for fish that consumed the CTRL diet.

Core microbial taxa (prevalence and detection thresholds of 90 and 20 %, respectively) of the posterior intestine were composed of the genera *Flavobacterium*, *Parcubacteria* and *Lactobacillus*. The most abundant phyla in the posterior intestine were Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. Compared to the CTRL fish, the inclusion of the tested algae, single or blended, led to a decrease or increase of specific groups of bacteria. The incorporation of the seaweed *G. gracilis* and the microalga *N. oceanica*, single or blended, led to a decrease in Acidobacteria (*Gp4*) and Firmicutes (*Clostridium sensu stricto* and *Exiguobacterium*); and an increase in Bacteroidetes (*Kordia*) and Proteobacteria (*Acinetobacter*). The diet GRA, in particular, led to a decrease in Acidobacteria (*Gp6*), Nitrospirae (*Nitrospira*), Proteobacteria (*Rhizobium*, *Roseateles* and *Sphingobium*) and Verrucomicrobia (*Opitutus*). The diet NAN was associated with a reduction in Bacteroidetes (*Muricauda*) and Proteobacteria (*Ruminobacter*); and an increase in Firmicutes (*Clostridium XI*) and Proteobacteria (*Massilia*). Concerning NANGRA, the decrease in the genera *Opitutus*, *Muricauda* and *Gp6*, and increase in *Massilia* reflected the differences observed with the inclusion of single algae *G. gracilis* or *N. oceanica*. The blend resulted in the decrease of only a single genus (*Pseudomonas*) that belongs to Proteobacteria, which is the predominant phyla in the gut microbiota of different marine fish species, including European seabass.

(Continued on next page)

Conclusion

Overall, results indicate that the inclusion of seaweed *G. Gracilis* and microalga *N. oceanica* leads to a reduction of the gut microbial alpha diversity, while the blend attenuates these effects. Further clarification on the fuction of each specific group of bacteria affected by these dietary treatments will allow for a better undertanding on the impact of the tested algae, single or in combination, on the gut microbiota of European seabass.

Acknowledgements

This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). M. Ferreira acknowledge Fundação para a Ciência e a Tecnologia (FCT) for grant SFRH/BD/144843/2019 (FCT/FSE). The support received from Bisa Saraswathy, Researcher, Nord University, for data analyses is acknowledged.

HISTOMORPHOLOGICAL CHANGES OBSERVED IN EUROPEAN SEABASS (*Dicentrarchus labrax*) INTESTINE FED INCREASING LEVELS OF A MICRO- AND MACROALGAE BLEND

Mariana Ferreira[1,2*], Beatriz Oliveira[1], Vera Sousa[1,2], Cátia Mota[2,3], Luisa M.P. Valente[1,2]

[1] CIIMAR, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal
[2] ICBAS, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3] REQUIMTE, LAQV, ICBAS, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
*Presenter author: marianaipf@gmail.com

Introduction

Current aquafeeds for carnivorous farmed fish species, such as European seabass (*Dicentrarchus labrax*), include moderate to low levels of fish meal and fish oil that are mostly replaced by high levels of plant products (PP). However, such formulations may compromise fish growth and have a negative impact on fish intestinal health. In this context, algae are natural products rich in bioactive compounds that may be included in feeds with high levels of PP to counteract the negative effects of such diets.

Intestinal integrity plays a crucial role in fish health, but detailed studies on the impact of novel formulations on fish intestine histomorphology are time-consuming and scarce. Here we applied both semi-quantitative and quantitative analysis to understand if the inclusion of a micro- and macroalgae blend in practical diets for European seabass provides some beneficial histomorphological changes in the intestine.

Materials and Methods

European seabass juveniles (11 g) were fed for 12 weeks with four diets in triplicate: a commercial-based diet (CTRL), that followed the current tendencies in aquafeeds, with already high levels of vegetable products and moderate levels of fish meal (12.5 %) and fish oil (13.2 %); and three experimental diets with the inclusion of increasing levels (i.e. 2, 4 and 6 %) of a commercial algae blend (blend 2, 4 and 6, respectively). The blend consisted of two microalgae (*Nannochloropsis oceanica* and *Chlorella vulgaris*) and two macroalgae (*Gracilaria gracilis and Ulva rigida*).

At the end of the feeding trial, anterior and posterior intestine samples from 9 fish per treatment (3 fish per tank) were collected and processed according to standard histological techniques. Sections were stained with hematoxylin and eosin for semi-quantitative scoring and Alcian blue/PAS (pH 2.5) for quantitative analysis. Micrographs of one section of each sample were evaluated using a light microscope with a camera. A continuous scale scoring system (1-5) was used to evaluate histomorphological changes in the intestine: submucosa and lamina propria width and cellularity; mucosal folds integrity; inflammatory infiltrates; and enterocytes nucleus position. For the quantitative analysis, Olympus cellSens Dimension Desktop imaging software was used to measure, in each section, the following parameters: cross-sectional perimeter (mm); *muscularis* thickness (μm); submucosa width (μm), lamina propria width (μm); total villus area (mm^2); villus length (μm); and goblet cells (GC) number and GC area. The *muscularis*, submucosa, and lamina propria were measured in eight selected points of each transverse section, and villus length was measured in the eight highest folds. Automatic counting of acid and neutral GC and villus area was performed on each cross-sectional area.

Results and Discussion

After the 12-weeks feeding trial, European seabass fed diets with the algae blend had a significantly higher final body weight and length compared to those fed the CTRL, with the highest values being registered for fish fed the blend 6. A larger perimeter was observed in both cross-sectional anterior and posterior intestinal sections in fish consuming blend 4 and 6 diets, compared to those fed the CTRL. A thicker *muscularis* was also observed in all fish fed the algae blend, but differences were only significant for the posterior intestine. Increased villus area and length were observed in the anterior intestine of fish fed the highest blend inclusion (6 %) compared to CTRL, but not in the posterior section. Such increased absorption area in the anterior part could explain the improved growth performance of those fish.

In both the anterior and posterior intestine, the number of goblet cells (GC) was higher in fish fed blend 6 diet compared to CTRL; the total number of acid GC increased significantly in the posterior intestinal section of these fish. Therefore, the blend diet (6 %) seems to be associated with increased mucus production that could favour nutrient digestibility, while the higher number of acid GC in the posterior intestine could be associated with improved resistance against pathogenic microorganisms.

(Continued on next page)

In the posterior intestine, the enterocytes of fish fed the CTRL showed a slight loss of their architecture, evidencing a minor nucleus displacement from their normal basal position, compared to those from blend 6. Therefore, the inclusion of the algae blend may allow a recovery of the normal intestinal structure often compromised by diets with high levels of plant products. In both anterior and posterior portions, the blend diets led to increased submucosa width, and increased cellularity could also be observed in the posterior intestine. Nevertheless, the level of inflammatory infiltrates remained similar among treatments.

Results indicate that the quantitative analysis could provide accurate information concerning lamina propria, submucosa, and *muscularis* width, as well as villus length. Also, the automatic counting of acid and neutral GC and villus area determination are fast and reliable procedures. However, the semi-quantitative evaluation seems a relevant complementary approach, providing important information regarding gut structure (enterocytes nucleus position) and the presence of inflammatory infiltrates.

Conclusion

Overall, results indicate that inclusion of a micro- and macroalgae blend, especially at the higher inclusion level (6 %), in diets for European seabass, results in some beneficial histomorphological changes in both anterior and posterior intestine that could partially explain the improved growth performance observed in fish fed the supplemented diets. Future studies on the topic of fish gut histomorphology should take advantage of quantitative analysis, coupled with a semi-quantitative approach, to have a deep understanding of the impact of novel diets on intestine structure and function.

Acknowledgements

This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). M. Ferreira and C. Mota acknowledge Fundação para a Ciência e a Tecnologia (FCT) for grants SFRH/BD/144843/2019 (FCT/FSE) and PD/BDE/150585/2020 (FCT/FSE), respectively.

DIFFERENTIAL LOCAL AND SYSTEMIC IMMUNE RESPONSES IN EUROPEAN SEABASS (*Dicentrarchus labrax*) FOLLOWING *Tenacibaculum maritimum* INFECTION

I. A. Ferreira[1,2,3,4*], P. Santos[1,5], M. Machado[1], F. A. Guardiola[1,6], A. do Vale[3,4], B. Costas[1,2]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto (Portugal)
[2]Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[3]Fish Immunology and Vaccinology Group, IBMC-Instituto de Biologia Molecular e Celular, University of Porto, 4200-135 Porto, Portugal
[4]i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal
[5]MARE - Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Peniche, Portugal
[6]Department of Cell Biology and Histology, Faculty of Biology, *Campus Regional de Excelencia Internacional* "*Campus Mare Nostrum*", University of Murcia, 30100, Murcia, Spain
*E-mail: ines.ferreira@ciimar.up.pt

Introduction

One of the most devastating bacterial diseases, associated with high mortality and economic losses, of wild and farmed marine fish is tenacibaculosis, caused by Gram-negative bacterium *Tenacibaculum maritimum* (Avendaño-Herrera *et al*., 2006). Knowledge regarding the pathogenesis of *T. maritimum* is scarce and can only be improved through the identification of the key virulence factors required for host colonization and disease progression and the host-pathogen interactions occurring during infection. The present study was conceived to evaluate European seabass (*Dicentrarchus labrax*) systemic and mucosal immune responses against *T. maritimum* infection. For that, seabass juveniles were bath-infected and changes in blood and plasma parameters as well as in the expression of immune-related genes in skin, distal gut and head-kidney were evaluated.

Materials and methods

A time-course trial was performed, in which groups of seabass (31.9 ± 6.9 g) were bath-challenged for 2 h in aerated seawater with 5 x 10^5 CFU mL^{-1} *T. maritimum* (challenged fish) or marine broth instead of bacteria (mock-challenged fish). Undisturbed fish randomly selected from the groups just before infection were used as controls (time 0). Following 4, 8, 24 and 48 h post-challenge, 8 fish from each treatment were randomly selected, euthanized and head-kidney (HK), distal gut and skin collected for total RNA extraction. RNA was retro-transcribed to cDNA and expression of immune-related genes was analysed by RT-qPCR and normalized with *40s* and *ef1b*. Blood samples were also collected for assessing haematological parameters (i.e., total white and red blood cells counts, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) and for collecting plasma to evaluate innate humoral parameters (i.e., peroxidase, protease, antiprotease, bactericidal and nitric oxide activities). To determine the severity of the challenge, a lethality trial was performed in parallel, using the same bacterial inoculum/ challenge protocol used for the time-course trial.

Results

Challenge with *T. maritimum* induced 40% mortality, whereas no mortality occurred in mock-challenged fish. The number of erythrocytes decreased in the blood of challenged fish at 8 h post-infection, when compared to control (time 0). Regarding leucocyte numbers, the levels of circulating neutrophils in challenged fish increased at 8 h post-challenge compared to control fish and mock-challenged individuals. Lymphopenia was observed at 8 h and 24 h in challenged fish compared to the controls and mock-challenged. However, no significant differences were observed for the remaining evaluated haematological parameters. Similarly, no significant changes were observed among the plasma humoral parameters evaluated. At the transcriptional level, an up-regulation of *il-1β* gene expression was detected in the distal gut and HK tissues at 8 h post-challenge, whereas *il-10* transcripts, were up-regulated at 8 h in the HK and at 24 h in skin and distal gut for the challenged fish. The expression of *mmp9* gene also increased at 8 and 24 h post-challenge in distal gut and skin, respectively. Moreover, *cxcr4* and *il-8* transcripts increased at 8 and 24 h in skin whilst in the HK the response was delayed for *il-8*, with an up-regulation at 48 h for the challenged fish.

(Continued on next page)

Discussion and conclusion

The up-regulation of some innate immune-related genes such as *il-1β*, *il-8*, *mmp9* and *cxcr4* in mucosal tissues (i.e. skin and gut) following *T. maritimum* infection by bath, suggest that a local response was triggered. This immune response can be explained by *T. maritimum* capacity to adhere, colonize and degrade mucosal tissues (Avendaño-Herrera *et al*., 2006). The abrasion and damage in the tissues can subsequently lead to a systemic infection. However, the absence of changes in the plasma innate humoral parameters supports the occurrence of a poor immune response at the systemic level. Nevertheless, it cannot be disregarded that cytokines *il-1β* and *il-10* were up-regulated in the HK as early as 8 h post-challenge, suggesting signs of a response at a systemic level. The neutrophilia in challenged fish is likely due to the migration of these cells to peripheral infected tissues. This interpretation is further supported by the enhancement of *mmp9* and *cxcr4* transcripts in distal gut and skin. The observed lymphopenia may be related with the immune response to *T. maritimum*. An acute challenge, such as an infection, can lead to the redistribution of lymphocytes, from the blood to other body compartments where they are needed (Davis *et al*., 2008), resulting in a decrease in circulating lymphocytes. Further work is required to disclosure the dynamics stablished between the local and systemic immune responses triggered by *T. maritimum* infection, as well as to better understand *T. maritimum* - host interactions.

References

Avendaño-Herrera, R., Toranzo, A. E., Magariños, B. (2006). A challenge model for *Tenacibaculum maritimum* infection in turbot (*Scophthalmus maximus*). Journal of Fish Diseases, 29, 1-4. https://doi.org/10.1111/j.1365-2761.2006.00712.x

Davis, A. K., Maney, D. L., Maerz, J.C. (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology, 22, 760-772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Acknowledgements

This work is partially supported by project BE4AQUAHEALTH (16-02-05-FMP-0013), funded by MAR2020 Operational Programme and the European Union through FEDER, and by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. I. Ferreira, A. do Vale and B. Costas benefited from grants by FCT (SFRH/BD/147750/2019, L57/2016/CP1355/CT0010 and IF/00197/2015, respectively).

DESIGN AND ENGINEERING CONSIDERATIONS TO SELECT MOST SUITABLE FISHFARMING SYSTEM TO MATCH AN OCEANIC WIND FARMMULTI-USE PLATFORM

A. Ferreira*[1,2], M. Ferreira[1,2], C. Navarro[3] and C. Andrade[4,5,6]

[1]CERENA – IST, Instituto Superior Técnico, Torre Sul, Avenida Rovisco Pais 1, 1049-001 Lisboa,Portugal
[2]IST, Instituto Superior Técnico, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal – Senioradvisor
[3]INNOVAKEME - Rua das Murças, n.°71, 3.0 andar, 9000-058 Funchal, Madeira, Portugal
[4]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto,Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208,Matosinhos, Portugal
[5]OOM – ARDITI, Observatório Oceânico da Madeira, Edifício Madeira Tecnopolo, Piso 0, Caminho da Penteada, 9020-105 Funchal, Portugal
[6]CMC, Calheta Mariculture Centre, Avenida D. Manuel I nº7, Vila da Calheta, 9370-135, Madeira,Portugal
Email: abilio.ferreira.2000@gmail.com

Introduction

The use and sharing of open ocean space by different activities of the Blue Economy is regarded by some as means to overcome difficulties and challenges from the high energyand dynamic environment, difficult logistics and cutting on high capital and operational costs. One of the classical examples is the multi-use of space by windfarms and aquaculture. The synergies of systems include the sharing of capital costs regarding mooring system and working platforms, plus a reduction in operational costs resulting from such as logistics and transports, maintenance teams and proximity of energy production – consumption. This presentation deals with the conceptual project OIPS for a multi-use platform of a defined floating windfarm, to be installed North Eastern Porto Santo Island (Portugal), at 70m depth single/double mooring and the choice of compatiblefish farming system, for the most efficient and sustainable production of both energy andfood.

Methods

We performed a internet survey of multi-purpose ocean platform projects associated withfish farming systems. The analysis included an assessment of technology readiness levels(TRL) of the different design systems proposed.

Results and Discussion

Several large concept projects co-financed by European funds have addressed this theme.Assuming an operation in offshore waters, interactions with the local environment and other social-economical activities are minimized and consequently, less stakeholders are involved. The interactive design and engineering of both energy and food production units depends upon the technical efficiency of systems and must have in account particularly, their operational compatibility to maximize and harmonize the outputs of the systems. The availability and quality of environmental data, particularly waves, wind and currents are critical for this purpose. The multi-use energy-producing platform provides for the reserve of energy to guarantee for its autonomy. Electronic sensors, video cameras and an AUV ensure the remote control and high automation of operations (including maintenance works), the online surveillance of the feed and livestock, and security of all facilities. TRLanalysis of similar projects shows the need to develop innovative equipment to cope withthe increment on operating loads of the windfarm platform (permanent fish farm cages and occasional service boat) under the site environmental conditions. These include the adoption of new materials and systems resistant to traction work, with small radius, such as cable and nets *dyneema*®. At low depths, separate mooring systems are advisable; thechoice of a single mooring point on the wind platform, and in a catenary in aquaculture, determines the choice of a heterogeneous type of anchorage.

AQUASENSE—A REAL-TIME PRECISION AQUACULTURE PLATFORM FOR INDUSTRY

Joao G. Ferreira[1,2,+], Rui Gomes Ferreira[1], Joao Lencart e Silva[1], Heather Moore[3], Matt Service[3], Fearghal O'Donncha[4], Roberto Pastres[5], Giulia Micallef[6], John D. Icely[7].

[1]Longline Environment Ltd., 63, St. Mary Axe, London, EC3A 8AA, United Kingdom
[2]DCEA, Faculdade de Ciencias e Tecnologia, NOVA, Qta Torre, 2829-516 Monte de Caparica, Portugal
[3]Agri-Food and Biosciences Institute (AFBI), 18a Newforge Lane, Belfast BT9 5PX, United Kingdom
[4]IBM Research – Ireland, Damastown Ind. Park, Mulhuddart, Dublin 15, Ireland
[5]Dipartimento di Scienze Ambientali, Informatica e Statistica, Ca' Foscari University of Venice, Via Torino 155, 30173, Mestre – Italy
[6]GIFAS, Inndyr, 8140 Inndyr, Norway
[7] Sagremarisco Lda., Apartado 21, 8650-999 Vila do Bispo, Portugal
[+]Corresponding author

Introduction

The EU Horizon 2020 Green Aquaculture Intensification in Europe (GAIN) project aims to promote eco-intensification of aquaculture by investigating several complementary strategies, including feed improvements, better use of secondary products, and precision aquaculture.

The development of precision aquaculture has four requirements: (i) deployment of sensors that supply environmental data on drivers of growth, welfare, disease, and mortality; (ii) detection of measurable response metrics from cultivated species—growth and mortality rates are the most significant; (iii) coupling and interpretation of these input and response data in order to obtain quasi- or real-time information on the cultivation process, providing farmers with meaningful indicators; and (iv) integrated platforms where industry stakeholders can easily access, process, and make use of data and information in order to optimise their activity in terms of production, environmental, and economic outcomes.

We present a blueprint of the AquaSense platform (https://aquasen.se), which was designed to meet these requirements, as part of the GAIN approach to increasing aquaculture yield in Europe by making better and more sustainable use of existing space and resources.

Methodology

AquaSense is a cloud-based platform that enables users to retrieve environmental data acquired using sensors deployed in or near cultivation structures such as cages, longlines, or trestles, and use those data to apply state-of-the-art mathematical models of growth and environmental effects.

AquaSense clients enter (or use stored) data on culture practice, including the duration of the culture period, initial stocking density, and mortality rates (Fig. 1), and the platform retrieves the relevant environmental data from a storage hub. For finfish, water temperature is the main driver of growth, and for bivalves such as mussels or oysters, data on the natural food supply are also retrieved, since theseorganisms are organic extractors and do not require additional feeding.

The well-tested (e.g. Ferreira et al, 2012; Saurel et al, 2014; Cubillo et al, 2016) 'Aqua' series of models (Fig. 2) is used to predict growth and environmental effects for key species grown in Europe, including Atlantic salmon (*Salmo salar*), Rainbow trout (*Onchorhynchus mykiss*), European seabass (*Dicentrarchus labrax*), gilthead bream (*Sparus aurata*), Pacific oyster (*Magallana gigas*), blue mussel (*Mytilus edulis*), Mediterranean mussel (*M. galloprovincialis*), and Manila clam (*Ruditapes philippinarum*).

Apart from growth, AquaFish (Fig. 2) provides outputs on feed ingestion and particulate waste, oxygen consumption, and excretory products.

The AquaSense platform simulates the growth of the selected culture organism based on the culture data and environmental drivers retrieved from *in situ* sensors and scales up the results to the cages (or other structures such as mussel longlines) defined by the farmer.

(Continued on next page)

These results at the farm and individual scale are displayedto the user in graphical form for easy interpretation. Any growth period, starting weight, etc can be simulated by the user.

Results and Discussion

Example results from the application of AquaSense to Atlantic salmon in Norway are shown below (Fig. 3).

In addition to these results, individual performance metrics are also provided, together with growth curves over the culture period (Fig. 4).

The outputs from AquaSense help farmers review how the harvested crop relates to changes in environmental conditions and evaluate the emissions footprint of their activity. The first component is important in terms of optimising production and understanding deviations from expected growth performance, and the second helps farm managers and water authorities assess the sustainability of the activity, particularly in light of co-use of water bodies, source apportionment of nutrient emissions, and legislative compliance.

In order to expand the reach of this system, GAIN has developed an Affiliate Farm Programme (AFP) for both finfish and shellfish farms. Implementation of the AFP will leverage data collection both at the farm and at the broader-scale level, and automatically ensure a clear and valuable legacy for the European aquaculture industry.

In a similar way to the connection of households to the electrical grid, farmers within the AFP will support the installation of relevant sensors, which will require an initial investment of about 10-20 k€, depending on choices, and will then be connected to the AquaSense platform—the incentive is to obtain access to a management system that will help industry stakeholders implement a precision aquaculture model for eco-intensification.

At a later stage, using data available from the Maritime and Environmental Thresholds for Aquaculture (META) platform (https://longline.co.uk/meta/), AquaSense will be extended to support traceability and environmental performance, including animal welfare, e.g. by identifying critical periods for parameters such as dissolved oxygen and water temperature for matches/exceedances to known species thresholds, thus providing consumers with greater confidence with respect to farmed products.

References

Cubillo, A.M., J.G. Ferreira, S.M.C. Robinson, C.M. Pearce, R.A. Corner, and J. Johansen 2016. Role of deposit feeders in integrated multi-trophic aquaculture - a model analysis. Aquaculture, 453, 54-66.

Ferreira, J.G., C. Saurel, J.M. Ferreira, 2012. Cultivation of gilthead bream in monoculture and integrated multi-trophic aquaculture. Analysis of production and environmental effects by means of the FARM model. Aquaculture 358–359 (2012) 23–34.

Saurel, C., J.G. Ferreira, D. Cheney, A. Suhrbier, B. Dewey, J. Davis, J. Cordell, 2014. Ecosystem goods and services from Manila clam culture in Puget Sound: a modelling analysis. Aquaculture Environment Interactions, 5, 255-270.

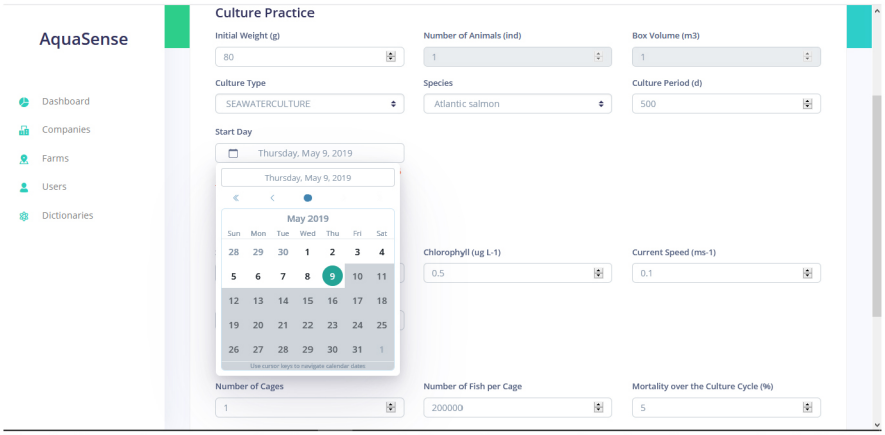


Fig. 1. Input data screen for farmers to define culture practice. AquaSense scales individual performance to the cultivated population.

(Continued on next page)

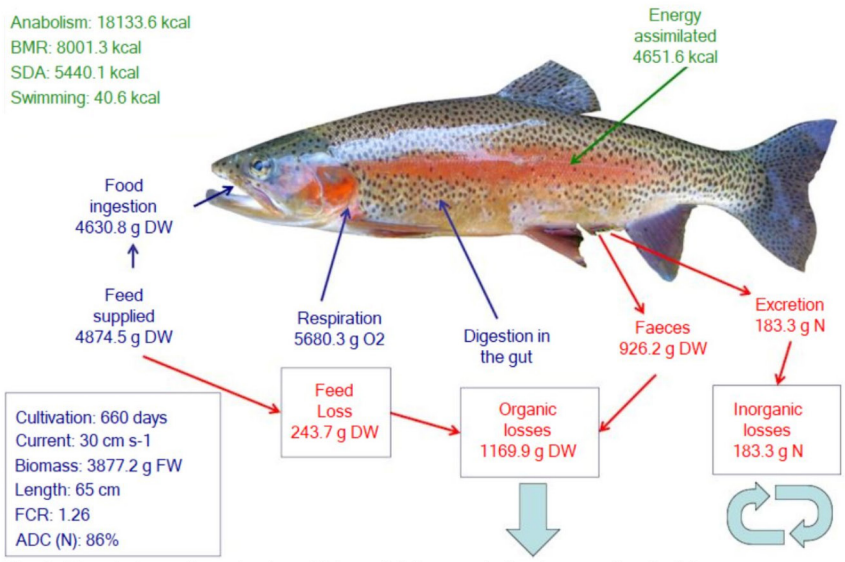


Fig. 2. Mass balance from the AquaFish model for a typical culture cycle of rainbow trout.

Fig. 3. Key indicators of precision aquaculture shown as AquaSense outputs.

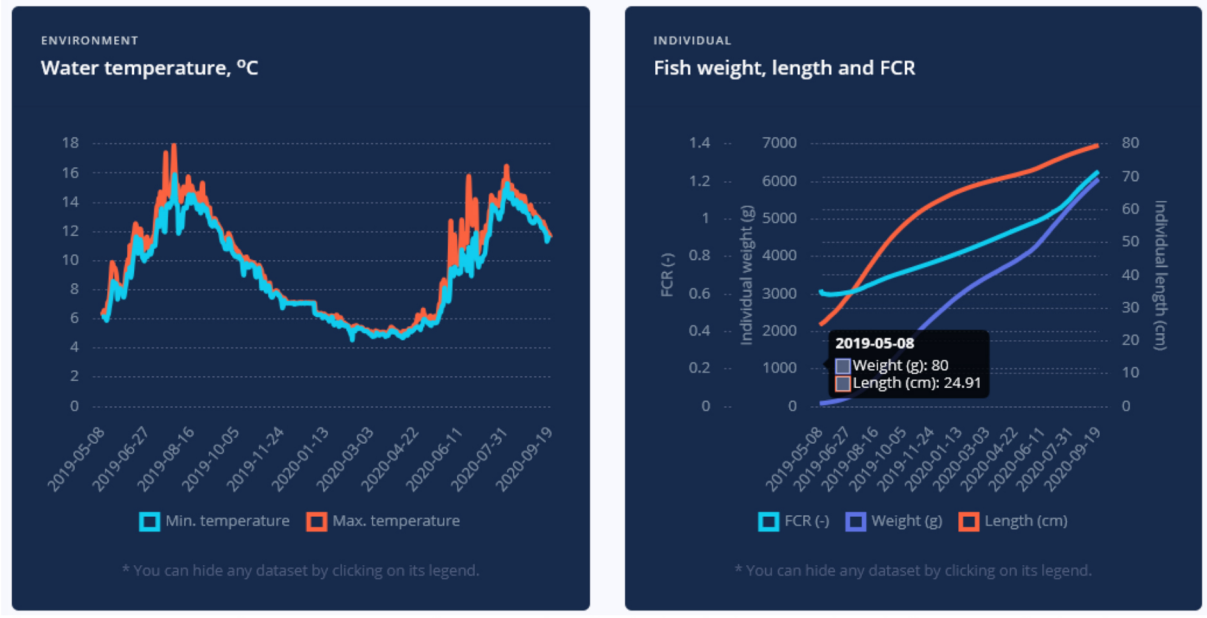


Fig. 4. AquaSense outputs for water temperature from sensor data, showing hourly changes, and standard output metrics for salmon growth simulated by the AquaFish model.

APPLICATION OF A HOLISTIC FRAMEWORK FOR ECOSYSTEM MODELLING— CATCHMENT TO COAST ANALYSIS OF DUNDRUM BAY, NORTHERN IRELAND

Joao G. Ferreira[1,2,+], Leonard Bernard-Jannin[1], Alhambra Cubillo[1], Joao Lencart e Silva[1], Heather Moore[3],t Matt Service[3], Joao Pedro Nunes[4]

[1]Longline Environment Ltd., 63, St. Mary Axe, London, EC3A 8AA, United Kingdom
[2]DCEA, Faculdade de Ciencias e Tecnologia, NOVA, Qta Torre, 2829-516 Monte de Caparica, Portugal
[3]Agri-Food and Biosciences Institute (AFBI), 18a Newforge Lane, Belfast BT9 5PX, United Kingdom
[4]Soil Physics and Land Management group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, the Netherlands

[+]Corresponding author

Introduction and Modelling Approach

The SUCCESS (System for Understanding Carrying Capacity, Ecological, and Social Sustainability) modelling framework was applied to Dundrum Bay, Northern Ireland. SUCCESS addresses (i) the partitioning of nutrient and bacterial loading from land; (ii) the circulation of water and water properties within Dundrum Bay and the exchange with the Irish Sea; (iii) the growth of bivalve shellfish in Inner Dundrum Bay; (iv) the biogeochemistry of Dundrum Bay, as it relates to ecosystem sustainability, in particular with respect to carrying capacity for bivalve cultivation. Each model has a number of uses *per se* and addresses different management challenges, and the linkages among models allow the whole set to be leveraged for improved decision-support.

Dundrum Bay is located on the southeast coast of County Down, Northern Ireland. Inner Dundrum Bay is a small, sheltered bay connected to the more exposed south facing Outer Dundum Bay by an inlet channel. Inner Dundrum Bay has two parts which extend on a SSW-NNE axis. The larger northern part (Inner Dundrum North) is approximately 3 km long and up to 1 km wide. The smaller southern part (Inner Dundrum South) extends for approximately 2 km to the SW and is up to 500 m wide, both drain to the Outer Bay via the Dundrum Outer channel. The Inner Bay is intertidal except for the inlet channel: it is a mesotidal embayment which is flushed through a single tidal inlet ~200 m wide and ~1 km long. This embayment exchanges most of its volume every tidal cycle due to the tidal oscillation at the mouth of the inlet linking the bay with the neighbouring shelf. This places a large emphasis on the circulation mechanisms of outer Dundrum Bay, where the greater part of the dispersion of land-based pollutants will take place.

The catchment area is approximately 150 km^2 and contains the four main rivers draining into the bay. Land use is approximately 80% agricultural, predominantly pastureland for sheep and cattle, but also pig and poultry farms. There are seven urban wastewater networks in the catchment. Up to 65% of the overall catchment's major freshwater sources may impact the Inner South area and 35% potentially affect the Inner North.

The designated shellfish waters in Dundrum cover an area of approximately 2.12 km^2 and are located in the inner bay (DOENI, 2009a). Aquaculture in the Inner Dundrum Bay area occurs in two licensed areas, where there is a history of shellfish cultivation since 1980. The licensed shellfish area covers 51.6 ha in the north (Pacific oyster and blue mussel) and 11.8 ha in the south (blue mussel), but shellfish culture is in the proximity of the main charted river channels: approx. 12 ha in the north side and 6 ha in the south side.

The well-tested EcoWin.NET (EWN) ecological model (e.g. Nobre et al, 2010; Bricker et al, 2018) was used within the SUCCESS framework to analyse the role of bivalves in top-down control of eutrophication symptoms.

Results and Discussion

Example results from the Soil and Water Assessment (SWAT) hydrological model (e.g. Nunes et al, 2017) and the EWN model application to eutrophication control are shown below.

(Continued on next page)

Shows the exports of nitrogen, phosphorus, and enteric bacteria from the catchment to Dundrum Bay. The nutrient input to the bay affects primary production, and the bacterial load is important in determining shellfish quality and restrictions to harvest. As expected, the exports are the most important from October to March, when rainfall and streamflow are the highest. During this period, bacteria and nutrient exports are more important in March and November compared to the December, January, and February. This can be explained by the stop in organic manure application between October 15th and February 1st. The bacteria and nutrient export peaks are observed one month after stopping and resuming slurry/manure application. This seems to indicate a delay of about one month between the input and the export of bacteria and nutrients. In addition, the period with no fertiliser application appears to be more efficient in reducing bacteria than nutrients exports. Finally, TP relative exports are the highest in summer, most likely due to the continuous nature of the final effluents that are the largest sources of TP.

Shows the effect of shellfish cultivation on the percentile 90 of chlorophyll in three EWN boxes: Box 2 and Box 4 are the surface boxes where shellfish are grown, and Box 6 is in the centre of the inlet channel.

The increase in the typical chlorophyll maximum ranges from 3.8 to 5.1%. For the surface boxes where cultivation takes place, the southern part of the inner bay shows a higher difference, but more interestingly, the inlet channel shows the highest difference—these results suggest that the effect of top-down control occurs in a broader area of the bay, since the benthic filter-feeders are removing food from the water passing through the cultivation sites. Changes to cultivation practice will thus be reflected in a more general way on bay-scale eutrophication.

Shows the change in chlorophyll concentration for the inner part of Dundrum Bay. Box 1 in the inner bay and box 29, the lower depth layer of the inlet channel, show the greatest differences when shellfish are switched off in the EWN model. Although the difference never exceeds 1 mg L^{-1}, partly due to the short water residence time, it reaches a 23% reduction in box 1 and a 30% reduction in box 29. The latter is particularly interesting because it shows a pronounced vertical gradient—box 6 only has a maximum reduction of 15%, and neither box 6 nor box 29 are shellfish cultivation areas.

The bay-scale assessment of differences in chlorophyll concentration—one of the primary symptoms of eutrophication—due to top-down control by farmed shellfish, can only be made by means of an ecosystem model. Models such as FARM (e.g. Saurel et al, 2014) or ABC (Ferreira et al, 2021) can determine food depletion within a farm, but they cannot predict what the resulting effect will be at the full bay scale.

Since phytoplankton abundance, biomass, and composition is one of the biological quality elements in the EU Water Framework Directive (2000/60/EC), and since both abundance and biomass are usually represented by chlorophyll as a proxy, system-scale models are a valuable management tool for considering different scenarios for eutrophication management.

The SUCCESS framework provides a model chain that allows managers to review options with respect to land use and coastal boundaries and analyse the consequences of different options, be they source control of emissions or complementary approaches such as enhanced shellfish culture for top-down control of eutrophication.

References

Bricker, S.B., Ferreira, J.G., Zhu, C., Rose, J. M., Galimany, E., Wikfors, G., Saurel, C., Miller, R. L., Wands, J., Trowbridge, P., Grizzle, R., Wellman, K., Rheault, R., Steinberg, J., Jacob, A., Davenport, E. D., Ayvazian, S., Chintala, M., Tedesco, M. A., 2018. Role of Shellfish Aquaculture in the Reduction of Eutrophication in an Urban Estuary. Environ. Sci. Technol. 52:173–183. https://doi.org/10.1021/acs.est.7b03970

Ferreira, J.G., Taylor N.G.H., Cubillo, A., Lencart-Silva, J., Pastres, R., Bergh, Ø., Guilder, J., 2021. An integrated model for aquaculture production, pathogen interaction, and environmental effects. Aquaculture 536:736438. https://doi.org/10.1016/j.aquaculture.2021.736438

Nobre, A.M., Ferreira, J.G., Nunes, J.P., Yan, X., Bricker, S., Corner, R., Groom, S., Gu, H., Hawkins, A. J. S., Hutson, R., Lan, D., Lencart e Silva, J. D., Pascoe, P., Telfer, T., Zhang, X., Zhu, M., 2010. Assessment of coastal management options by means of multilayered ecosystem models. Estuar. Coast. Shelf. Sci. 87:43–62. https://doi.org/10.1016/j.ecss.2009.12.013

Nunes, J.P., Jacinto, R., Keizer, J., 2017. Combined impacts of climate and socio-economic scenarios on irrigation water availability for a dry Mediterranean reservoir. The Science of the total environment. 584-585. 219-233. 10.1016/j.scitotenv.2017.01.131.

Saurel, C., Ferreira, J.G., Cheney D., Suhrbier, A., Dewey, B., Davis, J., Cordell, J., 2014. Ecosystem goods and services from Manila clam culture in Puget Sound: a modelling analysis. Aquac. Environ. Interact. 5:255–270. https://doi.org/10.3354/aei00109

(Continued on next page)

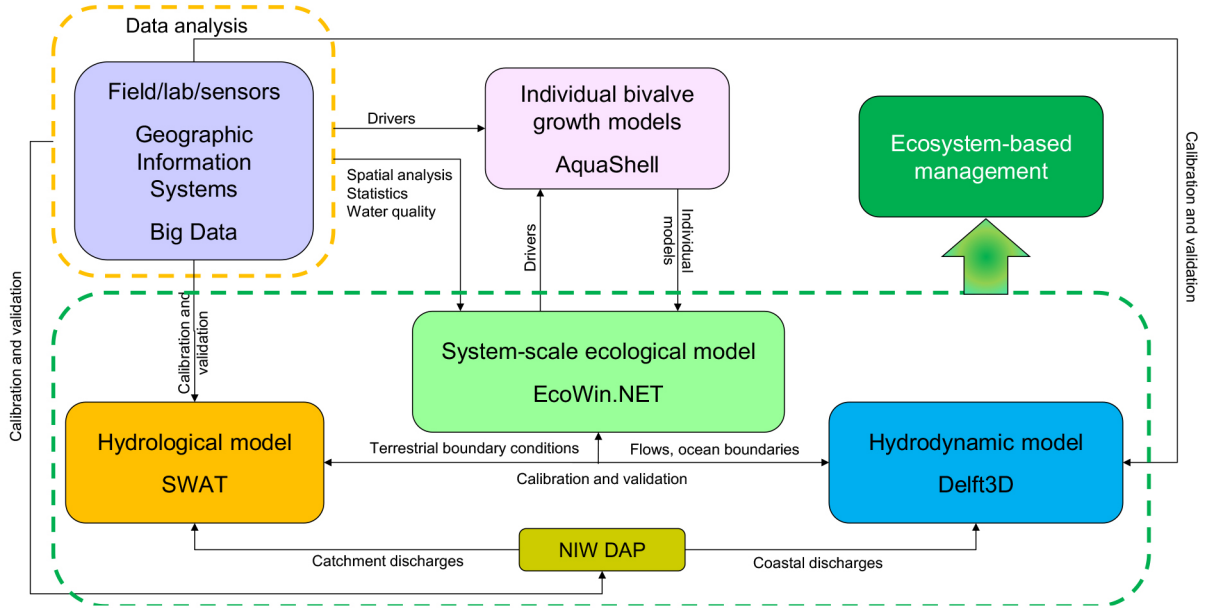


Fig. 1. SUCCESS ecological modelling framework applied to Dundrum Bay.

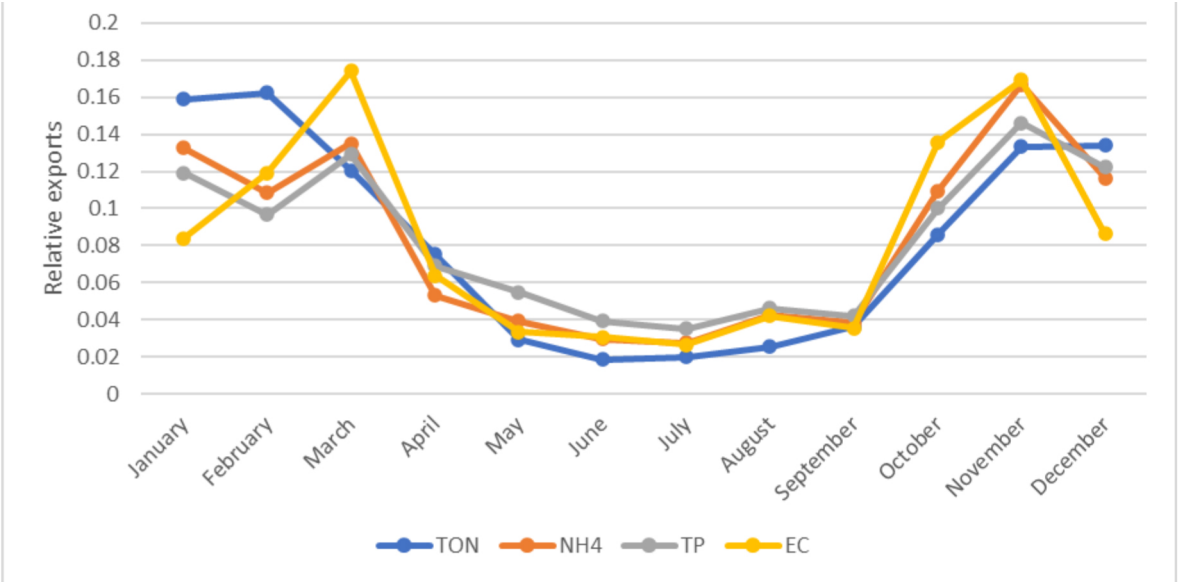


Fig. 1. Average monthly exports of TON, NH4, TP and E. coli (EC) as a proportion of annual export.

Table 1. Top-down control of eutrophication in inner Dundrum Bay and the inlet channel.

	Box 2	Box 4	Box 6
Standard model with shellfish P_{90} (µg chl L^{-1})	17.8	11.8	10.2
No top-down control by shellfish P_{90} (µg chl L^{-1})	18.6	12.2	10.7
Difference (%)	4.5	3.8	5.1

Table 1 shows the effect of shellfish cultivation on the percentile 90 of chlorophyll in three EWN boxes: Box 2 and Box 4 are the surface boxes where shellfish are grown, and Box 6 is in the centre of the inlet channel.

(Continued on next page)

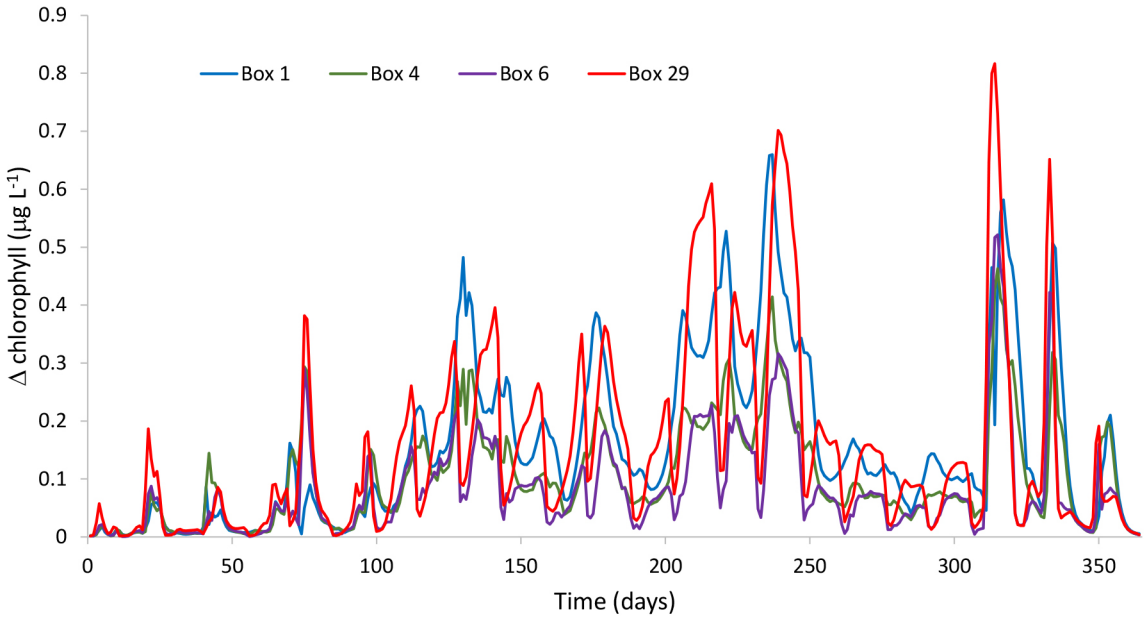


Fig. 1. Top-down control of eutrophication (phytoplankton with and without bivalves) in four model boxes (inner Dundrum Bay and inlet channel).

METAL-AMINO ACID COMPLEXES ARE A COST-EFFECTIVE STRATEGY TO HELP REDUCING FISH MEAL IN EUROPEAN SEABASS DIETS

A.C. Figueiredo-Silva[1]*, S. Chatzifotis[2]

[1]Zinpro Corporation, Eden Prairie, MN, USA; [2] HCMR, Gournes Heraklion, Greece
*Email: csilva@zinpro.com

Introduction

Comparison of the trace mineral (TM) composition of different protein sources to that of fish meal (FM), expose significant limitations in Zn, Se, and Fe, among other nutrients. In addition to lower TM content, antinutritional factors such as phytic acid, found in many plant meals used to replace FM, are not digested by fish. They have negative effects on mineral availability, causing difficulty in meeting TM needs. Trace mineral stability and absorption processes affect availability and ultimately animal performance. Metal-Amino Acid complexes (Metal-AA complexes, a mixture of a single specific metal complexed with different AAs in a 1:1 ratio) are taken up by AA-transporters, instead of metal ion transporters, reducing the risk for transport saturation and improving absorption efficiency. Furthermore, metal-AA complexes are more stable and minimally antagonized by other dietary ingredients, like phytic acid (Paripatananont and Lovell, 1995). Metal AA-complexes supplemented at half-rate of inorganic sources proved to maintain or even improve growth performance of European seabass and Atlantic salmon, respectively. Moreover, partial or complete replacement at 0.5x rate of inorganic minerals with metal-AA complexes reduced skin lesions in Atlantic salmon after infestation with *Caligus* and increased number of goblet cells in intestine and skin of European seabass, indicating enhanced barrier defense mechanisms against pathogens. In the latter study, conducted in collaboration with HCMR, evaluation included different TM premixes by source and level in 20% FM diets, reflecting FM inclusion levels practiced in commercial diets. A follow-up study at HCMR, co-funded by AQUAEXCEL EU programme, assessed how adjusting inclusion of a complete metal-AA complex premix contributes to a cost-effective reduction of FM from 20 to 10%, in European seabass diets.

Materials and methods

Quadruplicate groups of European sea bass with an initial body weight of 47 g were fed to apparent satiety for 12 weeks, one of 3 diets formulated to vary in their FM level (20 or 10%), and adjusted for their TM content. Control diet had 20% FM (FM20) and was supplemented with 50 ppm Zn as Availa®Zn, 40 ppm Fe as Availa®Fe, 12 ppm Mn as Availa®Mn, 3 ppm Cu as Availa®Cu, and 0.12 ppm Se as Availa®Se, (Zinpro Performance Minerals, Availa®Mins line). Two additional diets were formulated to reduce FM level in Control diet by 50% (10% FM) and supplemented with same premix at 1.5x (FM10, 1.5x) or 2x (FM10, 2x) used in the Control diet. Analyzed TM composition of FM20 and FM10 diets are shown in Table 1.

Results and discussion

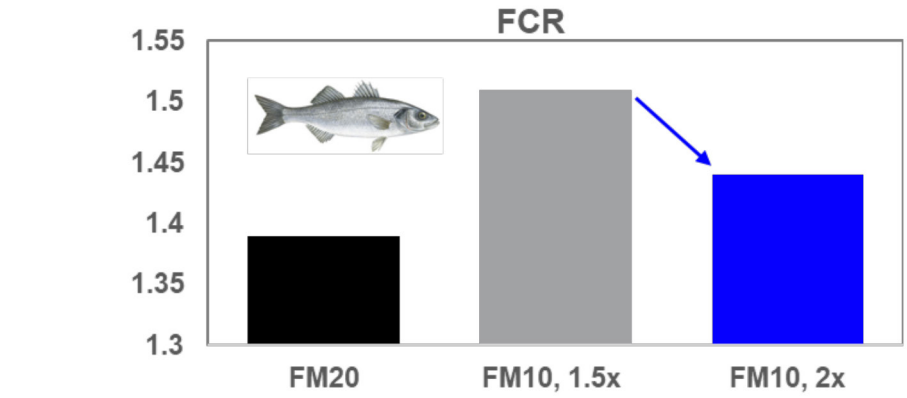
Adjusting mineral premix in FM10, at 1.5 or 2x the level used in FM20, maintained specific growth rate (between 0.74-0.77) and even slightly increased whole body Zn content (31ppm in FM20 vs 33ppm in diets FM10) of European seabass, though not statistically significant. Yet, specific growth rate (0.76 vs 0.74) and feed conversion ratio (FCR, 1.44 vs 1.51) were improved when TM premix was adjusted at 2x compared with 1.5x that in FM20 (Figure 1). Results indicate that adjusting the dietary TM content of FM10 to similar levels of that in FM20 may not be enough to sustain seabass performance Increasing the TM inclusion level from 1.5 to 2x of that used in FM20 improved SGR and FCR by 3 and 5%, respectively, reverting performance levels closer to that observed with FM20. This may be, at least partly, explained by likely lower nutrient availability in FM10, because of its higher plant protein content and antinutritional factors, compared to FM20 diet. A recent study shows performance and health of European seabass fed 10% FM or FMK-based diets could be kept similar, but required dietary levels of approximately 200–285 ppm Zn, 260–320 ppm Fe, 70–90 ppm Mn, and 0.8–1.0 ppm Se. While confirming the possibility of reducing FM in seabass diets, without negatively impacting performance, this scenario required Zn levels to surpass upper EU allowed levels in seabass diets (Table 1). In this study, it was shown that supplementation with metal-AA complexes contributes to a cost-effective reduction of FM from 20 to 10%, while respecting upper EU limits for TM supplementation, and TM content of seabass feeds. The exception was dietary Se content in the feed, that could not be kept at or below 0.5 ppm. Ingredient contribution to dietary Se content in aqua feeds, mainly contribution of FM and other marine ingredients, makes it practically impossible to keep Se levels in diets within allowed EU levels. Overall, it was shown that metal-AA complexes allow FM to be reduced from 20 to 10% without significantly affecting growth or FCR, while respecting upper EU limits for TMs in seabass diets. This strategy resulted in an 8.5% savings on feed cost, translating into a more sustainable and cost-efficient solution for the industry.

(Continued on next page)

Materials and methods

Table 1. Analyzed nutrient composition of the different diets

Nutrient Composition	FM20	FM10, 1.5x	FM10, 2x	EU Upper TM Limits
DM, %	94.8	95.7	95.0	
CP, % DM	48.1	47.7	47.6	
Fat, % DM	16.7	16.1	16.1	
Ash, % DM	7.3	6.3	6.3	
Energy, kJ/g, %DM	23.1	23.1	23.1	
Zn mg/kg	95.2	111.0	144.0	150
Cu mg/kg	8.3	10.6	12.7	25
Mn mg/kg	35.0	42.3	47.4	100
Fe mg/kg	391.0	418.0	483.0	750
Se mg/kg	0.86	0.76	0.88	0.5



Cost, Euros /MT	1008.83	921.85	923.84
Savings, Euros/MT		86.98	84.99

Fig. 1. Feed conversion ratio in European seabass at the end of the 12-week feeding study.

ASSESSING THE POTENTIAL FOR NUTRIENT TOXICITY ON SEAGRASS IN THE VICINITY OF AN AQUACULTURE SITE

R. Filgueira[1,*], T. Guyondet[2], P. Thupaki[3], G.K. Reid[4], L. Howarth[5], and J. Grant[5]

[1] Marine Affairs Program, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada
[2] Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, New Brunswick, E1C 9B6, Canada
[3] OMS Research and Consulting Ltd., Victoria, British Columbia, Canada
[4] Centre for Marine Applied Research, Dartmouth, Nova Scotia, B2Y 4T5, Canada
[5] Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada
Email: ramon.filgueira@dal.ca

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.

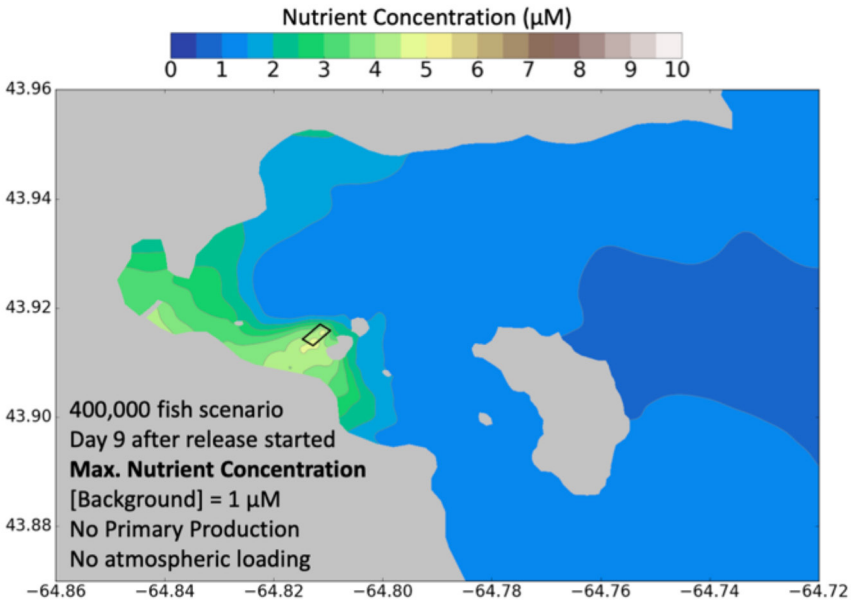


Fig. 1. Maximum predicted nutrient concentration (µM) in the 400,000 fish scenario after 9 days using as a background nutrient concentration of 1µM, no primary production, and no atmospheric loading.

(Continued on next page)

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.

References

Howarth LM, Filgueira R, Jiang D, Koepke H, Frame K, Buchwald C, … Grant J (2019) Applying the ecosystem approach to aquaculture: using δ15N to map the footprint of multiple effluent sources. Aquaculture Environment Interactions 11:671-684.

Jansen HM, Broch OJ, Bannister R, Cranford P, Handå A, Husa V, … Strand Ø (2018) Spatio-temporal dynamics in the dissolved nutrient waste plume from Norwegian salmon cage aquaculture. Aquaculture Environment Interactions 10:385-399.

Johnson C, Devred E, Casault B, Head E, Spry J (2018) Optical, Chemical, and Biological Oceanographic Conditions on the Scotian Shelf and in the Eastern Gulf of Maine in 2016. DFO Canadian Science Advisory Secretariat Research Document 2018/017. v + 58 p.

van Katwijk MM, Vergeer LHT, Schmitz GHW, Roelofs JGM (1997) Ammonium toxicity in eelgrass Zostera marina. Marine Ecology Progress Series 157:159-173.

POTENTIAL OF SOLID-STATE FERMENTATION EXTRACTS OBTAINED WITH WINERY AND OLIVE MILL BY-PRODUCTS AS IMMUNE SYSTEM ENHANCERS IN EUROPEAN SEA BASS DIETS

D. Filipe[1,2*], D. Ferreira-Martins[2], J. Salgado[3], I. Belo[3], A. Oliva-Teles[1,2], C. Castro[2], H. Peres[1,2]

1- Faculty of Sciences, University of Porto
2- CIIMAR- Interdisciplinary Center of Marine and Environmental Research. 3- CEB- Centre of Biological Engineering, University of Minho
Email: diogomoreirafilipe123@gmail.com

Introduction

Under a circular economy context, reutilization and recycling agro-industrial by-products are of utmost importance. The European Union produces 95% of world olive oil and 65% of wine, generating a vast waste prone to cause environmental detriment due to their high organic content and low biodegradability. The presence of important bioactive compounds in olive oil and wine wastes is well documented. Still, their high fiber and low protein levels are significant constraints for their use in animal nutrition. Solid-state fermentation (SSF) is a green biotechnological process that can utilize agroindustrial by-products like those from wineries and olive mills (grape pomace, vine-shoot trimmings, olive pomace) as substrates for microbial growth. SSF allows for the release of several value-added bioactive compounds, as phenolic antioxidants. In 2019, 263 215 tons of European sea bass were produced globally by the aquaculture industry. Intensive aquaculture conditions due to overcrowding and environmental conditions (water quality, hypoxia) and husbandry handling stressors (grading, transport, crowding, and vaccination) may induce fish inflammatory responses. Dietary fortification with natural immunostimulants may increase fish immunocompetence, contributing to decrease susceptibility of fish to stress. This trial aimed to evaluate the efficiency of extract produced through SSF of the olive mill and winery waste as dietary immunostimulants in European sea bass.

Materials and methods

A previously optimized mixture of olive mill and winery wastes (Filipe et al., 2019) was submitted to SSF by *Aspergillus ibericus* (MUM-01.29; Micoteca da Universidade do Minho (UM). After fermentation, an aqueous extraction was performed using a 1:5 ratio of solid/water (w/v) for 30 minutes with constant stirring. Another extract was also prepared from the same optimized mixture but without applying 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 (FWO4 diet); 1365 (FWO8 diet) and 683 (UWO diet) µmol Trolox/kg of diet. After the feeding trial of 66 days, fish were submitted to an inflammatory insult. Twenty-four fish per dietary treatment (60 g of mean body weight) were divided into two groups and intraperitoneally injected with a formalin-killed aqueous bacterin of *P. damselae subsp. piscicida* or with PBS solution (control group). 4h and 24h post-injection, the blood and head-kidney of six fish from each group were sampled. The activity of lysozyme, peroxidase, bactericidal capacity, protease, anti-protease, and ACH50 was determined in plasma samples. Immune gene expression of interleukin-1β beta (il-1β), interleukin 6 (il-6), interleukin 8 (il-8), caspase 3 (casp3), caspase 9 (casp9), cyclooxygenase 2 (cox2), and tumor necrosis factor-alpha (tnfα) was analyzed through q-RT PCR in head-kidney tissue samples.

Results

Screening the plasma innate humoral response showed an increase of ACH50 in fish fed with FWO 8 and UWO diets, at 4h post-injection, and a decrease in peroxidase activity with UWO diet at 24h, relative to the control diet.

Irrespective of the post-injection time, compared to the control diet FWO4 and UWO diets reduced pro-inflammatory cytokines (IL β1, IL 6, IL 8), casp3, casp9, and TNF-α, while the FWO8 diet increased COX2. Comparing the sampling points, in the control diet IL β1, IL 6, IL 8, and casp3 expression increased at 24h, while their expression was not affected with the test diets.

In the intestine, at both times post-injection, and relative to the control diet, IL-8, casp3, casp9, and TNF-α expression were increased in fish fed the FWO4 diet, while casp3 and casp9 were increased in fish fed the UWO diet.

(Continued on next page)

Conclusion

Regardless of fermentation, dietary fortification with olive mill and winery by-product extracts modulated fish response to an inflammatory insult.-

Present results underline the importance of further studying the potential of FWO and UWO extracts as a part of the circular economy strategy, contributing to more efficient use of resources for a greener and more competitive aquaculture.

Acknowledgements: Project SPO3 (ref. POCI-01-0145-FEDER-030377; FEDER-Operational Programme Competitiveness and Internationalization and FCT); project InovFeed (ref. MAR-02.01.01-FEAMP-0111; Programa Operacional Mar2020);

Bibliography:

Filipe, D., Fernandes, H., Castro, C., Peres, H., Oliva-Teles, A., Belo, I., & Salgado, J. M. (2019). Improved lignocellulolytic enzyme production and antioxidant extraction using solid-state fermentation of olive pomace mixed with winery waste. *Biofuels, Bioproducts and Biorefining*, *14*(1), 78–91. https://doi.org/10.1002/bbb.2073

Hassan K. Obied, †, Malcolm S. Allen, ‡, Danny R. Bedgood, †, Paul D. Prenzler, †, Kevin Robards, *,† and, & Stockmann§, R. (2005). Bioactivity and Analysis of Biophenols Recovered from Olive Mill Waste. *Journal of Agricultural and Food Chemistry*, *53*(4), 823–837. https://doi.org/10.1021/JF048569X

Soto, M. L., Conde, E., González-López, N., Conde, M. J., Moure, A., Sineiro, J., Falqué, E., Domínguez, H., Núñez, M. J., & Parajó, J. C. (2012). Recovery and Concentration of Antioxidants from Winery Wastes. *Molecules 2012, Vol. 17, Pages 3008-3024*, *17*(3), 3008–3024. https://doi.org/10.3390/MOLECULES17033008

EFFECTS OF PARTIAL SUBSTITUTION OF DIETARY PROTEIN SOURCES WITH DUCKWEED (*Lemna* sp.) MEAL ON THE RAINBOW TROUT (*Oncorhynchus mykiss*) GROWTH PERFORMANCES

Fiordelmondo E., Mariotti F., Magi G.E., Roncarati A.

School of Biosciences and Veterinary Medicine, Camerino University, Italy

Introduction

Feed's composition plays a crucial role in the rearing of the main fish species of interest to aquaculture due to the capacity to influence and shape composition and quality of fish meat. In farming conditions, fish need high quality feeds to obtain excellent growth and healthy animal status. In fact, raw materials used as feedstuff guarantee a balanced diet rich in essential active ingredients which improve fish healthy, fish welfare, and production performances in terms of yield. Aquaculture has been dependent on the capture of pelagic fish to use as feedstuff. Due to the rising prices of fishmeal and fish oil, they are substituted by alternative ingredients (Gasco et al., 2020) such as plant-based proteins (Parisi et al., 2020). In this context, duckweed (*Lemna* spp.) can be purposed as source of protein, lipid, and minerals (Chakrabarti et al., 2018). In an Italian rainbow trout farm, a trial was performed to evaluate if the duckweed meal can be used in partial substitution of the conventional feedstuffs used as protein source in fish feeding. An experimental diet including duckweed meal was formulated and compared with the control diet. At the end of the trial, zootechnical performances of rainbow trout fed with the duckweed inclusion were compared to those of fish fed with the standard diet.

Materials and Methods

Duckweed meal was included in the formulation of an experimental diet (LM) at 20% of the protein source represented by soybean meal, fish meal, wheat flour and gluten wheat meal. The protein content (41%) and lipid rate (20%) of LM diet were equal to the control diet (LC) formulated with the same feedstuffs except duckweed meal.

A total of 270 rainbow trout (mean body weight 124.5 ± 0.7 g) was randomly allocated in 6 tanks at the initial stocking density of 6.2 kg/m^3. At the end of the trial (90 days), fish were weighed, the final length was recorded, and the condition index was determined. Fish were administered twice a day (8 a.m. and 3 p.m.) by hand at the apparent satiation level collecting the unconsumed feed.

The following zootechnical performances were evaluated in the two different groups: final body weight (g); final mean length (cm); weight gain (g); survival rate (%).

Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS 25 (Version 25.0, Armonk, NY, USA) (IBM). Means of each value was calculated and considered significant with a value of $p < 0.01$ and compared using the Student-Newman-Keuls (SNK) test.

Sanitary conditions, such as fin erosion, parasites and bacterial diseases, were also investigated.

Results and discussion

At the end of the trial, fish fed with LM showed a mean value of final body weight of 340.53±4.3g, final length of 31.2± 1.3cm, weight gain of 216.03±2.8g, and survival rate of 98± 1%. Fish fed with LC showed a mean of final body weight of 31.0± 1.2cm, final length of 20.0± 0.6cm, weight gain of 224.3± 2.6cm, and survival rate of 99± 0%. All fish showed a good health condition, without signs of illness such as fin erosion, parasites or bacterial diseases.

Looking to these results, no significant statistic differences in terms of growth and survival were observed comparing the values obtained from fish fed with the inclusion of *Lemna* to those obtained from ones fed without *Lemna*. Our results are in agreement with previous studies concerning the investigation of the digestibility of duckweed in other fish species, such as carp (Pípalová et al., 2003) and tilapia (El-Shafai et al., 2004). In fact, in the present trial rainbow trout looked to respond well to the partial vegetable protein source administered. Based on these preliminary results, *Lemna* could be included in the rainbow trout diet without negative effects on fish growth and animal health.

(Continued on next page)

Acknowledgements
Research supported by EUREKA Project 2019. We are grateful the Erede Rossi Company for their valuable collaboration and technical assistance.

References

El-Shafai, S.; El-Gohary, F.; Verreth, J. A. J.; Schrama, J.W.; Gijzen, H.J. 2004. Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 35:574-586.

Gasco L., Acuti G., Bani P., Dalle Zotte A., Danieli P.P., De Angelis A., Fortina R., Marino R., Parisi G., Piccolo G., Pinotti G., Prandini L., Schiavone A., Terova G., Tulli F., Roncarati A., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Italian Journal of Animal Science 19:360-372.

Chakrabarti R., Clark W.D., Sharma, J.G., Goswami R.K., Shrivastav A.K., Tocher D.R. 2018. Mass Production of *Lemna minor* and Its Amino Acid and Fatty Acid Profiles. Front Chem. 6:479.

Parisi G., Tulli F., Fortina R., Marino R., Bani P., Dalle Zotte A., De Angeli A., Piccolo G., Pinotti L., Schiavone A., Terova G., Prandini A., Gasco L., Roncarati A., Danieli P.P. 2020. Protein hunger of the feed sector: the alternatives offered by the plant world. Italian Journal of Animal Science 19:1204-1225.

Pípalová I. 2003 Grass carp (*Ctenopharyngodon idella*) grazing on duckweed (*Spirodela polyrhiza*). Aquaculture International 11:325–336.

PHYTOGENIC BIOACTIVE COMPOUNDS SHAPE FISH MUCOSAL IMMUNITY

Joana P. Firmino*[1], Jorge Galindo-Villegas[2], Felipe E. Reyes-López[3], Enric Gisbert[4]

[1] TECNOVIT – FARMFAES, S.L., Alforja, Spain
[2] Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway
[3] Consorcio Tecnológico de Sanidad Acuícola, Ictio Biotechnologies S.A., Santiago, Chile
[4] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
* Email : jfirmino@farmfaes.com

Introduction

Aquaculture growth will unavoidably involve the implementation of innovative and sustainable production strategies, being functional feeds among the most promising ones. A wide spectrum of phytogenics, particularly those containing terpenes and organosulfur compounds, are increasingly studied in aquafeeds due to their growth promoting, antimicrobial, immunostimulant, antioxidant, anti-inflammatory and sedative properties (Reverter et al. 2021). Although the impact of such phytogenics upon fish mucosal immunity has been extensively evaluated, most of the studies fail in addressing the mechanisms underlying their pharmacological effects. Under this context, the present set of studies describe a holistic approach for evaluating the antiparasitic and antibacterial properties of a feed additive composed by microencapsulated carvacrol, thymol and garlic essential oil, as well as seeking to decipher its mode of action upon gilthead seabream mucosal tissues.

Materials and Methods

This presentation is based on recently developed results from our group. In particular, analyses performed in gills (Firmino et al. 2020), intestine (Firmino et al. 2021b) and skin (Firmino et al. 2021a) showed that these three tissues positively responded to the dietary administration of the phytogenics-based additive, although some variations in the mucosal transcriptional responses were observed among the different tissues. Besides, by combining the results obtained together with a profound literature review on the physiological and immunological mucosal responses of fish fed phytogenics, novel mechanisms are hypothesized to explain the mechanisms of cell activation that may be responsible for such mucosal immune-related responses.

Results and Discussion

From a global point of view, 759 DEGs were obtained in the gills of fish fed the phytogenics-supplemented diet, of which 53 of those DEGs were also modulated in the intestine and another 40 DEGs were also modulated in the skin. In the intestine, 581 DEGs were obtained; of them, 31 were modulated in the skin as well. Regarding the skin analysis, 534 total DEGs were obtained (*Figure 1*). Only 5 DEGs were shared among the three tissues analysed, suggesting tissue-dependent divergences in the mucosal responses. The different sets of common DEGs did not reveal noteworthy interactions when merged and submitted to an enrichment analysis. The fact that the modulation of genes that are differentially expressed in more than one of the studied tissues seems not to be connected and associated to specific biological processes reinforce the idea that each mucosal tissue responds in a distinct and singular way to the dietary administration of the functional feed additive. In this context, the gill was unexpectedly the tissue that revealed the highest number of DEGs promoted by the phytogenics-supplemented diet, rather than the intestine, which is the site of absorption of the feed additive and, *a priori*, the site where the major changes with regard to gene expression were expected. This difference in tissue susceptibility may be a consequence of the combination of several factors, such as the gills particular functionality and the mode of action of the phytogenics bioactive compounds.

The intrinsic physiology and anatomy of each mucosal tissue, the extrinsic environment that they are subjected to, their specific microbiota composition and/or its modulation are factors conditioning the different transcriptional profiles obtained (Cabillon and Lazado 2019). Additionally, the potential degradation of the bioactive compounds along the digestive tract and their different pharmacokinetics within each target tissue may also affect tissue-specific responses (Michiels et al. 2008). It is suggested that the terpenes and organosulfurs present in the studied feed additive display their mucosal immunomodulatory activity through the activation of Transient Receptor Potential (TRP) ion channels (Xu et al. 2006). In this sense, bioactive compounds may activate TRP channels leading to intracellular Ca^{2+} increase and non-canonical activation of the TAK complex. In parallel, stimulation by pathogen-associated molecular patterns (PAMPs) may facilitate the activation of TLR and TRP signalling pathways, which would explain the fish improved ability to cope with pathogenic challenges. This hypothesis opens interesting questions about at which extent gills, as a site of significant immunity and presence of ion channels due to its role in osmoregulation, may be susceptible to functional feed additives and recognized as promising target tissues for dietary therapeutic strategies.

(Continued on next page)

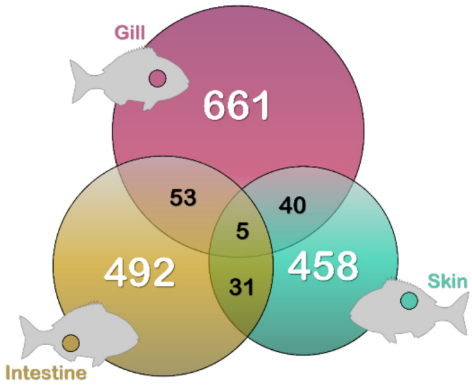


Fig. 1. Venn diagram for representing the exclusive and common DEGs in the gill, intestine and skin of seabream fed an additive composed by garlic essential oil, carvacrol and thymol (unpaired t-test, $P < 0.05$).

Acknowledgements: This work has been supported by the project DIETAplus funded by the MAPAMA (Spain) and FEMP funds (EU). FER-L thanks the support of Fondecyt regular Nb. 1211841. JF have been subsidized by the Industrial PhD program of Generalitat de Catalunya and TECNOVIT-FARMFAES S.L.

References

Cabillon, N.A.R. and Lazado, C.C. (2019). Mucosal barrier functions of fish under changing environmental conditions. *Fishes* 4(1): 2.

Firmino, J.P., Fernández-Alacid, L., Vallejos-Vidal, E., et al. (2021a). Carvacrol, thymol, and garlic essential oil promote skin innate immunity in gilthead seabream (*Sparus aurata*) through the multifactorial modulation of the secretory pathway and enhancement of mucus protective capacity. *Frontiers in Immunology* 12(559): 633621.

Firmino, J.P., Vallejos-Vidal, E., Balebona, M.C., et al. (2021b). Diet, immunity, and microbiota interactions: An integrative analysis of the intestine transcriptional response and microbiota modulation in gilthead seabream (*Sparus aurata*) fed an essential oils-based functional diet. *Frontiers in Immunology* 12(356): 625297.

Firmino, J.P., Vallejos-Vidal, E., Sarasquete, C., et al. (2020). Unveiling the effect of dietary essential oils supplementation in *Sparus aurata* gills and its efficiency against the infestation by *Sparicotyle chrysophrii*. *Scientific Reports* 10(1): 17764.

Michiels, J., Missotten, J., Dierick, N., et al. (2008). *In vitro* degradation and *in vivo* passage kinetics of carvacrol, thymol, eugenol and trans-cinnamaldehyde along the gastrointestinal tract of piglets. *Journal of the Science of Food and Agriculture* 88(13), 2371-2381.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., et al. (2021). Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture* 13(1), 537-555.

Xu, H., Delling, M., Jun, J.C. and Clapham, D.E. (2006). Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. *Nature Neuroscience* 9(5), 628-635.

EFFECT OF WATER TEMPERATURE AND PHOTOPERIOD ON BRAIN AND PITUTITARY MATURATION HORMONES DURING FRESHWATER PRODUCTION STAGE OF ATLATNIC SALMON (*Salmo salar L.*)

M.S. Fleming[b]*, E.P. Martinez[a, b], P. Balseiro[a], T. Hansen[b], S. O. Stefansson[b], S. O. Handeland[a, b]

[a]NORCE Environment, Norwegian Research Centre AS, Bergen, Norway
[b]Department of Biological Sciences, University of Bergen, Bergen, Norway
email: Mitchell.fleming@uib.no

Introduction

Early sexual maturation of Atlantic salmon during the freshwater phase of production is an ongoing economic and welfare issue for the industry. Early sexual maturation leads to risk of mortality after sea water transfer and/or the loss of production value due to change of energy utilization from body growth and sea water adeptness to gonad development.

The complex series of morphological and physiological changes that occurs during sexual maturation is driven through the activation of the brain-pituitary-gonad (BPG) axis. Recently, the understanding of how the BPG axis is activated to cause gonad development in mammals and birds has progressed drastically. Pars-tuberalis Thyrotrophin (PT-TSH) is stimulated either by melatonin (mammals; Klosen et al., 2013) or deep brain photoreceptors (birds; Yoshimura et al., 2013) which then causes a similar cascade of signaling events which leads to the release of follicle stimulating hormone (FSH) and Luteinizing hormone (LH) from the pituitary and subsequently gonad development. These gonadotrophs have been well studied in male Atlantic salmon yet the brain regulation of gonadotrophs release is less understood. The recent discovery of dorsal pituitary tshbb in Atlantic salmon suggests the possibility of a similar pathway which could lead to a better understand of the neuroendocrine regulation of sexual maturation (Fleming et al., 2019).

This study investigated the expression of brain Deiodinase 2 (*dio2b*), Gonadotropin releasing hormone (*GnRH*), and Kisspeptin receptor (*GPR54*) along with pituitary Thyrotropin b (*tshbb*), *FSH* and Luteinizing hormone (*LH*) as well as Gonadal somatic index (GSI).

Materials and methods

On October 28th, 2019, 1000 Atlantic salmon parr were randomly distributed among 8 0.5m^3tanks. Fish were raised under constant light (LD24:0) from first feeding. After transfer, fish were acclimated at 12.5°C for 1-week whereafter half the tanks were raised to 15°C while the remaining 4 tanks stayed at 12.5°C. The fish remained at their respected temperatures and continuous light until Feb 1st when a LD12:12 "winter-signal" was introduced to two of the 12.5°C and two of the 15°C tanks while the other tanks remained on continuous light (LD24:0). The winter signal lasted 5 weeks whereafter returned to continuous light. The experiment continued until May and all fish were fed in full ration using commercial feeds (Biomar).

8 samplings took place during the experiment where 6 males per tank were randomly selected and killed with an overdose of benzocaine (Benzoak vet.® 20%, ACD Pharma AS, Norway). Body length and weight were recorded and blood was collected from the caudal vein for sex steriod measuermets. Gonads were taken for GSI and gene expression measurements. Brain and pituitary were sampled for gene expression measurements. All gene expression analysis was done using qPCR.

Results

Sexual maturation was highest in the 15°C continuous light fish with an increase of GSI happening as early mid-February. The increase of GSI steadily increased until the end of the experiment where nearly 75% of the male salmon where sexually mature. The 15°C winter signal fish also had high occurrence of sexual maturation at the end of the experiment approaching 100% of the males sampled. Maturation at 12.5°C was lower with ~50% of the fish in the winter signal and ~25% of the fish in the continuous light became sexually mature. Majority of the sexual maturation in both 12.5°C conditions and the 15°C winter single condition occurred after the winter signal.

GnRH and GPR54 were both higher expressed in the 15°C fish while both 12.5°C conditions remained consistently low in comparison. This increase of GnRH and GPR54 expression was not seen in the 12.5°C fish. *Tshbb* had a robust response to the end of the winter with a sharp increase of expression which was not observed in the continuous light conditions. Nearly identical expression levels were observed between both winter signal conditions irrelevant of water temperature.

(Continued on next page)

Discussion

In the current study, high water temperatures resulted in a high occurrence of sexual maturation. Comparable levels of maturation have been observed in similar water temperatures in previous experiments and suggests that the use of high-water temperature during the freshwater stage of production leads to early sexual maturation (Fjelldal et al., 2018).

The two groups given the winter signal had an increase of *tshbb* after the winter signal ended. This increase of *tshbb* was not seen in the continuous light condition fish suggesting increasing day length after the winter signal stimulates the production of *tshbb*. Irachi et al, observed a similar increase of *tshbb* expression after returning to long day photoperiod (Irachi et al., 2021). These results suggest that *tshbb* is directly influenced by day length. The exact role of tshbb needs to be further studied however it is clear that continuous light inhibits the expression during the freshwater stage.

Genes expressed in the diencephalon region of the brain (*dio2b*, *GnRH*, *GPR54*) showed differential expression between temperatures. Little difference was observed between photoperiod conditions. Despite higher expression in 15°C than 12.5°C sexual maturation was still observed in all conditions. These results suggest that despite differential expression, the basal levels needed for maturation are already met at 12.5°C. Further experiments will be needed to better understand the threshold levels of certain genes which active the BPG axis to cause early sexual maturation and how temperature plays a role in this signaling pathway.

References

Fleming, M. S., Maugars, G., Lafont, A. G., Rancon, J., Fontaine, R., Nourizadeh-Lillabadi, R., ... & Dufour, S. (2019). Functional divergence of thyrotropin beta-subunit paralogs gives new insights into salmon smoltification metamorphosis. *Scientific reports*, *9*(1), 1-15.M

Fjelldal, P. G., Schulz, R., Nilsen, T. O., Andersson, E., Norberg, B., & Hansen, T. J. (2018). Sexual maturation and smoltification in domesticated Atlantic salmon (Salmo salar L.)–is there a developmental conflict? *Physiological reports*, *6*(17), e13809.

Klosen, P., Sébert, M. E., Rasri, K., Laran-Chich, M. P., & Simonneaux, V. (2013). TSH restores a summer phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. *The FASEB Journal*, *27*(7), 2677-2686

Nakane, Y., & Yoshimura, T. (2014). Universality and diversity in the signal transduction pathway that regulates seasonal reproduction in vertebrates. *Frontiers in neuroscience*, *8*, 115.

INTEGRATED PEST AND DISEASE MANAGEMENT IN AQUAPONICS

Ewumi Azeez Folorunso[1]*, Koushik Roy[1], Andrea Bohatá[2], Radek Gebauer[1], Jan Mraz[1]

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Na Sádkách 1780, 370 05 České Budějovice, Czech Republic
[2]University of South Bohemia in Ceske Budejovice, Faculty of Agriculture, České Budějovice 370 05, Czech Republic
E-mail: Efolorunso@frov.jcu.cz

Introduction
The growing human population has continued to intensify global food. Hence, there are crucial needs for sustainable food production systems that ensure a healthy food supply. Aquaponics is an innovative food production method that uses wastewater from fish culture to cultivate vegetables in hydroponics; in a confined coupled or decoupled system (Lennard & Goddek, 2019). However, pest and disease management, among other challenges, has limited the adoption of aquaponics for commercial purposes (Stouvenakers et al., 2019). The interactions in various aquaponics designs expose fish and biofilter units of aquaponics to pest and pathogen treatments. Hence, consensus efforts are urgently required to establish approaches that pose little or no adverse effects on fish and biofilter. We aimed to evaluate disease management steps and techniques in hydroponics to qualify as suitable techniques for different aquaponic designs. There are sufficient biocontrol and natural pesticides to deal with pests, though natural pesticides can slightly affect nitrification (Rašković et al., 2021). In contrast, there are scanty and less effective measures for fungi pathogens, causing considerable damages in aquaponics. Hence, we assessed the use of biological control against powdery mildew pathogen in aquaponics systems.

Materials and Methods
A meta-analysis of 168 peer-reviewed articles about pest and disease management in hydroponics and intensive fish culture systems was conducted to establish suitable steps and techniques for different aquaponics designs. The assessment of integrated pest and disease management steps and techniques was based on the potential of IPDM to have detrimental effects on fish and nitrifying bacteria.

To assess the use of biocontrol agents (BCA) against powdery mildew, detached cucumber leaves were pretreated with the suspension of conidia of *Lecanicillium lecanii* (LLE), *Isaria fumosorosea* (IFR), *Trichoderma virens* (TVI), and inoculated with *Podosphaera xanthii* under different relative humidity conditions (RH), ≥ 95%, 65-73%, and ≤40% RH. The domination and inhibition capacity of the BCAs was assessed by disease severity scored using a manipulated 12-grade scale (Horsfall and Barratt, 1945) and disease reduction percentage after three weeks.

Results
Adoption of integrated pest and disease management techniques (IPDM).

Non-chemical preventive measures in hydroponics and intensive aquaculture systems are highly proficient for all designs and do not affect fish and biofilter in decoupled and coupled aquaponics. While cultural and physical controls are primarily safe for all aquaponics designs, chemical control (pesticides) is unsuitable for coupled systems (where the wastewater is constantly recircled for reuse). For example, pesticides can have positive or negative effects on nitrification (Figure 1).

Biological control of powdery mildew
The three microbial control agents significantly suppressed the disease at a 10^7 CFU/ml concentration compared with the control. More importantly, under greenhouse conditions (65-73% RH), a significant disease reduction (DR) percentage of 85% against the powdery mildew disease was recorded in *L. lecanii*-pretreated leaves (Figure 2A).

Conclusion
Pest infestations in aquaponics systems can be primarily controlled using preventive, cultural, and biological control approaches. On the other hand, common pathogens such as powdery mildew are more problematic. Preventive inoculation of *L. lecanii, T. virens*, and *I. fumosorosea* in leaves can significantly reduce the outbreak of powdery mildew.

(Continued on next page)

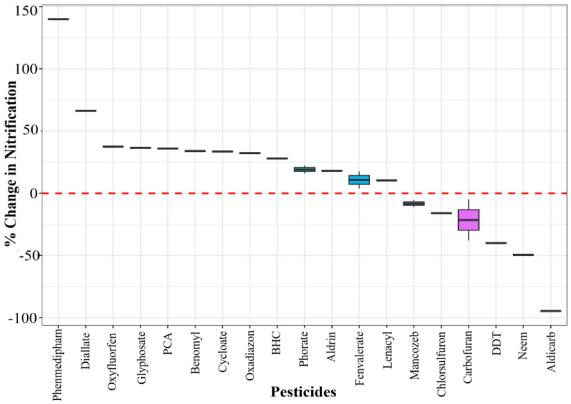


Figure 1: Effects of pesticides on percentage increase and decrease in nitrification

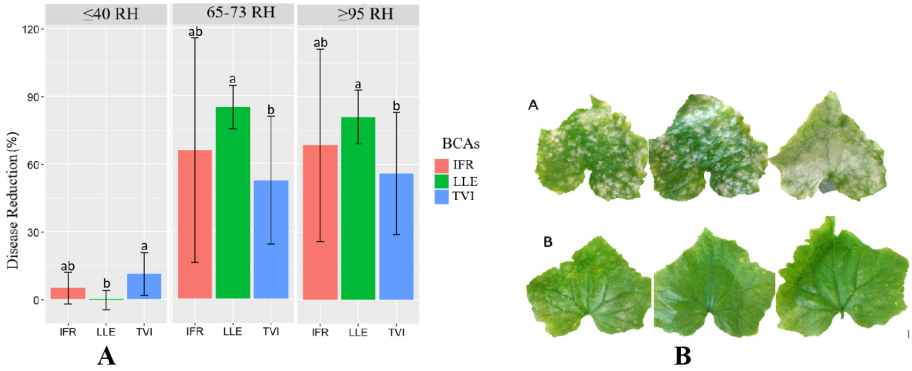


Figure 2: **A** is a Graph showing DR of LLE, IFR, and TVI under different relative humidities, and **B** shows the picture of (A) control and (B) LLE-treated leaves.

References

Horskfall, J. G., & Barrati, R. W. (1945). An Improved Grading System For Measuring Plant Diseases Vol-36. *Phytopathology*, *35*, 655. https://www.semanticscholar.org/paper/An-Improved-Grading-System-For-Measuring-Plant-Horskfall

Lennard, W., & Goddek, S. (2019). Aquaponics: The Basics. In S. Goddek, A. Joyce, B. Kotzen, & G. M. Burnell (Eds.), *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future* (pp. 113–143). Springer International Publishing.

Rašković, B., Dvořák, P., & Mráz, J. (2021). Effects of Biodegradable Insecticides on Biofilter Bacteria: Implications for Aquaponics. *Turkish Journal of Fisheries and Aquatic Sciences*, *21*(04), 169–177.

Stouvenakers, G., Dapprich, P., Massart, S., & Jijakli, M. H. (2019). Plant Pathogens and Control Strategies in Aquaponics. In S. Goddek, A. Joyce, B. Kotzen, & G. M. Burnell (Eds.), *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future* (pp. 353–378)

DOES SIZE MATTER? THE EFFECT OF TITANIUM DIOXIDE NANOPARTICLE SIZE OVER TIME ON GENE EXPRESSION IN TURBOT LIVER

E. Fonseca*, M. J. Araújo, M. L. Sousa, M. Vázquez, N. Mallo, S. Cabaleiro, L. Rodriguez-Lorenzo, M. Quarato, B. Espiña, A. Campos

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Av. General Norton de Matos s/n 4450-208 Matosinhos (Portugal)
E-mail: efonseca@ciimar.up.pt

Introduction

Nanotechnology has been widely used in medicine and in several industries, including aquaculture. Among the numerous nanoparticles (NPs) currently in production and use, titanium dioxide (TiO_2) NPs are emerging for their extensive application in consumer products, including textiles, paints or health-care products. As a new class of emerging pollutants with eco-toxicological impacts on marine ecosystems, there is a growing need to assess the risk of engineered nanoparticles (ENPs) to aquatic ecosystems and, consequently, to human health (Matranga et al., 2012). Most data in this field are the result of toxicity studies conducted mainly for short-term exposure periods in model organisms with no commercial interest (Khosravi-Katuli et al., 2017). To date, there is still paucity of knowledge of the molecular pathways involved in the mechanisms associated to NP exposure in aquatic organisms, especially in species with commercial value and produced in aquaculture facilities. The current study work aims to examine the expression of genes involved in several molecular pathways by long-term exposure to TiO_2 NPs with different sizes in one of the most valuable seafood products of European Atlantic coast aquacultures, the turbot *Scophthalmus maximus*.

Materials and Methods

Turbots (*Scophthalmus maximus*) were maintained under controlled conditions in 1 m^3 tanks (50 fish per tank, n=3) with constantly running TiO_2 NPs-free seawater. Citrate-coated titanium dioxide nanoparticles (TiO_2 NPs, Ø 5 nm and Ø 25 nm) were incorporated in the commercial dry pellet diet (0 and 1.5 mg/kg) and fish were fed daily with approximately a food/weight ratio of 1%, periodically adjusted during the experiment taking into account weight gain [average initial weight 45.0 g ± 1.0 g (NPs Ø 25 nm) and 73.0 g ± 0.5 g (NPs Ø 5 nm)]. Liver samples of 3 fish per tank were collected after 14 and 28 days of exposure and stored in RNAlater at -80 ºC. Total RNA was extracted and purified to further cDNA synthesis. Quantitative polymerase chain reaction (qPCR) was performed to analyse gene expression levels of *NRF2* (nuclear factor erythroid 2–related factor 2), *NFkB1* (nuclear factor kappa B subunit 1), *GR1* (glucocorticoid receptor 1), *THRαb* (thyroid hormone receptor alpha b), *PPARαb* (peroxisome proliferator-activated receptor alpha b) and *FASN* (fatty acid synthase). The changes in target gene expression levels in relation to two housekeeping genes [*18S* (18S rRNA) and *EF1α* (elongation factor 1-alpha)] were calculated using the Pfaffl method (Pfaffl, 2001).

Results and Discussion

Given its commercial interest, turbot was used as a model for bioaccumulation assays to study the effects of TiO_2 NPs in the expression of genes involved in several pathways. To assess any minor potential changes, turbots were exposed to TiO_2 NPs at higher concentrations than the expected in nature and European aquaculture systems. The analyses of gene expression levels in turbot liver, a key organ in detoxification processes, were conducted in transcription factors that regulate transcription of genes related to the response to oxidative stress (*NRF2*), immune response (*NFkB1*), endocrine system (*GR1*, *THRαb*), fatty acid β-oxidation (*PPARαb*), and in the fatty acid synthase gene (*FASN*) responsible for fatty acid synthesis. The relative expression levels of *NRF2* and *NFkB1* were higher in presence of Ø 25 nm TiO_2 NPs comparing with the exposure to Ø 5 nm TiO_2 NPs. These observations are in accordance with previous studies which have described a stimulation of immune system with the production of ROS and oxidative damage in fish exposed to TiO_2 NPs (Diniz et al. 2013; Tang et al., 2019; Bobori et al. 2020). Both *THRαb* and *GR1* relative expression levels increased after 14 days of exposure to Ø 25 nm TiO_2 NPs. After 28 days of exposure, the relative expression levels of these transcription factors were equivalent to the control group, suggesting the reversibility of alterations in metabolism and stress response, and an adaptation of fish to the system with the time. In terms of lipid metabolism, the presence of TiO_2 NPs led to a minor reduction of fatty acid β-oxidation activation. However, the production of energy through the breaking down of long fatty acids was stimulated after 28 days of exposure to Ø 5 nm TiO_2 NPs. The relative expression levels of *FASN* were slightly lower after 14 days of exposure, indicating a decrease of long-chain fatty acid production. However, after 28 days of exposure the relative expression levels of *FASN* are comparable to the control. During the Ø 25 nm TiO_2 NPs assay, a reduction in fish

(Continued on next page)

feeding rate was observed, probably due to changes in food palatability, which has affected fish weight gain. Nevertheless, in the Ø 5 nm TiO_2 NPs assay, no differences in fish weight increase were observed. No other behavioural changes were observed during both trials and no mortality or pathological signs were recorded. The exposure of turbots to smaller TiO_2 NPs (Ø 5 nm) resulted in less prominent changes in relative gene expression.

Conclusions

This study demonstrated that the effects on gene expression in turbot liver, the main site for regulating redox, lipid metabolism and detoxification, depend not only on the time of exposure to NPs but also on the NPs size itself. The recovered expression pattern points to a minor and late reactiveness of smaller TiO_2 NPs. Although our results suggest a potential alteration of hepatic function in turbot when exposed to high concentrations of TiO_2 NPs, further studies using metabolomic approaches are needed.

Acknowledgments

This work is funded by NANOCULTURE Project – INTERREG Atlantic Area Program (European Regional Development Fund, ERDF) – EAPA 590/2018.

References

Bobori, D., Dimitriadi, A., Karasiali, S., Tsoumaki-Tsouroufli, P., Mastora, M., Kastrinaki, G., Feidantsis, K., Printzi, A., Koumoundouros, G., Kaloyianni, M., 2020. Common mechanisms activated in the tissues of aquatic and terrestrial animal models after TiO_2 nanoparticles exposure. Environ Int.138:105611.

Diniz, M.S., de Matos, A.P., Lourenço, J., Castro, L., Peres, I., Mendonça, E., Picado, A., 2013. Liver alterations in two freshwater fish species (*Carassius auratus* and *Danio rerio*) following exposure to different TiO_2 nanoparticle concentrations. Microsc Microanal. 19:1131-1140.

Khosravi-Katuli, K., Prato, E., Lofrano, G., Guida, M., Vale, G., Libralato, G., 2017. Effects of nanoparticles in species of aquaculture interest. Environ Sci Pollut Res Int. 24:17326-17346.

Matranga, V., Corsi, I., 2012, Toxic effects of engineered nanoparticles in the marine environment: model organisms and molecular approaches. Mar Environ Res. 76:32-40.

Pfaffl, M. W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic acids research, 29:e45.

Tang, T., Zhang, Z., Xiaopeng Zhu, X., 2019. Toxic Effects of TiO_2 NPs on Zebrafish. Int J Environ Res Public Health. 16:523.

NUTRITIONAL PROPERTIES OF MARINE AND FRESHWATER MICROALGAE FOR BIVALVES' PRODUCTION IMPROVEMENT

Joana Fonseca*, Margarida Costa, Helena Cardoso, Gonçalo Espirito Santo, Inês Guerra, Pedro Cunha, Nádia Correia, Joana Teles, Joana Silva

ALLMICROALGAE Natural Products S.A., Rua 25 de Abril s/n, 2445-413 Pataias, Portugal
Email: joana.fonseca.95@gmail.com

Introduction

In the last years, natural stocks of bivalves have been decreasing due to overfishing, environmental stresses, and diseases. The production of these organisms through aquaculture has become crucial for natural stock recovery and to support human consumption - the main reasons for the high economic growth of this sector. To get an optimized production is essential to have an appropriate blend of microalgae adjusted to each stage of the bivalves' life cycle which represents 30% of the total seed production cost. Microalgae present high diversity of biochemical profiles, therefore, it is essential to explore the potential of different microalgae that can have beneficial biochemical features, key to bivalves' production. Besides the use of microalgae as a live feed, the use of microalgae concentrates and/or dried algae could also be an option that would reduce bivalves' production costs [1].

Material and methods

Five species of microalgae were produced at Allmicroalgae facilities. Marine microalgae (*Nannochloropsis oceanica, Phaeodactylum tricornutum* and *Tetraselmis chui)* were cultivated autotrophically in 35 m^3 outdoor industrial photobioreactors (PBR's). The freshwater microalgae *Chlorella vulgaris* prevenient from 5 m^3 industrial fermenter (heterotrophic culture) was grown autotrophically in 70 m^3 outdoor industrial PBR's and *Arthrospira platensis* were produced at 100 m^3 thin-layer cascade reactors (the only reactor available for *A. plantensis* production at Allmicroalgae). For all species, productivity rate was calculated. Microalgal cells were harvested by centrifugation and spray-dried for biochemical analysis. In order to determine the CHN composition, a Vario el III (Vario EL, Element Analyser System, GmbH, Hanau, Germany) was used following manufacturer instructions. Total protein content was achieved by multiplying the percentage of nitrogen by the 6.25 factor [2]. For the total lipid content, the Soxhlet method was used and carbohydrate content was calculated by subtracting the weight of proteins, lipids and ashes from the total dry weight of biomass. To estimate composition in eicosapentaenoic acid (EPA), the GC-FID method was used, and results are presented as % of Total Lipids.

Results

Among all species produced, freshwater microalgae *C. vulgaris* and *A. platensis* showed the best productivity values (0.083 and 0.077 g/L/day, respectively), however these microalgae were produced in different types of reactors with different cultivation methods which can justify these results. *T. chui* showed the best productivity achieved in the 35 m^3 tubular PBR's (0.058 g/L/day), followed by *N. oceanica* (0.042 g/L/day) and *P. tricornutum* that reached the lowest productivity value observed (0.028 g/L/day). The highest protein content was reached by *A. platensis* and *C. vulgaris* (60-70 and 54-65%, respectively), followed by *T. chui* and *P. tricornutum,* which presented similar values (35-46%). *N. oceanica* presented the lowest protein content (29-38%), however, this species revealed the higher lipid content (12-15%) and showed to be EPA-rich presenting it at 26-35% total lipids, the highest value observed after *P. tricornutum*, with 28-40% EPA. *T. chui* showed 7-9% EPA and the freshwater microalgae *C. vulgaris* and *A. platensis* did not present this polyunsaturated fatty acid in their composition. Regarding carbohydrates composition, *T. chui* showed the highest value (20-32%), followed by *N. oceanica*, which presented 15-20%. On the opposite, the lowest carbohydrate content was reached by *P. tricornutum* and *A. platensis* (3-7 and 2-10%, respectively).

Discussion and conclusions

The five species of microalgae produced at Allmicroalgae facilities showed to be biochemically very diverse. This variability of lipid and protein levels, fatty acids and carbohydrates, bring vast advantages for bivalves' production since they have different needs during their entire life cycle (from larval to adult stages).

Adults fed to obtain gametes for fertilization and posterior larvae development need a protein-rich diet. A possible diet including *T. chui* and *P. tricornutum* (already used for bivalves feeding), complemented with *A. platensis* and *C. vulgaris* could be applied in adult feeding since these are the microalgae with highest protein content. Although both algae are freshwater microalgae, they could be used as concentrates or dried algae [3] which avoid the salinity change of bivalves'

culture medium and promote an easier application of feed. On the other hand, after larvae hatching, *N. oceanica* could be an excellent option to include in a diet with *Tisochrysis lutea* (usually used for larval stages), since it has high EPA content, which is very important for clams such as *R. decussatus* [4]. Furthermore, *N. oceanica* is a marine microalga thus can be used as a live diet, as microalgae concentrate or as dried algae. After metamorphosis, at post-larvae and juvenile stages, the use of a diet composed by *T. chui* would be important for bivalves such as the oyster *Ostrea edulis* that presented higher growth when fed with a diet rich in carbohydrates [5].

To conclude, the production of different microalgae in a reproducible manner throughout the entire year could bring several advantages for bivalve producers. Using suitable microalgae species to each stage of bivalves' life cycle will undoubtedly reduce the costs of production, allowing a reduction of mortality and the achievement of clams, oysters and mussels with high quality and high economical value in a shorter term. However, experimental studies will be crucial to confirm the potential of the microalgae described.

References

1. Robert, R. & Trintignac, P. Substitutes for live microalgae in mariculture: a review. *Aquatic Living Resources* **10**, 315–327 (1997).
2. Richmond, A. & Hu, Q. Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition* 1–719 (2004). doi:10.1002/9781118567166
3. Aji & Parwadani, L. The use of algae concentrates, dried algae and algal substitutes to feed bivalves. *Makara Journal of Science* **15**, 1–8 (2011).
4. Aranda-Burgos, J. A., Da Costa, F., Nóvoa, S., Ojea, J. & Martínez-Patiño, D. Effects of microalgal diet on growth, survival, biochemical and fatty acid composition of *Ruditapes decussatus* larvae. *Aquaculture* **420–421**, 38–48 (2014).
5. Enright, C. T., Newkirk, G. F., Craigie, J. S. & Castell, J. D. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. *Journal of Experimental Marine Biology and Ecology* **96**, 1–13 (1986).

EVALUATION OF THE OPTIMAL DOSE OF THREE ANESTHETICS FOR GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES. EFFECTS ON PLASMA METABOLITES AND LIVER ENZYMES ACTIVITY

Fontinha, F.[1,2*], Magalhães, R.[1,2], Martins, N.[1,2], Pires, R.[1], Diaz-Rosales, P.[2], Oliva-Teles, A.[1,2], Peres, H.[1,2]

*filipafontinha@hotmail.com
[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, Edifício FC4, 4169-007 Porto, Portugal
[2]CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, 4450-208 Matosinhos, Portugal

Introduction

Concerns with animal welfare impose the adoption of practices that reduce stress. Several normal aquaculture practices such as weighing, sorting, transportation, and others provoke stress on fish. In this scope, anesthetic agents have been used to mitigate stress during several routine operations. The present study aimed to determine the optimal dose of three anesthetics (tricaine methanesulfonate, MS-222; 2-phenoxyethanol, 2-PE; and clove oil) to induce deep anesthesia in gilthead seabream (*Sparus aurata*) juveniles. The effects of anesthesia on hematology, plasma metabolites profile, cortisol levels, the hepatic activity of key enzymes of intermediary metabolism, and heat shock protein gene expression were evaluated and compared to non-anesthetized fish.

Material and Methods

Anesthesia induction and recovery time

The anesthesia induction and recovery time of the target anesthetics: 2-phenoxyethanol (2-PE; VWR), tricaine methanesulfonate (MS-222; Sigma-Aldrich), and clove oil (CO) extracted from the *Eugenia caryophyllata* tree, were measured.

MS-222 was mixed (1:2) with 95% sodium bicarbonate to make a stock solution of 1 mg/mL with a resulting pH of 7.3. The stock solution was added into 80-L tanks containing system water to achieve concentrations of 50, 100, 150, 200, and 250 mg/L. 2PE (Merck) was added to the 80-L tank to achieve concentrations of 0.15, 0.3, 0.45, and 0.6 mL/L. Clove oil (BioVer; 100% pure extract) was mixed (1:10) with 95% ethanol for the concentrations 0.2, 0.4, 0.6, 0.8, and 1 mL/L. To achieve the desired concentration, appropriate volumes of 2-PE and stock solutions of MS222 and clove oil were pre-solubilized into the water from the experimental system to facilitate its administration and then added to the anesthetic tank. Tanks containing only system water were prepared for assessing the recovery trial.

For each anesthetic and each concentration, 9 fish were randomly selected and transferred, one at a time, to the anesthetic tank. Fish were considered fully anesthetized (deep anesthesia) when showing a total loss of reactivity, no reaction to handling, and were lying on the bottom of the tank.

Effect of anesthesia on hematology, plasma metabolites, plasma cortisol, and hepatic enzymes activity

Gilthead seabream juveniles with an average body weight of 151g were unfed for 24h and randomly distributed (3 fish per tank) to triplicate anesthesia tanks containing the optimum concentration of each anesthetic (established in the previous trials). The same procedure was applied to the control group (without anesthesia).

As soon as fish reached deep anesthesia, blood was collected for plasma cortisol and metabolites analysis, and liver was collected for enzymes activity and gene expression.

(Continued on next page)

Results

Optimal doses for anesthesia for ongrowing gilthead seabream, were determined to be 0.45 mL/L for 2-PE, 150 mg/L for MS-222, and 0.6 mL/L for clove oil based on the necessary time to reach deep anesthesia and to be fully recovred.

For each anesthetic, induction time was directly related to the anesthetic concentration.

At the lowest concentration, 2-PE had the highest time for induction of deep anesthesia, while clove oil had the lowest anesthesia induction time.

Regardless of dietary treatment, deep anesthesia did not affect hematocrit and hemoglobin levels. Plasma glucose levels were higher in non-anesthetized than in anesthetized fish, regardless of the anesthetics used. Lactate and triglyceride levels were lower in clove oil anesthetized fish than in the other groups. No differences were observed in plasma protein, albumin, and cholesterol levels between non-anesthetized and anesthetized fish.

Alanine aminotransferase and aspartate aminotransferase activities in the control group were lower than in the 2-PE group, while glutamate-dehydrogenase was not affected by treatments. Hexokinase activity was lower in the clove oil group than in the other groups, while glucose-6-phosphate dehydrogenase activity was lower in the clove oil group than in the MS-222 group.

Gene expression of heat shock protein 70 kDa was not affected by the anesthetic treatments.

Conclusion

Relatively to the non-anesthetized fish, glycemia was reduced in all the anesthetized groups. Plasma glucose secretion is recognized as a secondary response to stress.So the reduction of glycemia, plasma cortisol level, lactate and RBC count observed in the fish anesthetized with clove oil, compared to the other anesthetics, suggests that clove oil was the most effective anesthetic tested, as it inhibits the primary and secondary stress responses and its by-products.

Acknowledgments

Present study was funded by FEDER-Operational Programme Competitiveness and Internationalization and FCT under the project SPO3 (ref. POCI-01-0145-FEDER-030377.

Fontinha, F., Magalhães, R., Martins, N., were supported by an FCT grant (2020.07212.BD, SFRH/BD/115870/2016, SFRH/BD/137919/2018, respectively).

STUDIES ON F1 PROGENY OF GYNOGENETIC SIBERIAN STURGEON *Acipenser baerii* FEMALE – APPLICATION OF MOLECULAR ANALYSIS AND GONADAL HISTOLOGY

D. Fopp-Bayat*, J. Szadkowska, M. Szczepkowski, B. Szczepkowska, K. Naumowicz, E. Ziomek

University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn (Poland)
E-mail: foppik@uwm.edu.pl

Introduction

The aim of the present study was application of molecular analysis and gonadal histology for analysis of F1 progeny of a gynogenetic Siberian sturgeon *Acipenser baerii* female. Additionally the gonad morphology and GSI index were analysed in studied fish.

Histological analysis was used to assess gonadal development, sex differentiation and the sex ratio in the progeny of the gynogenetic Siberian sturgeon female. Genetic analysis, based on microsatellite DNA loci genotyping, was carried out for identification of genotypes in Siberian sturgeon offspring. This study provides valuable information on the influence of meiotic gynogenesis on selected reproductive parameters in the progeny of subsequent generations.

Materials and methods

Ovulated eggs, obtained from gynogenetic Siberian sturgeon female were fertilized by sperm of Siberian sturgeon male. Obtained progeny was reared until gonad sampling (~20 months). Samples for histological analyses were collected from 30 randomly selected offspring of the studied gynogenetic Siberian sturgeon female. Different types of sex cells (characteristic for male or female gonads) were identified, and images were acquired. The sexual maturity of all sampled gonads was evaluated according to the procedure described by Sakun and Bucka (1968). The gonadosomatic index (GSI) was calculated based on the body weights and gonad weights of fish, determined upon sampling.

Fin clips from each randomly selected specimen were sampled for genetic analysis and segregation of alleles at five microsatellite loci were analyzed, according to the procedure described by Fopp-Bayat (2007).

Results and discussion

Both female and male individuals were identified in the examined offspring of gynogenetic Siberian sturgeon female. The sex of seven individuals was not identified based on the results of histological analyses, and they were regarded as "unidentified". Only sexually undifferentiated cells were identified in their gonads.

In sturgeon females, oogonia and previtellogenic oocytes were identified, which indicates that all females were in stages I and II of gonad development. Gonads of examined male were significantly more differentiated (in comparison to females gonads) and four developmental stages were identified: 1) stage I characterized by the presence of spermatogonia and undifferentiated gonocytes, 2) stage II, 3) stage II/III, and 4) stage III/IV where all types of male reproductive cells were observed.

Microsatellite genotypes of analyzed individuals were not linked to gender, therefore no sexual-specific genotypes were identified.

The present study set out to determine the degree of sexual maturity in the offspring of the gynogenetic Siberian sturgeon female. In the future, the results should be compared with the observations made in the progeny of other gynogenetic females. These findings will be used to identify potential defects in the gonadal development of the offspring of gynogenetic mothers.

References

Fopp-Bayat D 2007: Verification of meiotic gynogenesis in Siberian sturgeon (*Acipenser baeri*) using microsatellite DNA and cytogenetical markers. J Fish Biol 77: 478-485

Sakun, O.F., Bucka, N.A., 1968. Determinations of maturity stages and study of sexual cycles of fishes - Izd. Min. Ryb. Choz. SSSR, Murmansk. pp. 1-45 (in Russian).

Acknowledgments

This research was financially co-supported by the Minister of Science and Higher Education in the range of the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN".

EFFECTS OF PROCESSING AND EXOGENOUS DIETARY ENZYME INCLUSION IN PLANT MEAL BASED DIETS; PRELIMINARY RESULTS ON GROWTH PERFORMANCE, FEED UTILIZATION, AND NUTRIENT DIGESTIBILITY IN SEABASS *(Dicentrarchus labrax)*

Fountoulaki E.*[a], Vasilaki A.[a], Nikoloudaki Ch., Pyrenis G.[a], Henry M[a]. and Nengas. I.[a]

[a]Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Center for Marine Research, 46.7 Avenue Athinon-Souniou ,19013 Anavissos, Athens, Greece,
*e-mail: efoudo@hcmr.gr

Introduction

The limited availability of marine ingredients for aquafeeds led the feed industry to replace them with sustainable and cost efficient alternatives of different origin, plant or terrestrial. The major limitation in the use of plant raw materials is related to their high amount of anti-nutritional factors (ANF's) such as protease inhibitors, non-starch polysaccharides (NSP) and phytate (Francis et al., 2001), which affect growth parameters, feed utilization, nutrient digestibility etc. Processing technologies of the raw materials and feeds (heating, soaking, extrusion, enzyme addition etc) can be a valuable tool to enhance digestive utilization of nutrients (Kraugerud & Svilus 2011, de Vries et al., 2012). Exogenous enzymes have been investigated in fish (Lemos & Tacon 2015) where addition to diets has resulted in increased utilization of phytate phosphorus, other trace elements and protein, while the potential of NSP enzymes (xylanase) in fish diets as a way to improve growth and feed utilization is limited in fish and specifically in sea bass non existing. The aim of the present study was to investigate the effects of raw material processing as well as enzyme addition in plant meal based diets for seabass (*Dicentrarchus labrax*) on growth performance, feed utilization and nutrient digestibility.

Materials and methods

European seabass juveniles (~19.5g) were fed 5 experimental diets for 3 months at 24^{0}C water temperature, *at libitum*. Two control diets were formulated; a positive (CTRL+) containing 20% fish meal (FM), 24% soya protein concentrate (SPC), and 22% corn gluten (CGM), while the negative control (CTRL-) contained 10% of each of the following raw materials: rape seed meal, sunflower meal, and guar meal replacing almost half of the FM, SPC and CGM which were added at 15%, 12%, and 10% respectively. The raw materials were subjected to thermal processing prior their inclusion in the diet (diet Treated) while in the other two diets the enzymes phytase and xylanase were added by coating in diet Phy-Xyl and a commercial exogenous enzymes complex: Synergen™ (Alltech) in diet Synergen. All diets were isonitrogenous and isoenergetic containing 48% protein, 17% fat.

Results

After 83 days of feeding, fish significantly improved growth performance and feed utilization (SGR, FCR) in those diets where the thermal processing or the enzymes was added compared to the negative control diet (CTRL-). Furthermore Phy-Xyl diet showed significantly higher SGR than the other two diets, Treated and Synergen. Protein efficiency was also improved either by heat treatment of the raw materials or by the enzyme addition, the major effect being evident in diet Synergen. Concerning nutrient digestibility (ADC), protein was high for all diets but significantly higher in the Synergen diet, while fat ADC was significantly higher in the Phy-Xyl diet.

Discussion & Conclusions

The supplementation of exogenus enzymes as feed additives to improve growth, feed utilization, and nutrient digestibility of plant–based feedstuffs has been studied extensively in poultry and swine industry as a way to reduce the anti-nutritional effects of NSP and phytic acid. In the present study it was evident that exogenous enzymes supplementation has a great potential in sea bass by showing to improve significantly SGR and FCR as in other species (Atlantic salmon, Carp, Rainbow trout, Japanese sea bass, African catfish). The effectiveness of the enzymes used in the present study in reducing the anti-nutritional effect of NSP and phytate was more evident for Phy-Xyl diet compared to Synergen and Treated diet. Digestibility of protein was improved by the addition of Synergen as the product contains proteases which facilitate protein digestion while fat digestibility was the highest by the supplementation of xylanase & phytase. In fish endogenous digestive enzymes that hydrolyze NSP seems to be very low or not existing. One of the most important benefits of this enzyme is the reduction of the NSP induced digesta viscosity which result to a better digestibility of the fat.

(Continued on next page)

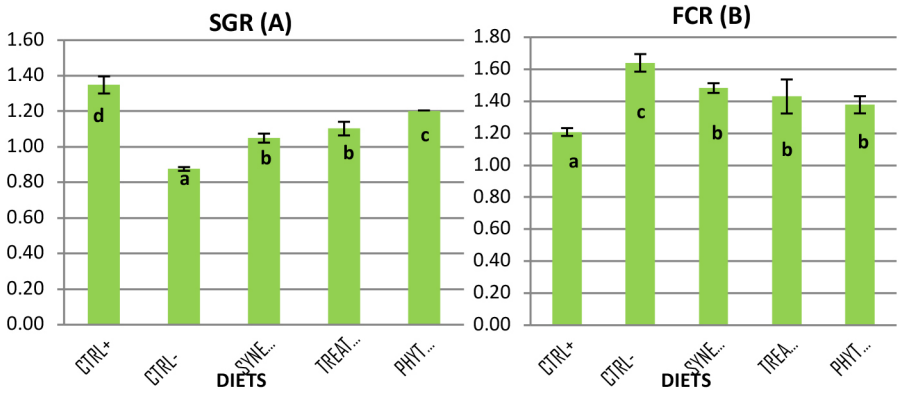


Fig 1 A) Specific Growth Rate and **B)** Feed conversion ratio (FCR) of sea bass fed the tested diets Different letters show significant differences between dietary treatments (ANOVA P<0.05,

References

De Vries, S., Pustjens, A.M., Schols, H.A., Hendriks, W.H., Gerrits, W.J.J., 2012. Improving digestive utilization of fiber-rich feedstuffs in pigs and poultry by processing and enzyme technologies: A review. Animal Feed Science and Technology 178, 123-138.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227. https://doi.org/10.1016/S0044-8486(01)00526-9

Kraugerud, O.F. & Svilus, B., 2011. Effects of online pretreatment of plant ingredients on processing responses and physical properties in extruded fish feed. Animal Feed Science and Technology 168, 250-256.

Lemos, D., & Tacon, A.G.J., 2015. Use of phytases in fish and shrimp feeds: a review. Reviews in Aquaculture 0, 1-17.

This project was funded from theEU Horizon 2020 research and innovation programme under grant agreement No 727610. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

RECOVERY OF HAEMAL LORDOSIS IN EUROPEAN SEA BASS *Dicentrarchus labrax* (LINNAEUS, 1758)

S. Fragkoulis[1]*, Ch. Kourkouta[1], A. Printzi[1], G. Geladakis[1], D. Kerasovitis[2] and G. Koumoundouros[1]

1, Biology Department, University of Crete, Heraklion, Greece.
2, Avramar S.A., PEO Patron-Athinon 55, Agios Vasilios, 26500 Rion, Greece
Email: stefanos_fragkoulis@hotmail.com

Introduction

Haemal lordosis is a frequent vertebral abnormality in finfish aquaculture, with significant and variable effects on fish externally morphology (Fragkoulis *et al*. 2019, 2021). In most of the fish species studied so far, haemal lordosis develops during the late metamorphosis and early juvenile period, mainly due to elevated fish swimming activity (Sfakianakis *et al*. 2006, Palstra *et al*. 2020, Printzi *et al*. 2021). Despite the significance abnormalities for the product quality, it was recently shown that haemal lordosis can substantially recover during the growth of seabream in sea cages (Fragkoulis *et al*. 2019).

In the present study we examined whether haemal lordosis may recover in European sea bass, the species where swimming-induced lordosis was first described (Divanach *et al*. 1997).

Material and Methods

Fifty-six sea bass juveniles (77±6 mm standard length, SL) with lordotic external morphology (dorsally shifted caudal peduncle) were selected from one reared population. All fish were anaesthetised, tagged electronically (FDX-B, Trovan Ltd, USA), photographed on their left side and returned to the sea cage until the end of the on-growing period (234±16 mm SL). At that stage, fish were anaesthetised, photographed and tag-identified. Subsequently, a representative sample of fish with a recovered and lordotic external morphology was radiographically examined.

Results & Discussion

At the end of the examined period (353 dpt), the 57% (32 out of 56) of the fish with severe lordotic morphology presented normal external phenotype, whereas 7% (4 out of 56) presented light lordotic morphology. The rest 36% of the fish (20 out of 56) continued presenting a lordotic external morphology. The radiographic examination of the fish, verified the macroscopically observed lordosis recovery. The 32% of specimens with recovered external morphology (28 out of 41) presented a completely normal vertebral column (Fig. 1Bi), whereas the 18% presented minor abnormalities of individual centra (Fig. 1Bii). The rest 50% of the recovered fish presented a light bending of the vertebral column (Fig. 1Biii).

Concerning severely lordotic fish that turned into fish with light lordotic external morphology, 40% presented light vertebral bending, whereas 60% presented either minor abnormalities of individual centra, severe lordosis, or a counterbalancing kyphosis anteriorly to lordosis (Fig. 1A, 1Bii-Bv). Finally, all fish with severe lordotic external morphology, presented a severe vertebral bending (Fig. 1Bv).

In the present study we showed that the external morphology of lordotic sea bass juveniles may completely recover during the on-growing period. The recorded recovery rate of lordosis in sea bass (57%) is higher than that previously reported for seabream (44%, Fragkoulis *et al*. 2019). Fragkoulis *et al*. (2019), assumed that the rearing in the sea cages, might be responsible for the recovery, due to the adaptability of the vertebrae in the less intense water current velocities, compared to conditions in the tanks. Our results do not contradict this hypothesis, since the recovery was observed during the on-growing period. Similarly, our radiographic findings on the anatomy of the lordosis recovery are in total agreement with Fragkoulis *et al*. (2019), with the same phenotypes observed in both studies. Our results show that lordosis recovery is not species-specific and has to be taken into consideration during the quality control at the end of the hatchery phase. Further morphometric analysis in the future, could provide useful indices for the accurate discrimination between abnormal fish, on the basis of their recovery potential.

(Continued on next page)

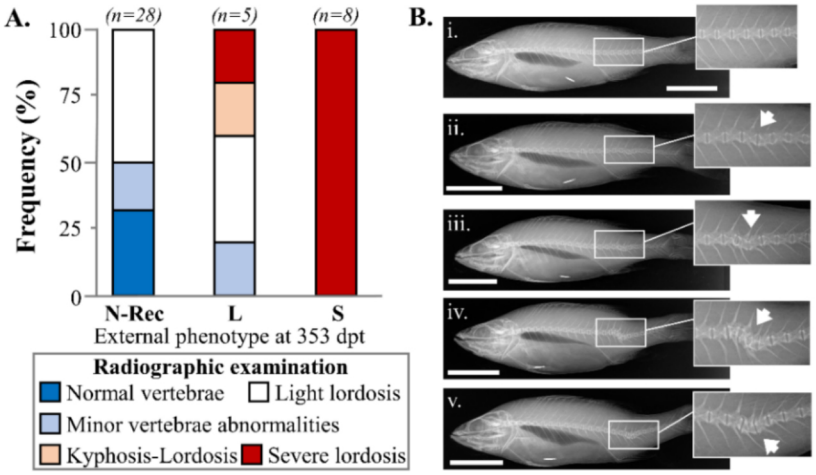


Fig. 1. (**A**) Radiographic categorization of the initially lordotic fish at the end of the trial (353 dpt). N-Rec, L or S, fish with recovered, light-lordotic or severe-lordotic external morphology respectively. (**B**) Representative photographs of the observed radiographic phenotypes. i, normal. ii, abnormalities of individual centra. iii, light internal lordosis. iv, kyphosis and lordosis. v, fish with abnormal external morphology and severe internal lordosis. Scale bars= 5 cm.

Acknowledgments

This study was financially supported by EU (European Maritime and Fisheries Fund (EMFF) and national (Greek) funds (NSRF 2014-2020, call Novelty in Aquaculture, Project No. 5010952) of the Ministry of Rural Development and Food, Greece.

References

Divanach P, Papandroulakis N, Anastasiadis P, Koumoundouros G, Kentouri M (1997) Effect of water currents during postlarval and nursery phase on the development of skeletal deformities in sea bass (*Dicentrarchus labrax* L.) with functional swimbladder. Aquaculture 156:145-155.

Fragkoulis S, Printzi A, Geladakis G, Katrimpouzas N, Koumoundouros G (2019) Recovery of haemal lordosis in Gilthead seabream (*Sparus aurata* L.). Sci. Rep. 9, 9832.

Fragkoulis S, Koumoundouros G (2021) Simple morphometrics for predicting lordosis-induced deviations of body shape in reared Gilthead seabream (*Sparus aurata* L.). J Fish Dis. 2021; 00:1–3.

Sfakianakis DG, Georgakopoulou E, Papadakis I, Divanach P, Kentouri M, Koumoundouros G (2006) Environmental determinants of hemal lordosis in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquaculture 254:54-64.

Palstra AP, Roque A, Kruijt L, Jéhannet P, Pérez-Sánchez J, Dirks RP (2020) Physiological effects of water flow induced swimming exercise in seabream *Sparus aurata*. Front. Physiol. 11:610049.

Printzi A, Fragkoulis S, Dimitriadi A, Keklikoglou K, Arvanitidis C, Witten PE, Koumoundouros G (2021) Exercise induced lordosis in zebrafish *Danio rerio* (Hamilton, 1822). J. Fish Biol. 98:987–994.

INTEGRATION OF DIFFERENT DATA SOURCES FOR MANAGEMENT OF OFFSHORE BIVALVE AQUACULTURE AT SAGRES, PORTUGAL

Bruno Fragoso[1,2*], John Icely[1,2], Priscila Goela[2], Tegan Blount[2], Sonia Cristina[2], Sergei Danshenko[2], Carla Freitas[3], Gerald Moore[4]

[1]Sagremarisco Lda., 8650-999 Vila do Bispo, Portugal
[2]Centre for Marine and Environmental Research (CIMA), University of Algarve, 8005-139 Faro, Portugal
[3]Aquaexam Lda., Centre for Regional Innovation of the Algarve (CRIA), University of Algarve, 8005-226, Faro, Portugal
[4]BioOptika, Crofters, Gunninslake, PL18, UK
E-mail: fragoso.b@gmail.com

Introduction

There is an increasing effort to integrate environmental data obtained from diverse sources to improve management systems for aquaculture. As part of this effort, the EU Horizon 2020 programmes has funded research projects to develop technological innovations for sustainable growth in the aquaculture sector as a contribution to precision aquaculture in the blue economy. Green Aquaculture Intensification in Europe (GAIN) is one of these projects which includes as one of its study sites, an offshore longline system in the SW of Portugal for the aquaculture of bivalves.

In the case of the offshore aquaculture industry, there are substantial benefits to continuous monitoring of conditions for water quality and productivity. On one hand, regular manual sampling allows for an accurate and assessment of the status of the water quality at the moment of collection, offering the possibility of having additional measurements, if required for a specific parameter. However, recent studies indicate that low frequency data collection in aquaculture might not be sufficient to provide an adequate image of the relevant biogeochemical processes for an improved production (Sampaio *et al*., 2021)producers should adopt tools and protocols for environmental monitoring and management of these enterprises. There are currently issues concerning the efficacy of data collection procedures and limnological sampling at low frequency, which is widely used by managers and aquaculture surveillance agencies. In this context, the present study evaluated the effectiveness of high-frequency (HF. Continuous monitoring with data logger-based sensors deployed at the bivalve aquaculture sites overcome this limitation but such equipment requires frequent maintenance and are often subject to adverse weather conditions which might cause severe interruption in data acquisition. Satellite remote sensing data, with its synoptic view of neighbouring phenomena, is especially useful to forecast short-term adverse or beneficial conditions for the cultured species, however its accuracy is significantly decreased by adjacency effects and periods of cloud cover, especially if offshore aquaculture is located near the coast (Icely et al.,2013). Blending the three approaches might be best for periods with cloud-cover to overcoming the caveats for the individual monitoring techniques, thereby, providing enhanced view of the biogeochemical processes responsible for cultured species production and to manage and mitigate risks with adverse oceanographic conditions.

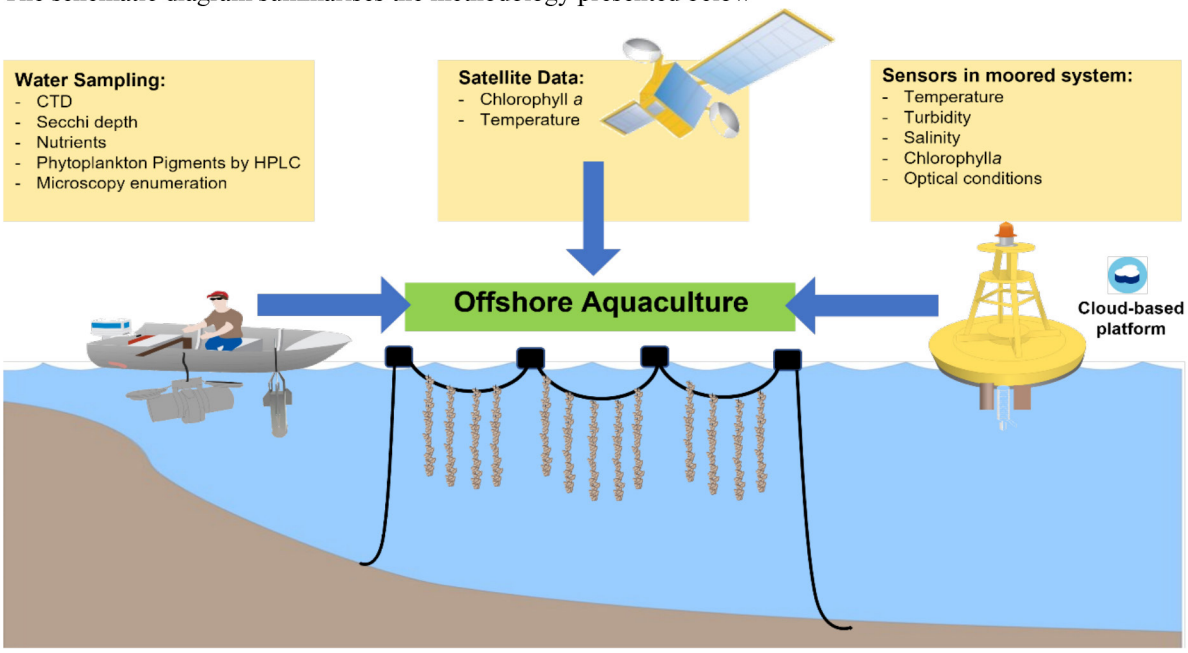
This poster shows how historical data, as well a sampling programme funded at an offshore longline for mussel aquaculture at Sagres SW Portugal, have contributed to the objectives of the project.

Methodology

Data was collected from June 2018 till October 2020. In the case of offshore longlines for mussel aquaculture at Sagres, SW Portugal, the data are supplied from a solar powered data logger with sensors installed on a signal buoy for offshore mussel aquaculture to estimate sub-surface temperature, salinity, fluorescence, turbidity, and optical conditions. Data from these sensors are transmitted every two hours to a cloud-based platform or downloaded manually and subsequently transferred to the platform. Classical oceanographic sampling has also been implemented to calibrate and validate the sensor's data, with the deployment of: a CTD for conductivity and temperature profiles; a Secchi disc for water clarity; and a Niskin flask for water samples to estimate chlorophyll, total suspended matter, nutrients, as well as pigment and microscopical analysis to determine the microplankton community. This *in situ* data has also been complemented with satellite data, again from different sources such as the Copernicus Marine Service (EU) and the National Oceanic and Atmospheric Administration environmental data access programme (USA).

(Continued on next page)

The schematic diagram summarises the methodology presented below



Results and Discussion

With this approach, it has been possible to compare the quality of data obtained from different sources. For example, how does chlorophyll obtained from near real time fluorescence measurements compare with chlorophyll products from satellite images, and chlorophyll estimates with spectrophotometric and High-Performance Liquid Chromatographic (HPLC) techniques for *in situ* water samples; there is generally good agreement between datasets, with spectrophotometric measurements overestimating Chlorophyll *a* values for higher ranges, as expected (Santos *et al*., 2003). These data are now available to reduce uncertainties associated with models that are available for farmers to improve their farming practices.

Microscopic analysis revealed interesting results, especially concerning HAB groups. *Pseudo-nitzschia* blooms were detected in August 2019, June and August 2020, both coinciding with high levels of Chlorophyll a (2-5 $mg.m^{-3}$). Alert levels of *Gymnodinium catenatum* were found in the end of October 2020 (960 $cell.l^{-1}$), whereas values of Chlorophyll *a* were of 0.8 $mg.m^{-3}$. The relation of these communities with other pigments such as peridinin is in progress. These observations confirm that the relationship between Chlorophyll *a* (the *proxy* for phytoplankton community used by optical sensors) and HAB is complex, reinforcing the importance of maintaining *in situ* sampling in aquaculture monitoring programs to complement optical sensors (buoy and or satellite based) information.

Conclusion

The data from the Sagres site has contributed to precision products developed over the duration of the GAIN project, some of which are being presented in oral contributions at this conference. For example, Fearghal et al "A cloud platform for precision aquaculture" led be IBM Research and Ferreira et al. "AquaSense – a real -time precision aquaculture platform for industry" led be Longline Environment Ltd.

References

Icely, J.D.,*et al*., 2013. Contribution of remote sensing products to the management of offshore aquaculture at Sagres, SW Portugal. Ed. H. Ouwehand Proceedings of ESA Sentinel-3 OLCI/SLSTR and MERIS/(A)ATSR Workshop European Space Agency 6pp.

Sampaio, F. G. *et al*. (2021) 'Unveiling low-to-high-frequency data sampling caveats for aquaculture environmental monitoring and management', *Aquaculture Reports*. Elsevier, 20, p. 100764. doi: 10.1016/J.AQREP.2021.100764.

Santos, A. C. A. *et al*. (2003) 'Comparison of three methods for Chlorophyll determination: Spectrophotometry and Fluorimetry in samples containing pigment mixtures an spectrophotometry in samples with separate pigments through High Performance Liquid Chromatography. | Instituto de Botân', *Acta Limnologica Brasiliensia*, 15(3), pp. 7–18.

IMPROVEMENT OF THE LARVAL REARING PROTOCOLS FOR SEABASS (*D .labrax*) THROUGH THE EARLY INTRODUCTION OF A DRY DIET, IMPROVING SURVIVAL RATES, GROWTH AND FRY QUALITY

G. Franchi[1*], T. De Wolf [1], V. Carbone[1], S. Lenzi[1], D. Troiano, S. Debono[2], J. Teske[2] and G. Rombaut[2]

[1] Inve Aquaculture Research Centre, Via P.Gigli snc, 57016 Rosignano Solvay (LI), Italy
[2]INVE Technologies NV, Hoogveld 93, 9200 Dendermonde, Belgium
E-mail: g.franchi@inveaquaculture.com

Introduction

The European Seabass, *Dicentrarchus labrax*, is the second most important marine finfish species produced in the Mediterranean area with and annual fry production of over 500million. The majority of the fry are produced using the Green Water Technique (algae, rotifers and Artemia), but around 20% is produced without algae and rotifers, keeping the larvae in the dark until around 9dph and starting first exogenous feeding with Artemia instead of rotifers. This technique is normally resulting in a slower growth of the larvae and lower robustness compared to the green water technique.

In this study, new feeding protocols were developed to improve the performance of Seabass during larval rearing without the use of rotifers, introducing a rotifer substitution diet from the time of mouth opening. For the classic green water technique, a variant was studied introducing the new diet at 5dph and reducing the normal rotifer quantities with around 50-60%.

Materials and methods

Seabass experiment

Nearly hatched Seabass larvae, originating from the same pool of eggs, were stocked at the a density of around 80 larvae.l^{-1} in 1,000l larval rearing tanks. Four different feeding protocols were used:

1. Full live food Control using a standard diet from 18dph onwards
2. Improved Live food Control with 50% Rotifer Substitution and using the new diet from 4dph
3. Clear water technique without rotifers using the Rotifer substitution diet
4. Green Water technique without rotifers using the Rotifer substitution diet

In all treatments, *Artemia* has been fed from 10dph onwards. A photoperiod of 14L/10D was used and the treatments were done in triplicate.

During the larval rearing period, weekly biometrics were carried out to compare growth rates. A salinity stress test was done at the end of the trial to determine the stress resistance. At 56dph, larval survival, produced biomass per tank and deformity levels were evaluated.

Results

The growth of the Seabass larvae during the period of 56days showed a superior growth up to 14dph for the Full Live Food Control. Later, the growth accelerated in the treatments where the rotifer substitution diet was used. At 56dph, the treatment with 50% rotifer substitution had the lowest weight, even if the difference with the other treatments was not significant.

Average survival rate was highest for treatment 4, without rotifers, using the rotifer substitution diet and green water (65%). The lowest survival rate was obtained in the clear water treatment (45%). The survival rate of the improved LFC was slightly higher than the standard LFC (55 versus 52%). Combining the data of survival and average weights, tank biomasses were compared and lowest values were obtained for the Clear water technique. The highest biomass was obtained in the Green water technique without rotifers, using the new diet at the start of exogeneous feeding. Tank biomasses for the other two treatments showed intermediate and similar values.

Regarding the stress resistance, treatments without rotifers or with low amounts of rotifers, but adding the dry diet from the early beginning, were performing like the LFC.

Deformity levels showed a higher percentage of head deformities in the Clear water treatment, where no algae were used. The treatment showing the highest percentage of fish without any deformity was the improved Live Food Control, using the new diet fed from mouth opening. In the treatments where no rotifers were fed, the green water treatment showed significant less deformities compared to the clear water treatment.

(Continued on next page)

Conclusions

This study shows that Seabass larvae can be reared obtaining a high survival rate and good fry quality when the rotifer substitution diet is used from the first days of exogeneous feeding. The treatments where no rotifers were used, but in which high-quality marine algae were added during the larval rearing water until 11days post hatch, resulted in the best treatment. The treatment with 50% of rotifer substitution performed less in terms of growth compared to the standard LFC, but the quality of the produced fry improved in terms of deformities. The lower growth could be explained by the fact that the Seabass larvae preferred rotifers above the rotifer substitution diet when administered in the same period. As such, a lower amount of dry feed was ingested, resulting in a suboptimal growth.

The use of high-quality marine algae is fundamental to obtain optimal growth and quality in the larval rearing of Seabass. Even in the absence of rotifers, but using algae and the rotifer substitution diet, results are excellent. This allows the simplification of larval rearing protocols for Seabass without compromising the growth, neither the quality of the produced fry.

WHO IS THE BEST SURROGATE FOR GERM STEM CELL TRANSPLANTATION IN FISH?

R. Franěk[*1], Y. Cheng[1], M. Fučíková[1], V. Kašpar[1], X. Xie[1], M.A. Shah[1], O. Linhart[1], I. Šauman[2,3] and M.Pšenička[1]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25, Vodňany, Czech Republic
E-mail: franek@frov.jcu.cz
[2]Biology Center of the Academy of Sciences of the Czech Republic, Institute of Entomology, Branišovská 31, 370 05, České Budějovice, Czech Republic
[3]Faculty of Science, University of South Bohemia, Branišovská 31, 370 05, České Budějovice, Czech Republic

Introduction
Surrogate reproduction technology in fish has potential for aquaculture as well as endangered species preservation and propagation. Species with 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. One of the general prerequisites for the successful surrogacy and the pure donor-derived gamete production is the sterility of the host. Various sterilization methods have been developed and used in fish surrogacy; however, a direct comparison of available methods is missing. Such a knowledge gap hinders choice for the surrogate in various fish species, including those in high commercial demand such as tuna or sturgeons, where is a particular limitation from the point of the live material availability and difficulty to perform a high throughput assessment of different surrogates. Yet, large sturgeons or tuna species are one of the most prominent candidates for surrogacy.

Materials and methods
Transgenic zebrafish expressing EGFP protein in germ cells exclusively was utilized in this study as a model species to answer whether and to which extent different sterilization strategies can affect the surrogacy. Germ cell-depleted recipients (produced using knockdown of *dead end* gene) (MO), triploid recipients (3n), and zebrafish x pearl danio hybrid (H) recipients were tested as they represent the most frequently used types of surrogates. Spermatogonia isolated from vas::EGFP transgenic strain were intraperitoneally transplanted into swim-up 5-day old zebrafish. Transplantation success, survival, gonadal development, and reproductive output of the fish was analysed.

Results
Post-transplantation survival in MO T group was comparable to the controls, while survival performance of 3n T, H T and control groups was slightly lower. Altogether, overall survival from transplantation to 6 months of age was in all groups (included transplanted groups) from 65 to 85 %. The transplantation success evaluated two-week post-transplantation showed consistent results across different sterilization methods of the EGFP positive cells in recipients. Most of the EGFP positive cells were located in the posterior or medial part of the body cavity. The highest incidence of adult germline chimeras was observed in the MO T group, followed by 3n T group. Interestingly, % of adult germline chimeras in H T and AB T group were almost equal Intraperitoneally transplanted GSCs were capable of establishing donor-derived spermatogenesis in all tested sterilization treatments as well in non-sterilized AB recipients. Observed gross gonadal development was prominent MO T group which gained the largest increment in gonadal development (comparing transplanted group with their respective sterilization control). In MO T group, transplanted GSCs were able to frequently reconstitute spermatogenesis unilaterally or even bilaterally into fully developed testes in term of length and width.

The sperm concentration and total amount of produced sperm in germline chimeras was influenced by the fact that the testes comprised of donor-derived germ cells are not reaching their full size compared to controls. MO recipients males produced highest volume of sperm, concentration of spermatozoa, total number of spermatozoa and finally also total motile spermatozoa among tested sterilized recipients (Fig. 1).

Comparison of transplanted recipients with AB and vas::EGFP controls showed poor performance of H T recipients, in semi-artificial fertilization trials, while MO T and 3n T males showed performance comparable to one of the controls (AB C or vas::EGFP). In vitro fertilization resulted in higher progeny production in all groups including controls. Importantly, the percentage of swim-up larvae was statistically comparable amongst all groups except the H T group.

(Continued on next page)

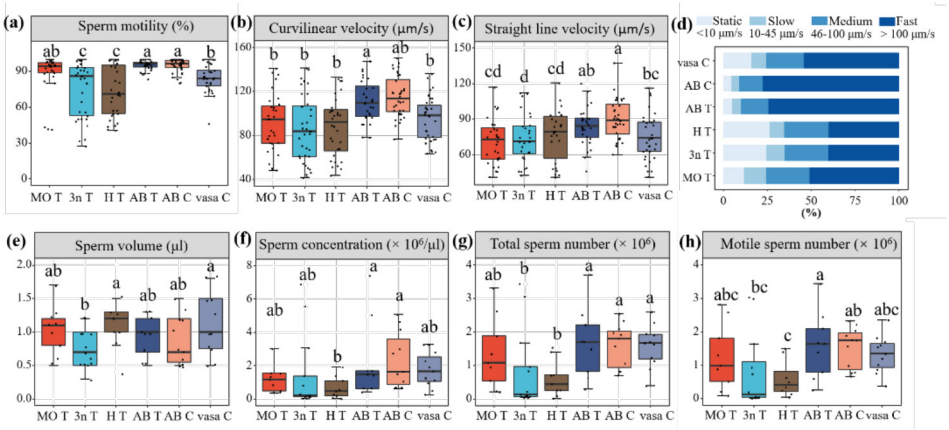


Figure 1. Sperm and spermatozoa motility parameters in germline chimeras and controls.

Discussion

GSCs 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 identify best sterilization treatment - essential factor influencing the surrogacy success rate. The presented study assessed various sterilization treatments in fish for surrogates preparation and their influence on gonadal development and reproductive output in germline chimeras. Of the utmost importance, germ cell-free gonads were identified as the best environment for transplanted cells yielding the highest transplantation success and gonadal development. Importantly, reproductive performance of males including quantity and motility parameters and fertilization rate clearly favours germ cell depleted recipients. The use of triploid and hybrid males from the point of view of the production of sufficient quantity and quality sperm proves to be risky to achieve stable results. Moreover, only germ cell depleted recipient retained reproductive characteristics of the donor strain. Presented findings should help in decision on what type of sterilization should be used prior to transplantation and surrogacy induction, especially in non-model fish species.

Funding

The work was supported by National Agriculture Agency project number QK1910428, by the Ministry of Education, Youth and Sports of the Czech Republic - project Biodiversity (CZ.02.1.01/0.0/0.0/16_025/0007370).

LEARNING FROM THE DEVELOPMENT OF MARICULTURE IN GREECE

K. Frangoudes*[1] and A. Conides[2]

[1] UMR AMURE, IUEM, Université de Brest, rue Dumont d'Urville, 29280 Plouzané, France
Katia.Frangoudes@univ-brest.fr
[2] Hellenic Center for Marine Research, Greece

Introduction
The development of mariculture in Greece started in the 80's. This new activity appeared as a financial opportunity and attracted investors among which few fishers. Private investments supported by EU subsidies contributed to the creation of a high number of aquaculture units of different size in the whole country. The creation of each aquaculture unit was subject to multiple authorizations given by different ministries (fisheries, archaeology, maritime transport, economy, etc.) and an acceptance by the municipality. Since then all aquaculture units were created without any specific marine planning and they often caused negatives reactions from local residents.

Methodology
Through interviews realised in the west coast of Greece with mariculture farmers, civil servants of district and regional fisheries authorities, natura 2000 employees, touristic activity and fishers we tried the capture the nature of the arguments used in favour and against aquaculture during the first period and nowadays. Additional interviews with national authorities (ministries of environment and agriculture and fisheries) and aquaculture experts brought more light concerning expectations about the law related to marine spatial planning which is still not implemented in the country despite the high demand of farmers.

Results
The analysis of this qualitative data will discuss first the current perspectives of development of mariculture in Greece in relation to marine spatial planning and second, based on the Greek example, provide information to others Mediterranean countries wishing to develop mariculture about how to overcome and mitigate social protests.

GENOMIC SELECTION OF RESISTANCE TO *Flavobacterium columnare* IN RAINBOW TROUT USING LOW DENSITY SNP PANELS

C. Fraslin[1]*, A. Kause[2], A. Nousiainen[2], H. Koskinen[2], R.D, Houston[1]

[1] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh United-Kingdom
[2] Natural Resources Institute Finland (Luke)
Email: clemence.fraslin@roslin.ed.ac.uk

Introduction

Columnaris disease (CD), caused by *Flavobacterium columnare* is an emerging disease affecting rainbow trout (*Oncorhynchus mykiss*) aquaculture worldwide. With no commercial vaccine available so far, selection to improve host resistance is of major importance for trout farmers. In aquaculture breeding, genomic selection has been increasingly used for traits that are difficult to measure on candidate fish (such as disease resistance traits). Previous studies suggested that rainbow trout resistance to CD could be improved by selective breeding, and that genomic selection was a useful approach to speed up this process (Silva et al., 2019). However, genomic selection can be prohibitively expensive as it requires genotyping of a large number of fish with high density genotyping arrays. The aim of this study was to assess the efficiency of genomic selection using low-density SNP panels for this disease resistance trait.

Material and methods

In May 2019, 105 rainbow trout families (from 33 dams and 48 sires) were produced from the Finnish national breeding programme maintained by Luke and their eggs were pooled. In June 2019, around 30,000 fry were separated into three fingerling tanks at the farm of Hanka-Taimen Oy (expected average of ~100 fish per family per tank). The fish were monitored daily for mortality, and when suspected signs of *F. columnare* were observed, the dying fish were sent to a veterinarian for a diagnosis. Once the presence of *F. columnare* was confirmed, the fish were treated with an approved treatment against CD to stop the outbreak. Simultaneously, about 510 fish with clear signs of CD per tank were randomly sampled among fish that died during the first 5 days of the disease outbreak. A piece of tail was sampled from those 1,531 fish for DNA extraction and genotyping. Thereafter, the fish rearing continued in the tanks, and in September-October 2019 tissue sample of 1,519 live randomly sampled fish were collected among the surviving fish. In total, 3,054 challenged fish and 81 parents were genotyped using the 57K SNP Axiom™ Trout Genotyping Array. After quality controls (QC), 27,907 SNPs were retained and used as the HD panel.

Resistance was analysed as a binary trait (0=alive; 1=dead) with the rearing tank as a fixed effect in the statistical model. The (genomic) estimated breeding values [(G)EBV] of fish were obtained using pedigree-based BLUP and genomic BLUP (GBLUP) computed with BLUPF90 software (Misztal et al., 2002). The efficiency of pedigree-based or genomic prediction was estimated using Monte-Carlo "leave-one-group-out" method by removing the known phenotype from 20% of the fish, and then using the remaining 80% fish and their information to predict the (G)EBVs of the 20% validation fish group. This was repeated 20 times. Accuracy of prediction was computed as the mean over the 20 replicates of the correlation between the (G)EBV and the true phenotype of fish in the validation group, divided by the square root of the genomic based heritability.

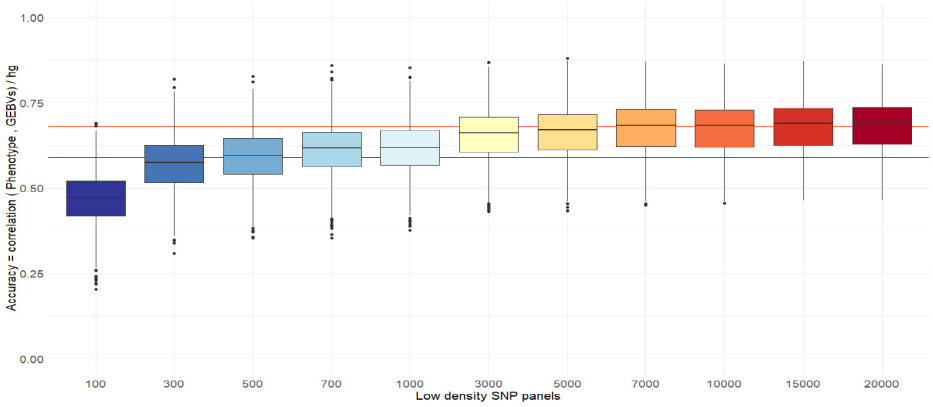


Figure 1. Accuracy of genomic prediction for different low-density SNP panels. The red line is the average accuracy for GBLUP using the HD panel, the blue line the average accuracy for PBLUP.

(Continued on next page)

The impact of reducing the SNP density on genomic prediction was tested with eleven low to medium density *in silico* SNP panels. For each panel, SNPs were sampled from the 28K QC-filtered SNPs from the HD panel using the CVrepGPAcalc package (Tsairidou et al., 2020). The SNPs were randomly sampled within each chromosome without replacement, with the number of SNP sampled from a given chromosome being proportional to the physical length of the chromosome in the *O. mykiss* reference genome (Omyk_0.1, (Gao et al., 2018)). For each target density, 10 replicate panels were generated, which were allowed to overlap by chance. Genetic parameters and GEBVs were estimated as described above for each low-density panel.

Results and discussion

Pedigree based heritability was estimated to be 0.18 (± 0.038) on the observed scale, and genomic heritability was estimated to be 0.21 (± 0.029) on the observed scale. Pedigree-based prediction accuracy was 0.59 (± 0.080) and the use of genomic evaluation increased the prediction accuracy by 14% for the GBLUP (0.68 ± 0.076). Decreasing the number of SNPs used for the prediction tended to decrease the accuracy of genomic prediction (Figure 1). Accuracies obtained with 300 to 1000 SNPs were close to the accuracy of PBLUP. However, prediction accuracies obtained with SNP density panels from 3K and above were close to the accuracy obtained with the HD panel, suggesting that 3K may be a cost-effective SNP density at which to genotype in future studies and commercial practice.

Conclusion

In this study, we showed that using low density SNP panels (down to 5K or even 3K) would results in comparable accuracy than with the 28K HD SNPs panel. Reducing the SNP density is one way towards a more affordable genomic selection without losing efficiency. Approximately 3K SNP panels may provide a more affordable route to genomic prediction of breeding values with small compromise in accuracy.

Acknowledgment

This study is part of the AquaIMPACT project and was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 818367. The skilled staff of Savon Taimen Oy and Hanka-Taimen Oy are thanked for their expertise in data collection and fish rearing.

References

Gao, G., Nome, T., Pearse, D.E., Moen, T., Naish, K.A., Thorgaard, G.H., Lien, S., Palti, Y., 2018. A New Single Nucleotide Polymorphism Database for Rainbow Trout Generated Through Whole Genome Resequencing. Front. Genet. 9. https://doi.org/10.3389/fgene.2018.00147

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D.H., 2002. BLUPF90 AND RELATED PROGRAMS (BGF90). Presented at the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, p. 2.

Silva, R.M.O., Evenhuis, J.P., Vallejo, R.L., Gao, G., Martin, K.E., Leeds, T.D., Palti, Y., Lourenco, D.A.L., 2019. Whole-genome mapping of quantitative trait loci and accuracy of genomic predictions for resistance to columnaris disease in two rainbow trout breeding populations. Genet. Sel. Evol. GSE 51, 42. https://doi.org/10.1186/s12711-019-0484-4

Tsairidou, S., Hamilton, A., Robledo, D., Bron, J.E., Houston, R.D., 2020. Optimizing Low-Cost Genotyping and Imputation Strategies for Genomic Selection in Atlantic Salmon. G3 GenesGenomesGenetics 10, 581–590. https://doi.org/10.1534/g3.119.400800

MASS FINGERPRINT-BASED APPROACH FOR ORIGIN DISCRIMINATION OF MADEIRA AQUACULTURED *Sparus aurata*

Jorge Freitas[1*], Pedro Silva[1], Rosa Perestrelo[1,4], Paulo Vaz-Pires[2,3], José S. Câmara[1,4]

[1] CQM – Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal
[2] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3] CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros de Leixões, Av. General Norton De Matos, S/N, 4450-208 Matosinhos, Portugal
[4] Departamento de Química, Faculdade de Ciências Exatas e Engenharia, Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal

Email: jsc@staff.uma.pt

Introduction:
Even though considered illegal and several regulatory guidelines are in enforcement, food fraud is still a recurrent practice through all food supply chain. In the case of seafood products, missing the identification of species and repackaging of products, constitute the most common frauds. Therefore, the development of appropriate analytical approaches, to be used against food fraud is necessary[1]. The aim of the present study is to evaluate, for the first time, the possibility to differentiate between fish from two different mariculture farms located in Madeira island, using mass fingerprint of fish mucus combined with multivariate analysis.

Material and Methods:
Sparus aurata mucus was collected at two different farms both explored by IlhaPeixe SA. One located at Baia D'abra, Caniçal (32°44'31.4"N 016°41'26.7"W), and the other at Campanário, Ribeira Brava (32°39'38.1"N 017°03'22.1"W). A volume between 5-10ml of mucus were collected at fish capture. Mucus samples were cleaned with a chloroform/methanol precipitation method. The aqueous layer and pellet were analyzed using a MALDI-TOF MS. The MALDI full spectra were exported and analyzed using MASS-UP software, for biomarker discovery and multivariate analysis.

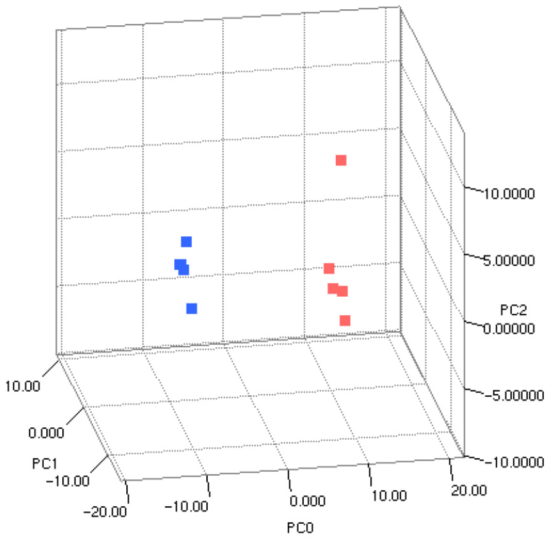


Figure 2 - PCA results for mucus samples analysis. Blue – Caniçal. Red - Ribeira Brava

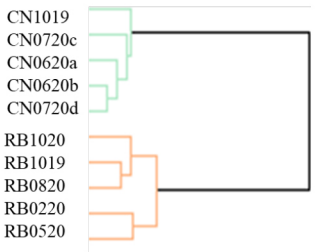


Figure 1 - HCA results of mucus samples. Code represents location, month, and year.

(Continued on next page)

Results:
Figure1, is the representation of the PCA analysis results for the data containing samples from Caniçal (blue) and Ribeira Brava (red). The eigenvalue for the three first components are PC0-395.4, PC1- 63.0 and PC2-60.0. The variances were 0.51, 0.082 and 0.078, respectively. The figure 2 shows the hierarchical cluster analysis (HCA) obtained from the analysis of the peaks lists. The results of the agglomerative HCA were expressed as dendrograms, for better results representation. It was also possible to obtain a list of potential biomarkers for each location, a total of 35 peaks with 17 for Caniçal and 18 for Ribeira Brava.

Conclusion:
The results indicate that there are differences between the mass peaks of samples from different origins. The discriminant peaks are good candidates' biomarkers to differentiate between the two sea farm sites. Further analysis must be done in order to access if the discriminant peaks are still detected at selling points or if others will arise, mostly influenced by the product degradation.

Acknowledgements:
This work was supported by FCT-Fundação para a Ciência e a Tecnologia through the CQM Base Fund - UIDB/00674/2020, and Programmatic Fund - UIDP/00674/2020, and by ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, through the project M1420-01-0145-FEDER-000005 - Centro de Química da Madeira - CQM+ (Madeira 14-20 Program). The authors also acknowledge the financial support from FCT - Fundação para a Ciência e Tecnologia and Madeira 14-2020 program to the Portuguese Mass Spectrometry Network through PROEQUIPRAM program, M14-20 M1420-01-0145-FEDER-000008). The authors also acknowledge ARDITI and IlhaPeixe S.A., through the support granted under the M1420 Project-09-5369-FSE-000001 - for PhD grant to Jorge Freitas.

References:
1 Freitas, J., Vaz-Pires, P., Câmara, J.S, Aquaculture., p 734857, (**2020**). https://doi.org/10.1016/j.aquaculture.2019.734857

SOLUTIONS TO A BETTER FUTURE FOR AQUACULTURE AT MADEIRA ARCHIPELAGO

A. Friedrich*

The Oceanum Portugal Project,
E-mail: arthurfriedrich@gmail.com

The future of Aquaculture at Madeira Island

The consumption of dried codfish is a long time tradition in Portugal. Codfish is the most frequently consumed fish at Madeira Archipelago. Curiously, codfish does not inhabit Madeira's seawaters. Norway is the source of the majority of codfish consumed on Madeira, where it grows in aquaculture farms.

Although Madeira's population is a large consumer of aquaculture products, raise fish in the archipelago's waters is not simple. Besides the open water challenges, aquaculture producers face political and social pressures. Accordingly to local newspapers, popular associations and political oppositions parties declare themselves against aquaculture sea-cages. The protesters claim the removal of aquaculture sea-cages assuming they harm the sea view and are prejudicial to tourism and the environment, while few jobs are generated. Opposition to aquaculture is not exclusive of Madeira's Island, but at the archipelago, it affects the picture policymakers, entrepreneurs and scientists want to draw as the future of aquaculture, not permitting the activity to achieve its full potential.

The future of aquaculture is only possible if objections are considered but also contested in a process to construct the desired outcome. The social process of finding consensus is strategy making. The stakeholders must create the strategic decision process, which will guide the implementation of actions necessary to create the best version of aquaculture.

Strategy making as a tool

To create strategy, though, the stakeholders must be on the same page, which is only possible throughout education. A variety of initiatives have been developed in recent years to promote ocean literacy and promote educational activities. One of them is the Young4Ocean Forum.

The Young4Ocean Forum is an online platform supported by the European Commission for young EU changemakers passionate about the sea. The common goal is to shape a future with a healthy ocean that sustains us all. The Young4Ocean is an opportunity for the youth to speak up for their generation, share ideas and present their projects. It also permits the accreditation as an EU Young Ocean Advocate, which provide access to additional benefits such as coaching & mentoring and resources to develop a project.

Platforms as the Young4Ocean Forum are essential to reinforce the connection between youngsters. Credible information is available to members to deepen their knowledge and apply it to their projects. Access to high-quality data and specialists empower the youth to actively discuss and build the strategy for the sea in their local community. Defend aquaculture on Madeira Island is an example.

Cohesion and divergence are critical to effective strategy making. According to the book *Making Strategy*, written by Fran Ackerman e Colin Eden, professors at Strathclyde University Business School, sometimes strategy making needs to encourage divergence of thinking rather than risk too much cohesion. Conflict, to a certain extension, generate the energy necessary to boost creativity.

Madeira's aquaculture conflict brings people out of the status quo zone, and consequently, boosts creativity. People search for alternatives to change the status quo in two scenarios, to survive throughout crises or to improve and achieve better results. Madeira's economy faces a crisis due to covid-19, and its society needs to leave the status quo. The Oceanum Portugal project was born to help Madeira solve this problem while promoting aquaculture.

The Oceanum Portugal project mission is to integrate Madeira Island Sea Nature tourism, aquaculture and local restaurants to improve their business results under the triple bottom line concept. The triple bottom line concept defines that businesses should not aim for profit only but also care about the social and environmental outcomes of their actions. Therefore, the project's main objective is to integrate enterprises under the blue economy concept of profitably, which means exploring the sea and, at the same time, respecting and preserving the environment.

(Continued on next page)

Oceanum Portugal aims to integrate local business (Nature Tourism and Local Restaurants) with the aquaculture sector that has the potential to be an alternative to make Madeira Island economically heterogeneous and resilient, generating jobs and improving R&D investments in the industry. The project will analyze the companies' performances and then make a strategic formulation to integrate them while promoting them on social media.

Oceanum Portugal team will evaluate the relationship between the company's mission with the actual activities they promote. Second, the team will assess its resources and capabilities (Resource-Based View) defended by R. Grant and J. Barney and make an external analysis to evaluate how the companies face the five environmental forces proposed by Porter. These will lead to the strategic options that will drive the action plan to integrate the enterprises, always focusing on achieving the triple bottom line. Finally, the project will also promote the companies on social media to improve their visibility.

Who are the bene iciaries?
The project targets business owners or managers from the three sectors: Sea nature tourism, aquaculture and local restaurants placed on Madeira Island.

Enterprises from the three sectors will benefit from receiving management consulting services and social media visibility when associated with Oceanum Portugal. Therefore, the association created between the aquaculture sector, nature tourism and local restaurants will aggregate value to their products and improve their resilience.

Next Steps
Promote aquaculture and define its future is a matter of establishing consensus and act. Join the Youth for Ocean Forum is a perfect opportunity to gain knowledge and contact the best people in the field. In addition, it is possible to submit your project to become a Young Ocean Advocate and receive mentoring and coaching from field specialists.
Join at the Maritime Forum on European Commission Official Website:
https://webgate.ec.europa.eu/maritimeforum/en

If you have a company and want to improve its results while preserving the sea, Oceanum Portugal will help you. Contact us on our social media channels to understand how we can help.

Webpages:
https://www.instagram.com/oceanum_portugal/
https://www.linkedin.com/company/oceanumpt

Bibliography
Ackerman, F., & Eden, C. (2015). *Making Strategy - Mapping Out Strategic Sucess*.
Hermida, M., & Costa, S. (2020). Between Tradition and Taste: Fish Consumption Habits in a Small Portuguese Archipelago. *Journal of Aquatic Food Product Technology*, *29*(4), 335–349. https://doi.org/10.1080/10498850.2020.1734892
Rocha, L. (2020). Associação "Cosmos denuncia aquicultura ao Ministério Público. *Funchal Notícias*.
Rocha, L. (2021a). Junta de Freguesia da Ribeira Brava salienta aspectos negativos da piscicultura. *Funchal Notícias*.

COMMERCIAL FEED REPLACEMENT FOR A SUSTAINABLE AND SELF-SUFFICIENT INTEGRATED MARINE AQUAPONIC PRODUCTION

Lorenzo Rossi[1], Carlo Bibbiani[1], Alberto Pardossi[2], Chiara Sangiacomo[1], Baldassare Fronte[1*]

1 Department of Veterinary Science, University of Pisa, Pisa, Italy
2 Department of Agriculture Food and Environment, University of Pisa, Pisa, Italy.

* baldassare.fronte@unipi.it

Introduction

Since Aquaculture is asked to produce sustainable animal proteins for the future growing world population, the large use of commercial feeds may represent a relevant bottleneck. Grains such as soybean, corn, wheat, barley, and even more precious raw materials such as fish oil and fishmeal, are commonly produced far away from the consumption sites. Moreover, their intensive production represents itself a severe hazard in terms of environmental pollution, soil and sea overexploitation, and biodiversity reduction. Yet, the overseas transportation of large quantities of raw materials and final products is characterized by a very high Carbon Footprint. In this context, an environmentally sustainable alternative to intensive aquaculture might be the Integrated Multi-Trophic Aquaculture approach. Stemming from IMTA, the Self-sufficient Integrated MultiTrophic AquaPonic system (SIMTAP, H2020 PRIMA-Programme) encompasses four consecutive trophic levels, starting from microalgae, deposit- and filter-feeder organisms (DFFO), fish and plants, reared in a recirculating system using saltwater.

In the SIMTAP system, micro- and macro-algae are raised for feeding DFFO, which in turn are harvested for feeding (or partially sold) fish; finally, solid fish wastes "feed-back" DFFO, while soluble wastes such as nitrogen and phosphorous are absorbed by hydroponically grown halophytes, salt-tolerant glicophytes and macro-algae, with that bio-remediating the recirculating water.

The DFFO are heterotrophic species such as polychaetes, bivalves, and echinoderms, which may represent a sustainable, nutritionally valuable, alternative to fish oil and fish meal in fish nutrition.

To evaluate how the use of these organisms may affect the growth performances of carnivorous fish species, two different trials have been carried out so far. The first consisted in the diet inclusion of increasing rates of mussels, until totally replacing the commercial feed. In the second trial, the commercial feed was fully replaced by a mixture of mussels and clams.

Material and Methods

All the experimental procedures were approved by the Organism for Animal Welfare of the University of Pisa and the Italian Ministry of Health (authorization code: B290E.N.AHZ). The trials were carried out using six cylindric 420 L tanks of the SIMTAP system located at the Department of Agricultural Food and Environment of the University of Pisa (Pisa, Italy).

Juveniles of Gilthead Sea Bream (*Sparus aurata*) were used and daily fed at 3% (on dry matter base) of their live body weight (BW). The diet ingredients used were the following: INVE© O. range P15 as commercial feed; frozen mussels (*Mytilus platensis*) and clams (*Chamelea gallina*) as feed replacers. The fish biomass of each tank was weighed every week in order to adjust the amount of diet to be supplied; then, Feed Conversion Rate (FCR), Condition factor (Kf) and Specific Growth Rate (SGR) were calculated per each tank and dietary treatment. On day 0 and at the end of the experiment, fish were weighed and measured individually (total length, TL). At the end of the experiments, 25 fish from each tank were also euthanized with an overdose of tricaine methanesulfonate (MS222©), dissected for abdominal viscera and liver weight determination, and calculating Viscera-Somatic (VSI) and Hepato-Somatic (HSI)
indexes.

Experiment 1: 1,243 fish (mean weight 4.95±1.120 g; mean total lengths 7.39±0,600 cm) were distributed in the 6 dietary treatments: F100M0 (100% feed), F80M20 (80% feed, 20% mussels), F60M40 (60% feed and 40% mussels), F40M60 (40% feed, 60% mussels), F20M80 (20% feed, 20% mussels) and F0M100 (100% mussels). After tawing, mussels were minced, and diets supplied 4 time per day.

Water temperature, salinity and pH approximately maintained at 24 °C, 32 g L^{-1}, 7.6, respectively, and DO above 6 mg L^{-1}.

Experiment 2: 1,255 fish (mean weight 6,78±1,41 g; mean total lengths 8,06±0,65 cm) were used. Again, fish were divided into 2 dietary treatments and 3 replicates: F100 (100% feed) and M100, this latter consisting of a mixture of 50% and 50% tawed and minced mussels and clams, respectively. Water temperature, salinity and pH were kept at 22 °C, 25 g L^{-1}, 7.5, respectively, and DO above 6 mg L^{-1}.

Statistical analysis: One-Way ANOVA followed by Tukey-Kramer HSD (Honestly Significant Difference) for the Experiment 1 and Student's test for the Experiment 2 were used for statistical analysis of growth performance parameters. Differences were considered significant at $P<0.05$.

Results

Experiment 1: thawed minced mussels showed significantly higher palatability than dry commercial feed, with fish intensively competing for catching mussel particles. On day 0, groups F100M0, F0M100 showed significantly lower ($P<0.05$) initial BW (4.76±1.074 and 4.76±1.042 g, respectively) than groups F80M20, F60M40 and F40M60 (4.86±1.076, 5.10±1.216 and 5.17±1.078, respectively). These differences were not anymore significant on day 28 and 35 while on day 42 the group fed 100% mussels (F0M100) showed the lowest BW (13.02±2.608), significantly different ($P<0.05$) from the group fed 100% commercial feed (14.69±3.498). Moreover, this latter group showed a significantly lower ($P<0.05$) BW than the group fed 60% commercial feed and 40% mussels (15.57±3.327). The group F0M100 showed the highest cumulative FCR (1.11), followed by the group F80M20 (1.06), F20M80 (1.04), F40M60 (1.02), F100M0 (1.01), and F60M40 (0.98).

Experiment 2: as observed in Experiment 1, fish fed on thawed minced mussels and clams showed higher competitiveness for the diet than those fed on commercial feed. Regarding initial BW and TL no significant differences were observed among treatments: 6.82±1.446 g and 8.08±0.638 cm for F100 and 6.75±1.378 g and 8.04±0.657 cm for M100. On day 48 of the experimental period, treatment F100 showed significantly higher BW and TL than M100: 23.62±4.471 g and 11.66±0.837 cm for F100, 20.83±3.943 and 11.40±0.704 cm for M100. Mean weight gain was significantly higher for treatment F100 than for M100: 3,522.12±90.015 g and 2,884.16±150.177 g, respectively. Also, FCR was significantly different among treatments: 0.88±0.050 for F100 and 1.05±0.062 for M100. Statistical analysis of TL, SGR, Kf, VSI and HSI data of both experiments is in progress.

Discussion and Conclusion

In general, results suggest better Gilthead Sea Bream growth performances when mixed diets are used. In fact, the use of mussels as only diet ingredient reduced fish growth by 11.3% and 16.4% in comparison to commercial feed and to a diet consisting of a mixture 60% commercial feed and 40% mussels, respectively. Similar results were observed also when a diet consisting of 50% mussels and 50% clams rather than mussels only was used. Probably, the enrichment of the wet diet mixture with the introduction of additional ingredients such as polychaetes and/or echinoderms, may improve its nutritional value and fish growth performances enhanced.

Acknowledgments

The Authors thanks Blue Resolution® association (Monsummano Terme – Italy) for kindly supplying frozen mussels and clams. SIMTAP is part of the PRIMA Programme supported by the European Union.

SOIL-BASED AQUAPONICS: THE EFFECTS OF SOIL ADDITION AS INVESTIGATED IN FOUR DIFFERENT TRIALS

Lorenzo Fruscella*, Benz Kotzen, Sarah Milliken

School of Design, University of Greenwich, Park Row, London SE10 9LS
l.fruscella@gre.ac.uk

Introduction

Aquaponics is an innovative and sustainable food production technology, identified by the European Commission as one of the ten technologies that will change our lives. In the EU, because of the lack of soil, aquaponics is considered a type of hydroponics, and it is therefore 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. This research explores ways through which different soil substrates (inert potting soil, nutrient-rich potting soil, certified top soil, artificial soil for raised beds) can be included in coupled and de-coupled aquaponics, thus taking it one step closer to organic certification. By including soil in aquaponics, this research can ultimately help the technology with its marketability, commercialisation, public acceptance, and popularity. The research is done through four different trials, and is to take place between April and August 2021. The trials aim at investigating the effects that soil has on the health, growth, and quality of the plants and the fish, while examining how the soil microbiome is affected by the use of aquaponic water and processed sludge. This presentation will include results from the trials, and possible analyses and conclusions, depending on their development. The four trials and their description are listed below.

Trial 1: Basil cultivation in pots

This trial investigates the effects of aquaponic water and processed sludge on plant growth and quality and soil microbiome in basil (*Ocimum basilicum*) cultivated in pots. Five treatments are used: basil in pots filled with inert potting soil watered with aquaponic water, basil in pots filled with inert potting soil watered with aquaponic water and sludge, basil in pots filled with nutrient-rich potting soil watered with tap water, basil in pots filled with nutrient-rich potting soil watered with aquaponic water, basil in pots filled with nutrient-rich potting soil watered with aquaponic water and sludge. The effects of the use of the different substrates and water type on plant health, quality, and growth, as well as soil microbiome composition are investigated. Each treatment is replicated three times.

Trial 2: Basil and Nile tilapia coupled aquaponics

This trial investigates the effects of aquaponic water on the growth and quality of basil (*Ocimum basilicum*) grown in soil-filled pots, as well as potential effects of soil addition to fish health. The growth of plants in soil-based aquaponic systems is compared to that of conventional aquaponics. The design of the soil-based aquaponic systems is inspired by conventional coupled aquaponics, however the plant units are designed to allow the plant roots in soil filled pots continuous access the aquaponics water. Four different treatments are used, all involving tanks will fish with a tray at the top, where water is recirculated between the fish and plant units. Treatments are the following: conventional soil-less aquaponics, soil-based aquaponics with inert soil in pots, soil-based aquaponics with nutrient-rich soil in pots, pots filled with nutrient-rich soil and watered with tap water. Each treatment is replicated three times.

Trial 3: Onions cultivation in raised beds

This treatment investigates the effect of watering onions (*Allium cepa*) cultivated in raised beds and watered with water from different sources; the aim of the study is to compare the effects of manure, currently allowed in organic production, and aquaponic water and aquaponic water with sludge as fertiliser on the growth and quality of the plants. Four outside raised beds are divided into four sub-units, for a total of 16 sub-units. Each subunit is devoted to the cultivation of onion sets. The four treatments, each replicated four times, are 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.

(Continued on next page)

Trial 4: Bok Choy cultivation in outside containers

This treatment investigates the effect of watering bok choys (*Brassica rapa*) cultivated in raised beds and watered with water from different sources; the aim of the study is to compare the effects of manure, currently allowed in organic production, and aquaponic water and aquaponic water with sludge as fertiliser on the growth and quality of the plants. A total of twelve 20L rectangular containers are used, for a total of four treatments. The treatments, each replicated four times, are 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 with sludge.

Trials estimated timeline:

- Basil cultivation in pots: April – June 2021(trial started)
- Basil and Nile tilapia coupled aquaponics: May – July 2021(to begin on May 10th)
- Onions Cultivation in Raised Beds: April – August 2021(trial started)
- Bok Choy Cultivation in outside containers: May – July 2021(to begin on May 17th)

IN VITRO TOOLS TO UNDERSTAND THE DIETARY EFFECTS N THE INTESTINAL INTEGRITY AND PHYSIOLOGY OF GILTHEAD SEA BREAM *Sparus aurata*

R. Robles[1] , J. Fuentes*[2] , L. Bermudez[1], S.F. Gregório[2], W. Nuez[3].

[1]Testing Blue S.L., Puerto Real, Spain.
[2]CCMar, Faro, Portugal.
[3]Adisseo, France.
Email: rociorobles@testingblue.eu

Introduction
Diet formulation and the inclusion of new ingredients are fundamental for performance indexes in fish aquaculture. However, there is a lack of tangible and reliable diagnostic tools to assess the animal's dietary effects on protein processing from digestion to absorption. The gut works as a selective barrier allowing nutrients in and denying entry to pathogens. It is possible to analyze the functions of the intestine ex-vivo if the tissue is kept alive and reflects in vivo functionality. When the tissue is opened flat and mounted between the two compartments of the Ussing chamber, transport, and epithelial properties can be studied using epithelial electrophysiology.

Material and methods
48 sibling seabream juveniles (189.96 ±10.01 g initial average individual body weight) were distributed in 4 tanks of a marine (32 psu) recirculating aquaculture system (RAS) held at 20ºC. A group of 12 animals were stocked per tank. Fish were fed either a control diet (diet A) or the same diet to which a digestive enhancing additive (Aqualyso, Adisseo) was added (diet B). Both diets were prepared following the same protocol except for the addition of the feed additive. Fish were fed manually twice per day 6 days per week. Trial lasted 4 weeks.

Epithelial electrophysiology in Ussing chambers
The anterior intestine, was isolated and mounted as previously described (Gregorio et al., 2013) with apical (luminal) and basolateral (blood side) sides of the tissue identified on a tissue holder of 0.25-0.30 cm^2 and positioned between two half-chambers containing 2 ml of physiological saline. Bioelectrical parameters for each tissue were recorded continuously during the *in vitro* period onto Labscribe3 running in a MacIntosh computer using IWorx188 and Lab-Trax-4 data acquisition systems, from the time of mounting for 90 min. Epithelial resistance (Rt, $\Omega.cm^2$) was manually calculated (using Ohm's law) from the current deflections induced by a bilateral +2 mV pulse of 3 s every minute. e.

Electrogenic amino acid transport
The mid intestine of sea bream juveniles was isolated and mounted as described above for the anterior intestine. In this subset of experiments the apical side of the preparation is stimulated with an amino acid mixture (M 5550 MEM [50X], Sigma-Aldrich). The amino acid pool consists of a complex mixture of essential amin acids

The principle of the test is that the epithelium is stimulated by the presence of amino acids, thus generating a change in the current due to the cotransport of amino acids with ions (Broer, 2008). In the seabream mid-intestine, the response is concentration dependent, relatively quick and plateaus within 30 min of amino acid addition. Therefore, sequential effects of different concentrations can be quantified in each epithelial preparation. Here sequential concentrations of 8 and 16 mM were used.

Results & conclusions
The time-response in vitro in the Ussing chamber experiment shows a typical progression to plateau values in both Diet A and Diet B. Dietary manipulation was without significant effect on tissue resistance, indicating that tissue selectivity was not compromised by dietary composition manipulations.

Electrogenic, transcellular, ion dependent amino acid transport is significantly a consistently increased in the mid intestine of fish fed Diet B. Our system provides a functional in vitro picture of tissues conditioned in vivo. The interpretation of these results points to a better capacity of dietary amino acid transport in fish fed Diet B, and the likely incorporation for tissue a muscle growth. However, we may need to exercise caution with this set of results, as only essential amino acids were present in the amino acid pool tested.

(Continued on next page)

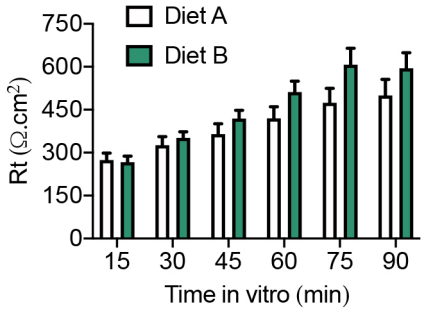


Figure 1. Tissue resistance (Rt, Ω cm^2) in the anterior intestine of sea bream juveniles recorded *in vitro* during experimental testing of 90 min. Each column shows the average + SEM (n=12 fish per diet).

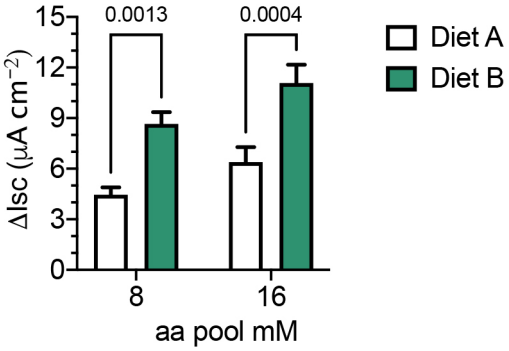


Figure 2. Electrogenic essential amino acid transport in the mid intestine of sea bream juveniles recorded in vitro in Ussing chambers. Changes in short circuit current (Delta Isc, μA.cm^{-2}) are evoked by sequential apical addition of essential amino acid pool of 8 and 16 mM. Each column represents the average + SEM. ((n=12 fish per diet).)

References

Broer, S (2008). Amino acid transport across mammalian intestinal and renal epithelia. Physiol Rev 88, 249-86

Gregório SF, ESM Carvalho, S Encarnação, JM Wilson, DM Power, AVM Canário, J Fuentes. (2013). Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus auratus* L.). Journal of Experimental Biology 216 (3): 470- 9 http://dx.doi.org/10.1242/jeb.073742

PERACETIC ACID AS A TREATMENT FOR AMOEBIC GILL DISEASE: EFFICACY AND PHYSIOLOGICAL RESPONSES OF ATLANTIC SALMON

Francisco Furtado[1*], Mette W. Breiland[2], David Strand[3], Lars-Flemming Pedersen[4], Carlo C. Lazado[2]

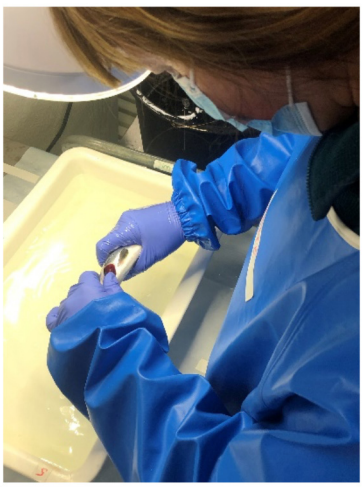
[1]CIISA, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal
[2]Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, 9019 Tromsø, Norway
[3]Fish Health Research Group, Norwegian Veterinary Institute, 4045 Oslo, Norway
[4]Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, PO Box 101, 9850 Hirtshals, Denmark
Email: francisco96.furtado@gmail.com

Introduction

Amoebic gill disease (AGD) is a parasitic infestation affecting Atlantic salmon, particularly during the seawater phase of production. It is caused by the amphizoic marine amoeba *Neoparamoeba perurans*. Upon attachment on the gills, the amoeba trophozoites elicit a localised tissue response including epithelial hyperplasia, hypertrophy and lamellar fusion and the gross pathology is characterised by raised white mucoid lesions. These white plaques are used to grade the severity of AGD infection through visual inspection. Freshwater and oxidative therapeutics are used to treat AGD, the former being the most commonly used. Despite offering alternative options for freshwater bathing, oxidative therapeutics often have conflicting treatment resolutions. In addition, there is a significant knowledge gap on the underlying physiological processes on how salmon respond to these treatments. In this study, we explored the efficacy of peracetic acid (PAA) in treating AGD and the physiological responses of salmon smolts during the treatment.

Materials and methods

AGD in smolts was induced by bath exposure to the parasites for 1 hr. Thereafter, the disease was allowed to develop with routine monitoring of the gill score (GS 0 = clear of lesions → GS 5 = extensive lesions). When the gill score (GS) reached 1-2, fish were treated with PAA via bath exposure. Three commercial PAA products were tested (Perfektoxid, AQUADes and ADDIAqua) at a concentration of 5 ppm. Two exposure durations were evaluated – 30 mins and 60 mins. Uninfected fish were likewise treated similarly. Sampling was performed 1 day and 2 weeks after treatment. The disease status of the fish was assessed by visual pathology of the gills and qPCR quantification of the parasites (gill swabs and water samples). The gills and olfactory organs were subjected to histological evaluation and gene expression analysis of key biomarkers for oxidative stress. Systemic oxidative stress was determined by quantifying the level of reactive oxygen species and total antioxidant capacity in plasma.



Gill scoring of salmon experimentally infected with AGD.

(Continued on next page)

Results and Discussion

Gill score and parasite load: The infected-untreated group had an average GS of 2.8 two weeks post-treatment. Except for the group treated with ADDIAqua, groups treated with PAA for 1 hr had an almost similar GS with the infected-untreated group. Fish treated with PAA for 30 mins registered lower GS than the infected-untreated and 1 hr-treated groups. The lowest GS (1.9) was in AQUADes treated group, followed by ADDIAqua (2.1), then with Perfektoxid (2.4) for 30 mins. qPCR analysis of the parasite on the gills did not provide a clear tendency either among the groups or between sampling points. The level of the parasite in the rearing water increased through time in all PAA-treated groups, which might indicate an ongoing parasite shedding post-treatment.

Histology: AGD-infected fish displayed the typical pathology, which was increased hyperplasia. Two weeks post-treatment, the number of lamellar hyperplasia cases in the 30-min treated group was lower than the infected-untreated and 1-hr PAA-treated groups. This increased hyperplasia likewise resulted in increased lamellar fusion. Epithelial lifting was prevalent in all groups. The mucous cell number in the lamella but not in the filament increased following treatment, and neither inter-treatment nor temporal differences were found to influence this profile. PAA-treated fish exhibited a compromised olfactory organ architecture two weeks post-treatment. There was a tendency for the mucosal tip of the olfactory lamella to increase in size especially in Perfektoxid-treated group, regardless of the exposure duration. The thickness of the lamellar epithelium and *lamina propia* did not demonstrate substantial changes among treatment groups and through time.

Gene expression: Thirteen biomarkers for oxidative stress were evaluated in the gills and olfactory organs. Overall, PAA treatments resulted in the modulation of the expression of the marker genes and the changes were pronounced 1 day after than at 2 weeks post-treatment. This indicates that mucosal oxidative stress was triggered, though not chronic. Unlike in other response variables where treatment duration elicited strong differential responses among the groups, the gene expression profile in the gills and olfactory organ revealed no such general distinction.

Systemic oxidative stress: ROS levels in the plasma increased through time, especially in the group treated for 30 mins. The groups exposed to AQUADes and Perfektoxid for 30 mins had higher ROS levels than the infected-untreated group two weeks post-treatment. There was a large fish-to-fish variation in the total antioxidant capacity (TAC) in the plasma of the experimental fish. No significant differences in plasma TAC were found among treatments and between sampling points.

Conclusions

This study demonstrated that PAA treatment of AGD in salmon smolts resulted in quite varied response profiles. Disease resolution was not fully established as assessed by visual pathology, histopathology and qPCR, though there were indications that the type of PAA product might play a role in its treatment efficacy. Future studies should be directed at standardising the PAA treatment protocol. From physiological aspects, salmon were able to respond to PAA treatments despite being in a diseased state. AGD however, could trigger changes in the expression PAA was demonstrated to trigger local oxidative stress, though the heightened state did not last long.

Acknowledgment

This research is part of the PERAGILL project funded by the Norwegian Seafood Research Fund (ref. 901472).

HOW MACHINE LEARNING AND ARTIFICIAL INTELLIGENCE HAVE SIGNIFICANTLY IMPROVED OPEN OCEAN FISH FARMING

Langley Gace *

Innovasea Systems, Inc.
425 Ericksen Avenue NE
Suite 101
Bainbridge Island, WA 98110
LGace@InnovaSea.com

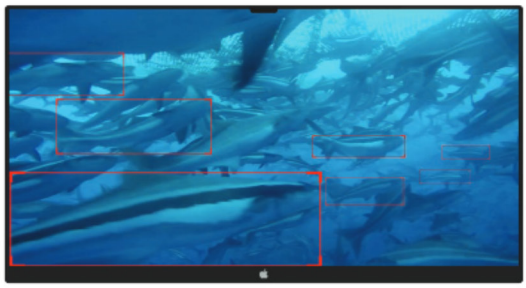
Open ocean fish farming has changed dramatically in the past few years. The industry is embracing the significant advantages that machine learning and artificial intelligence (AI) have to offer as well as affordable high definition underwater cameras. Feeding submerged pens is one operation that has benefited significantly from these technological advancements.

Efficient feeding is critical to a farm's profitability as well as reducing its impact on the local environment. Feed-related expenses are often responsible for 50% or more of a farm's operating costs. The 10% improvement in the FCRe (2.0-1.8) can potentially equal more than $1.1 million savings annually on a 3000 ton farm.

Until recently, all feed was delivered via forced air from the feed barge to the pen located at the surface. With the advent of submerged pens, feed then had to be delivered via a slurry of water. In addition to requiring less power, there are several other advantages, such as providing a cushion of fluid to deliver more of the pellet to the pen.

High-definition underwater cameras are no longer cost-prohibitive, allowing the farmer to observe feeding and other activities in all the pens from the safety and comfort of their office on the feed barge or remotely on land. Video from these cameras are analyzed by AI algorithms to identify pellet waste and fish satiation. These programs help the feed manager to reduce feed waste, optimize feed rate, and to determine when the fish have been adequately fed.

Stereoscopic biomass estimation cameras constantly measure fish dimensions within the pen. The onboard algorithms then provide any near real-time biomass estimation within 3% accuracy. This data is critical to the farm managers enabling them to optimize feed schedules as well as predict harvest mass and schedules.



ANTIMICROBIAL PEPTIDE ANALYSES OF SELECTED AND NAÏVE *Crassostrea gigas* OYSTERS TO *VIbrio aesturianus* PATHOGEN

A. Garcia[1,3]*, G. Vilella[1,2], J. Estêvão[1,3], A. Cunha[1,3], B. Costas[1,3], L. Dégremont[4], S. Fernandéz-Boo[1]

[1] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR). Matosinhos, Portugal
[2] University of Porto, Faculty of Sciences, Portugal
[3] Abel Salazar Biomedical Sciences Institute (ICBAS-UP), Porto, Portugal.
[4] SG2M, LGPMM, Ifremer, Avenue Mus de Loup, 17390 La Tremblade, France
*Email: afgarcia@ciimar.up.pt

Introduction

Oysters are filter-feeder animals and as a result they are in permanent contact to diverse communities of microorganisms [1]. Bacterial and virus outbreaks represent a major threat to the oyster industry, being responsible for severe diseases that often leads to massive oyster mortality and harsh economic losses [2, 3]. Pathogen recognition and disease control in oysters are mediated by an innate immune system, in which antimicrobial peptides play a key rule. In this study, the susceptibility of naïve or selected oysters *Crassostrea gigas* to infection with *Vibrio aestuarianus* has been approached, regarding mortality and expression of genes which codify for antimicrobial peptides.

Material and Methods

Six families of *C. gigas*, 3 naïve and 3 selected for their higher resistance to *V. aestuarianus*, were tested for infection with *V. aestuarianus*. 12 oysters per family were distributed in each tank (72 oysters/tank). All oysters for three of the tanks were injected with 100 μl of bacterial suspension (10^6 bacteria/oyster) into the adductor muscle (infected condition), while oysters of the three other tanks were injected with artificial sea water (control conditions). Mortality was recorded every day during 30 days, and for each tank, three live oysters of each family were sampled at 24h, 48h and 72h post-infection, for haemolymph, mucus and tissue collection. Haemolymph was centrifuged and haemocytes were kept on trizol for gene expression studies by real-time PCR. Plasma was used for protein and NO quantifications. Also, mucus was kept at -80ºC for further studies of antimicrobial activity and tissue will be used for *V. aestuarianus* quantification to verify the infection progression.

Results

Results of cumulative mortality reveal higher mortality in the infected conditions (mean = 51%) than of control condition (mean = 6%). Then, when infected with *V. aestuarianus*, selected families showed a lower mortality (mean = 32%) than naïve oysters (mean = 70%) (figure 1). Regarding protein content and NO, no significant differences were found among resistant and naïve oysters in plasma; still, results seem to point to higher protein content of the resistant animals even in control conditions. Four families were selected for gene expression analysis based on mortality results (the two best and the two worst). Overall, resistant and naïve families show different patterns of gene expression. For control conditions, in general, genes were higher expressed in resistant oysters. Additionally, either facing a bacterial challenge or in control conditions, some genes involved in host defense, as for instance defensin (Cg-Defhs), exhibited a higher expression in resistant families, at all sample time points.

Discussion

The ability to defend against a pathogen can be affected by environmental, genetic factors or combination of both [4]. It is also known that recognition of microbial products leads to production of a large range of antimicrobial molecules [5]. Therefore, in our study, families of naïve and selected oysters were chosen to be submitted to a bacterial challenge with *V. aesturianus*. Results of mortality are in agreement with the selection scheme developed, indicating that the selected families have a higher resistance to the infection by *V. aestuarianus*. Moreover, it appears to be in accordance with expression results of genes related with defense against pathogens. The higher levels of defensins in resistant families and other genes involved in host defense can be associated with a more efficient response to infection. Antimicrobial activity studies are also being performed in mucus samples of all families in order to understand if there is a different profile among resistant and naïve families.

(Continued on next page)

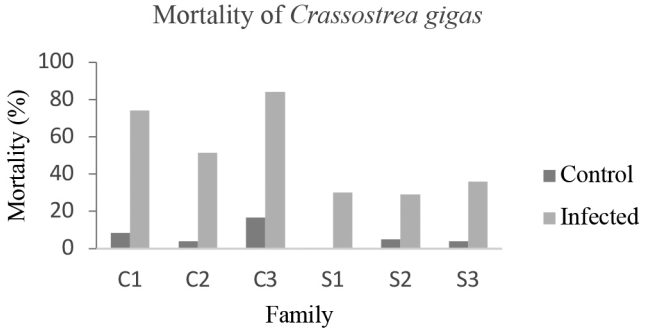


Figure 1: Cumulative mortality of six families of oysters *Crassostrea gigas*, naïve (C1, C2 and C3) or selected (S1, S2 and S3), after infection with *Vibrio aesturianus* (light grey). For control conditions (dark grey) oysters were injected with artificial seawater.

Acknowledgments

This work was also supported by the project CRAGIAMP-POCI-01-0145-FEDER-030232, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF. Ana Garcia is supported by a BYT-FCT PhD Grant (UI/BD/150907/2021).

Bibliography

[1] E. Bachère *et al*., "The new insights into the oyster antimicrobial defense: Cellular, molecular and genetic view," *Fish Shellfish Immunol*., doi: 10.1016/j.fsi.2015.02.040.

[2] L. Dégremont, C. Garcia, and S. K. Allen, "Genetic improvement for disease resistance in oysters: A review," *J. Invertebr. Pathol*., 2015, doi: 10.1016/j.jip.2015.05.010.

[3] M. Leprêtre *et al*., "Comparative Proteomics of Ostreid Herpesvirus 1 and Pacific Oyster Interactions With Two Families Exhibiting Contrasted Susceptibility to Viral Infection," *Front. Immunol*., 2021, doi: 10.3389/fimmu.2020.621994.

[4] P. Azéma, J.-B. Lamy, P. Boudry, T. Renault, M.-A. Travers, and L. Dégremont, "Genetic parameters of resistance to Vibrio aestuarianus, and OsHV-1 infections in the Pacific oyster, Crassostrea gigas, at three different life stages," *Genet. Sel. Evol*., 2017, doi: 10.1186/s12711-017-0297-2.

[5] M. Gonzalez *et al*., "Evidence in oyster of a plasma extracellular superoxide dismutase which binds LPS," *Biochem. Biophys. Res. Commun*., 2005, doi: 10.1016/j.bbrc.2005.10.075.

WHAT ARE THE MOST IMPORTANT DRIVERS FOR BUYING NEW AQUACULTURE FISH PRODUCTS? THE CASE OF FRENCH AND GERMAN CONSUMERS

Sonia García Muñoz[1]*, Raquel Llorente[1], Irene Peral[1]

[1]AZTI, Basque Research and Technology Alliance (BRTA), Food Research, Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160 Derio, Bizkaia, Spain
*E-mail: sgarcia@azti.es

Introduction

Fish consumption in the European Union (EU) shows a growing tendency in the last decade (FAO, 2020). However, as wild fish cannot meet the fish global demand, the aquaculture has become the most suitable alternative to fill these growing needs (Carrillo *et al*., 2020). Thus, new food products based on aquaculture products are required on the market to fill the consumers needs. For this reason, AZTI (Spain) has successfully developed four new food products based on aquaculture species (seabass, gilthead seabream and meagre) in the frame of H2020 MedAID project.

The new fishery products from aquaculture were designed following a consumer-centred approach, since involving consumers during the development of new products has been proved to be the best alternative for increasing market success rate (Banović *et al*., 2016). In fact, these products were successfully evaluated by Spanish consumers (García-Muñoz et al., 2021). However, the most important drivers for buying these new products in a European context were unknown. Hence, these products were evaluated in France and Germany, since the household expenditure per capita for fishery and aquaculture products of these two countries are between the highest in the EU (EUMOFA, 2019).

The aims of this study were (i) to validate the new aquaculture products in a European context, (ii) to know the consumers fishery and aquaculture consumption habits and (iii) to analyse the most important drivers for buying new fishery products from aquaculture.

Materials and methods

This cross-sectional study was developed in two European countries (Germany and France) involving 1,000 fish consumers (500 per country). Fish consumers (50% female, 50% males) between 25 and 65 years old (mean age ± SD: 44.51 ± 11.14) evaluated pictures and information about the new fish products (Grilled seabass with lemon, Sea and mountain burger, Seabream breaded bites and Organic seabream with couscous). The questionnaire included questions regarding fish consumption habits, products associations, purchase intention, drivers for buying fishery and aquaculture products, and socio-demographic characteristics.

Results

Differences were observed between French and German consumers regarding frequency of fish consumption, the most consumed fish at home, the origin of the fish purchased and consumed (i.e., wild, aquaculture), and the fish cooking method used by participants at home. It should be considered that around 25% of the participants had never consumed fish from aquaculture. Regarding the products validation, French and Germans consumers preferred Seabream breaded bites and Organic seabass with cous-cous. These products were also selected for increasing consumers day-to-day fish intake.
Organic seabass with cous-cous obtained the highest purchase intention in France, meanwhile Seabream breaded bites obtained it in Germany.

When information about the products was provided to consumers (i.e., percentage of aquaculture fish in the final recipe, storage conditions, servings and packing weight, product sensory description, Nutri-Score label, recipe suggestion and cooked products pictures), the purchase intention increased up to 13%.

Finally, the consumers identified taste, healthy product and percentage of fish contained as the most important drivers for buying fishery and aquaculture products. In addition, when consumers felt both that they would like to taste the products and they would like the product taste, higher purchase intention was obtained. Also, similar results were achieved when the products were familiar for consumers and when they felt that they could easily prepare them. Moreover, the purchase intention was positively related to product tasty appeal, packaging that fits with consumer needs, and the good image of the product.

(Continued on next page)

Conclusions

Consumption habits for new aquaculture products seem to be related with product associations and purchase intention. Moreover, based on the differences detected between French and Germany consumers, it is highly recommended to follow a consumer-centred approach to better understand fishery and aquaculture consumer needs and consumption habits in each market niche.

Finally, the insights found in this study regarding the identification of fish consumption habits, preferences and purchase drivers might increase market success rate and purchase intention percentage of the new aquaculture products. In this way, the competitiveness and growth of the Mediterranean aquaculture industry might be improved.

Acknowledgments

This study has received funding from the European Union in the frame of Horizon 2020 - MedAID (Mediterranean Aquaculture Integrated Development), grant agreement number 727315 (http://www.medaid-h2020.eu/).

References

Banović, M., Krystallis, A., Guerrero, L., & Reinders, M. J. (2016). Consumers as co-creators of new product ideas: An application of projective and creative research techniques. Food Research International, 87, 211-223.

EUMOFA. (2019). *The EU fish market*. Retrieved from Luxembourg: https://www.eumofa.eu/documents/20178/314856/EN_The+EU+fish+market_2019.pdf/

FAO. (2020). The state of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

García-Muñoz, S., Llorente, R., López-Mas, L., Guerrero, L., Peral, I. (2021). Is there room in the fish market for the development of new aquaculture products? Conference, Eposters & e-Market. Aquaculture Europe 2020, pag. 237. Available online: https://www.aquaeas.eu/images/stories/Meetings/AE2020/AE20_Abstracts_FINAL.pdf.

DIETARY EFFECTS ON GROWTH, SURVIVAL AND BEHAVIORAL FEEDING RESPONSES IN LUMPFISH (*Cyclopterus lumpus* L.) LARVAE

Ibon García-Gallego*, Elin Kjørsvik

Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, University of Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Las Palmas, Spain
Department of Biology, NTNU Norwegian University of Science and Technology, Trondheim, Norway
E-mail: ibon.garcia101@alu.ulpgc.es

Introduction
Deployment of lice-eating cleaner fish in salmon cages is considered one of the most effective and sustainable methods to control sea lice infestations. The lumpfish (*Cyclopterus lumpus*) is the most used and commercially produced species of cleaner fish in Norway (Nordland, 2017). The Norwegian lumpfish production is currently characterized by variable growth and survival during the larval stages (Powell et al. 2017). Little is still known about nutritional requirements and the functional behavior during development of lumpfish larvae. How larval and juvenile behavioral responses are affected by the nutritional quality of the first feed is also not well known, and this may be critical for larval quality and the juvenile's capacity to eat the lice off the salmon when it is transferred to the sea cages. The objective of this study was to describe and evaluate the ontogenetic development of feeding behavioural responses in lumpfish larvae from hatching to 33 days post hatch (dph), in relation to larval size and nutritional quality of the first feed, based on growth, survival, swimming and feeding activity.

Material and methods
Larval groups received five different dietary treatments: 1) formulated diet (control), 2) cirripedia (frozen preserved plankton from Planktonic AS), 3) enriched *Artemia*, 4) copepods (*Acartia tonsa*) (from CFEED AS) and 5) a combination of *Acartia tonsa* and cirripedia (2-22 days post hatch). The larvae were fed formulated diet either for the full period or weaned from *Artemia* and cirripedia nauplii to the formulated diet between 20-22 days post hatch. All groups were fed the same formulated diet until the experiment ended at 33 days post hatch. All feeding behaviour observations were recorded by video. At 16 dph, 22 dph and 28 dph, 10 larvae from each tank were randomly sampled into individual 1litre plastic bottles and starved for 3 hours. After 3 hours an amount of live prey (350 cirripedia/ml + 350 *Artemia* nauplii/ml) were added. For 2 minutes larvae were recorded. The parameters observed during the records were: number of seconds each larva swam, number of second attached to a substrate, orientating itself in the tank, number of successful and unsuccessful attacks, type of attack performed (swimming or attacked) and number of seconds of the swimming attacks.

Results
Feeding behaviour was found to be different both through the ontogeny development as well as among the treatments, especially at 22 days post hatch (dph) and 28 dph. Lumpfish larvae showed more swimming activity at 16 dph compared with 22 dph and 28 dph. Larvae fed more at 22 dph and 28 dph and they used more time for the swimming attacks at 28 dph. Larvae fed on *Artemia* were more active swimmers and they also orientated more. However, at 28 dph, cirripedia treatment larvae showed more swimming activity and performed more successful attacks compared with the other treatments ($P < 0.05$). Lumpfish larvae from copepods and formulated diet treatments swam less, spent more time attached and less time orientating. Also, they performed a smaller number of successful attacks ($P < 0.05$) showing a lower predatory instinct.

Artemia treatment resulted in significantly better growth and survival than other diets. Cirripedia diet resulted in initial slow growth, catching up with *Artemia* group after weaning to formulated diet. Formulated diet presented low growth and poor survival compared to other diets.

Feeding enrichment *Artemia* during the start feeding resulted in better results based on growth, survival, and swimming and feeding activity of lumpfish larvae than other treatment diets. However, after the weaning period those lumpfish larvae fed cirripedia showed similar growth and more successful attacks.

(Continued on next page)

Conclusions

The difference in feed quality of the diet treatments during the 33 dph start feeding of lumpfish larvae, affected the larvae behaviour activity and performance. The results of this study indicate a high correlation between live feed quality and the effects on growth, survival and feeding behavioural responses in lumpfish larvae, suggesting the high importance of early nutritional quality on the larval feeding behaviour, which is currently not well known in lumpfish.

Funding

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL2020).TNA project ID number: AE170011

References

Nordland E (2017). Erlend Waatevik, EWA Consulting. [cited 29 Mar 2018.] Available URL: https://ilaks.no/forventer-en-produksjon-pa-30-millioner-rognkjeks-i-2017/. iLaks [in Norwegian.]

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture, 10(3), 683-702.

DIETARY EFFECTS ON GROWTH, SURVIVAL AND BEHAVIORAL FEEDING RESPONSES IN LUMPFISH (*Cyclopterus lumpus* L.) LARVAE

Ibon García-Gallego*, Tu Anh Vo, Frank Mlingi, Luciana Musialak, Cecilie Miljeteig, Marte Solli Lindskog, Saba Akbar, Sunniva Kværnø, Andreas Hagemann, Arne Malzahn, Elin Kjørsvik

Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, University of Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Las Palmas, Spain
Department of Biology, NTNU Norwegian University of Science and Technology, Trondheim, Norway
SINTEF Ocean, Trondheim, Norway
E-mail: ibon.garcia101@alu.ulpgc.es

Introduction

Deployment of lice-eating cleaner fish in salmon cages is considered one of the most effective and sustainable methods to control sea lice infestations. The lumpfish (*Cyclopterus lumpus*) is the most used and commercially produced species of cleaner fish in Norway (Nordland, 2017). The Norwegian lumpfish production is currently characterized by variable growth and survival during the larval stages (Powell et al. 2017). Little is still known about nutritional requirements and the functional behavior during development of lumpfish larvae. How larval and juvenile behavioral responses are affected by the nutritional quality of the first feed is also not well known, and this may be critical for larval quality and the juvenile's capacity to eat the lice off the salmon when it is transferred to the sea cages. The objective of this study was to describe and evaluate the ontogenetic development of feeding behavioural responses in lumpfish larvae from hatching to 33 days post hatch (dph), in relation to larval size and nutritional quality of the first feed, based on growth, survival, swimming and feeding activity.

Material and methods

Larval groups received five different dietary treatments: 1) formulated diet (control), 2) cirripedia (frozen preserved plankton from Planktonic AS), 3) enriched *Artemia*, 4) copepods (*Acartia tonsa*) (from CFEED AS) and 5) a combination of *Acartia tonsa* and cirripedia (2-22 days post hatch). The larvae were fed formulated diet either for the full period or weaned from *Artemia* and cirripedia nauplii to the formulated diet between 20-22 days post hatch. All groups were fed the same formulated diet until the experiment ended at 33 days post hatch. All feeding behaviour observations were recorded by video. At 16 dph, 22 dph and 28 dph, 10 larvae from each tank were randomly sampled into individual 1litre plastic bottles and starved for 3 hours. After 3 hours an amount of live prey (350 cirripedia/ml + 350 *Artemia* nauplii/ml) were added. For 2 minutes larvae were recorded. The parameters observed during the records were: number of seconds each larva swam, number of second attached to a substrate, orientating itself in the tank, number of successful and unsuccessful attacks, type of attack performed (swimming or attacked) and number of seconds of the swimming attacks.

Results

Feeding behaviour was found to be different both through the ontogeny development as well as among the treatments, especially at 22 days post hatch (dph) and 28 dph. Lumpfish larvae showed more swimming activity at 16 dph compared with 22 dph and 28 dph. Larvae fed more at 22 dph and 28 dph and they used more time for the swimming attacks at 28 dph. Larvae fed on *Artemia* were more active swimmers and they also orientated more. However, at 28 dph, cirripedia treatment larvae showed more swimming activity and performed more successful attacks compared with the other treatments ($P < 0.05$). Lumpfish larvae from copepods and formulated diet treatments swam less, spent more time attached and less time orientating. Also, they performed a smaller number of successful attacks ($P < 0.05$) showing a lower predatory instinct.

Artemia treatment resulted in significantly better growth and survival than other diets. Cirripedia diet resulted in initial slow growth, catching up with *Artemia* group after weaning to formulated diet. Formulated diet presented low growth and poor survival compared to other diets.

Feeding enrichment *Artemia* during the start feeding resulted in better results based on growth, survival, and swimming and feeding activity of lumpfish larvae than other treatment diets. However, after the weaning period those lumpfish larvae fed cirripedia showed similar growth and more successful attacks.

(Continued on next page)

Conclusions

The difference in feed quality of the diet treatments during the 33 dph start feeding of lumpfish larvae, affected the larvae behaviour activity and performance. The results of this study indicate a high correlation between live feed quality and the effects on growth, survival and feeding behavioural responses in lumpfish larvae, suggesting the high importance of early nutritional quality on the larval feeding behaviour, which is currently not well known in lumpfish.

Funding

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL2020).TNA project ID number: AE170011

References

Nordland E (2017). Erlend Waatevik, EWA Consulting. [cited 29 Mar 2018.] Available URL: https://ilaks.no/forventer-en-produksjon-pa-30-millioner-rognkjeks-i-2017/. iLaks [in Norwegian.]

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture, 10(3), 683-702.

DOES DIETARY LINOLENIC/LINOLEIC ACID RATIO AFFECT INTESTINAL OXIDATIVE STRESS BIOMARKERS IN GILTHEAD SEA BREAM?

I. García-Meilán*[1], I. Agredano, [1], A. Profitós [1], R. Fontanillas [2], E. Capilla[1], I. Navarro[1], and A. Gallardo [1]

[1] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 643, 08028, Barcelona, Spain
[2] Skretting Aquaculture Research Centre (ARC), Sjøhagen 3, Stavanger, Norway
E-mail: irene.garcia@ub.edu

Introduction

Among vegetable oils, soybean oil, which is rich in n-6 fatty acids, mainly linoleic (C18:3 n-6) is commonly used to partially replace fish oil in fish feeds, while others like linseed oil are less employed. Despite this, linseed oil is one of the richest plant sources of n-3 fatty acids with 75% of polyunsaturated fatty acids (PUFA), mainly α-linolenic acid (ALA, C18:3 n-3); and it seems to be a good alternative as total or partial fish oil replacement, since linseed oil use has been successful in some species. Dietary lipid profile affects membrane composition and modifies lipid peroxidation (LPO) levels, which could negatively affect membrane structure and fluidity, and intestinal permeability. In this sense, LPO production and the antioxidant status could affect digestion and absorption processes, modifying therefore nutrient availability. The aim of the study was to investigate the effect of increasing the dietary linolenic/linoleic ratio on LPO and antioxidant capacities at intestinal level in gilthead sea bream (*Sparus aurata*).

Materials and methods

Two isonitrogenous (46%) and isolipidic (22.2%) diets were formulated and produced by Skretting ARC (Norway) in which a 25% of dietary oil came from fish oil to meet eicosapentaenoic and docosahexaenoic acids requirements and 75% was vegetal oil. The vegetable oils used were soybean (S) and linseed (L) oils and diets were named accordingly. Diet S contained soybean oil as the only vegetable oil, and diet SL contained both oils with a S/L ratio of 3.8. The linolenic/linoleic ratio was 0.12 for S diet and 0.32 for SL diet.

One-hundred ten gilthead sea bream with an initial weight of 81.52g were distributed in tanks and fed *ad libitum* twice a day with the corresponding diet for 18 weeks. No differences in final weight were found between S and SL fish; being on average 255.7 ± 7.7 g. Once the growth trial finished, fish were sacrificed at 24h post-feeding and samples of pyloric caeca and proximal intestine were collected. LPO levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) activities were determined. Gene expression of antioxidant enzymes (*sod1, sod2, cat, gpx1, gpx4, gr, gst3*) was analysed by quantitative PCR.

Results

The partial dietary substitution of soybean oil for linseed oil (diet SL) resulted in increased activities of the antioxidant enzymes SOD (x1.8), CAT (x1.5), GR (x1.7) and GST (x2.1) in pyloric caeca (Fig. 1), while GPx activity was not modified. Instead, in the proximal intestine, only SOD activity increased (x1.8) (Fig. 1). No changes in gene expression of the antioxidant enzymes were found in pyloric caeca or proximal intestine. Up-regulation of these enzymes could not deal with the increase in oxidative stress, and LPO levels were 51.4 fold higher in pyloric caeca and 3.4 fold higher in proximal intestine of SL fed fish than in S fed ones (Fig. 1). The SL group fed the diet with a higher linolenic/linoleic ratio, showed a high individual variability in LPO levels. Thus, in pyloric caeca 67% of fish showed LPO levels up to 0.150 nMol · mg prot^{-1}, whereas the remaining 33% showed a of LPO level above 1. SL fed fish were subdivided into two groups according to their level of LPO (L-SL, low LPO and H-SL, high LPO). H-SL animals showed superior CAT (x1.9) and GR (x2.0) activities in pyloric caeca, and a lower relative intestinal length (-38.2%) in comparison to L-SL group (Table 1).

Discussion and conclusion

Data showed that the partial dietary substitution of soybean oil by linseed oil, which leads to an increase in linolenic/linoleic ratio, could negatively affect oxidative stress control at intestinal level, especially in pyloric caeca of those fish that were not able to adapt its relative intestinal length, suggesting that its use in fish feeds formulation should be limited.

(Continued on next page)

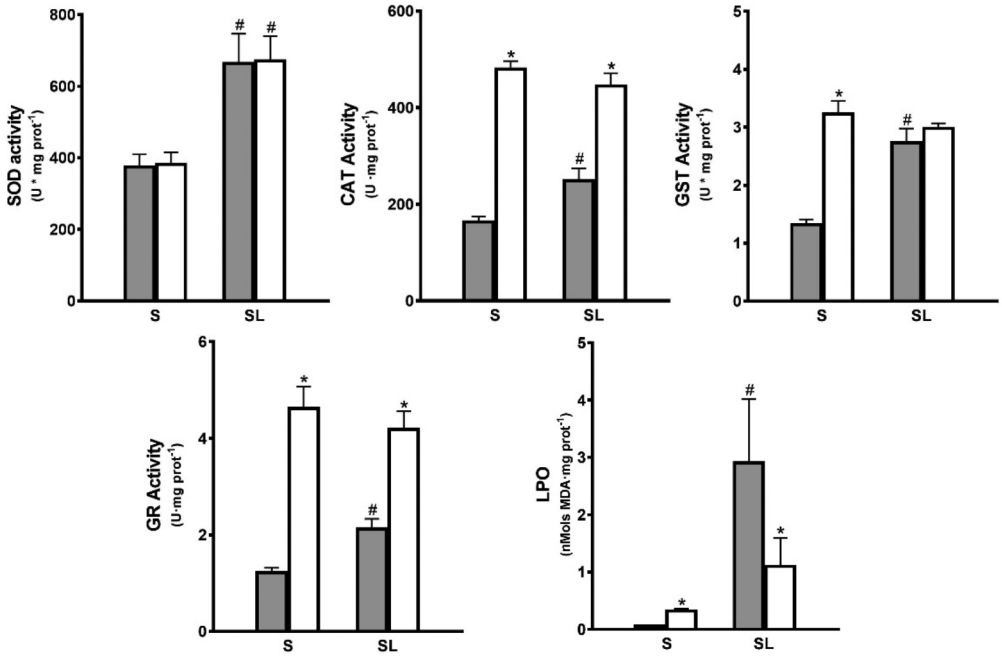


Figure 1. SOD, CAT, GST and GR activities and lipid peroxidation (LPO) levels at 24h post-feeding in pyloric caeca (grey bars) and proximal intestine (white bars) of gilthead sea bream fed experimental diets. Values are presented as mean ± SEM. (n=6 for S fed fish; n=9 for SL fed fish). Significant differences between the two experimental conditions are shown by #, and between intestinal regions with *.

Table 1. Lipid peroxidation (LPO), CAT and GR activities in pyloric caeca and relative intestinal length of L-SL and H-SL gilthead sea bream at 24h post-feeding.

	L-SL	**H-SL**
LPO (nMols MDA· mg prot^{-1})	0.107 ± 0.01	9.4 ± 2.8$^{\#}$
CAT Activity (U · mg prot^{-1})	191.9 ± 9.0	365.8 ± 69.4$^{\#}$
GR Activity (U · mg prot^{-1})	1.57 ± 0.17	3.08 ± 0.34$^{\#}$
Relative intestinal length (mm · g fish)	0.68 ± 0.06$^{\#}$	0.42 ± 0.04

INTESTINAL OXIDATIVE STATUS OF GILTHEAD SEA BREAM FED WITH DIETS OF DIFFERENT FATTY ACID PROFILE

I. García-Meilán*[1], I. Agredano, [1], A. Profitós [1], R. Fontanillas [2], E. Capilla[1], I. Navarro[1], and A. Gallardo [1]

[1] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 643, 08028, Barcelona, Spain.
[2] Skretting Aquaculture Research Centre (ARC), Sjøhagen 3, Stavanger, Norway.
E-mail: irene.garcia@ub.edu

Introduction

Palm oil, rich in saturated and monounsaturated fatty acids (MUFA) (50 and 40%, respectively) and rapeseed oil, rich in MUFA (57%), mainly oleic acid, are commonly used to partially replace fish oil in fish diets due to their price, availability, and good growth results in most fish species. Moreover, linseed oil, is one of the richest plant sources of n-3 fatty acids with 75% of polyunsaturated fatty acids (PUFA), mainly α-linolenic acid (ALA, C18:3 n-3), and although less frequently used, fish oil replacement by linseed oil has been successful in some fish species. Dietary lipid profile affects membrane composition and modifies lipid peroxidation (LPO), which could negatively affect membrane structure and fluidity, and intestinal permeability. In this sense, LPO production and the antioxidant status could affect digestion and absorption processes, modifying therefore nutrient availability. The aim of the study was to investigate the effect of different dietary fatty acid profiles on LPO and antioxidant capacities at intestinal level in gilthead sea bream (*Sparus aurata*).

Materials and methods

Five isonitrogenous (46%) diets were formulated and produced by Skretting ARC (Norway) in which 25% of dietary oil was provided as fish oil, to meet eicosapentaenoic and docosahexaenoic acids requirements, and 75% was vegetal oil. The vegetable oils used were palm (P), rapeseed (R) and linseed (L) oils and diets were named based on the oil they contained. Diet P contained palm oil as the only vegetable oil, diet R rapeseed oil, and diets PL, RL and PRL a combination of vegetable oils. These last three diets contained a similar amount of n-3 (0.88%), higher than in the P diet (0.4%) and the R diet (0.72%). P and PL diets were rich in saturated fatty acids and R and RL in MUFA.

Two-hundred eighty-six gilthead sea bream with an initial weight of 81.8g were distributed in tanks and fed *ad libitum* twice a day with the corresponding diet for 18 weeks. Once the growth trial finished, fish were sacrificed at 24h post-feeding and samples of pyloric caeca and proximal intestine were collected. LPO levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) activities were determined. Gene expression of antioxidant enzymes (*sod1, sod2, cat, gpx1, gpx4, gr, gst3*) was analysed by quantitative PCR.

Results

Gilthead sea bream fed the mono- and di-substituted diets showed higher LPO levels in the proximal intestine versus the pyloric caeca (x 2.9–5.6) (Fig. 1). This pattern was also observed for CAT, GR and GST activities, but not for SOD and GPx ones, which were similar in the two intestinal segments of these fish (Fig. 1). On the other hand, PRL animals showed similar LPO levels in both intestinal segments, showing the highest LPO amount in pyloric caeca and the lowest in the proximal intestine (Fig. 1). PRL fed fish showed differences between segments for SOD and GPx activities, being SOD activity lower in proximal intestine and that of GPx in pyloric caeca (Fig. 1). In addition, PL fed animals presented the highest LPO production (Fig. 1). SOD and GR activities were higher in fish fed mono-substituted diets, whereas CAT activity was higher in animals fed di-substituted diets (Fig. 1). GPx activity tended to be higher in animals fed P and PL diets (Fig. 1). The ratio of GST activity between proximal intestine and pyloric caeca was maintained between the different groups studied (range of 1.8-2) (Fig. 1). The gene expression of antioxidant enzymes was not significantly affected by diet, except for *gpx4* expression that was modulated in PI. Finally, animal growth did not show significant differences between groups either (average weight of 264.9 ± 6.24 g).

(Continued on next page)

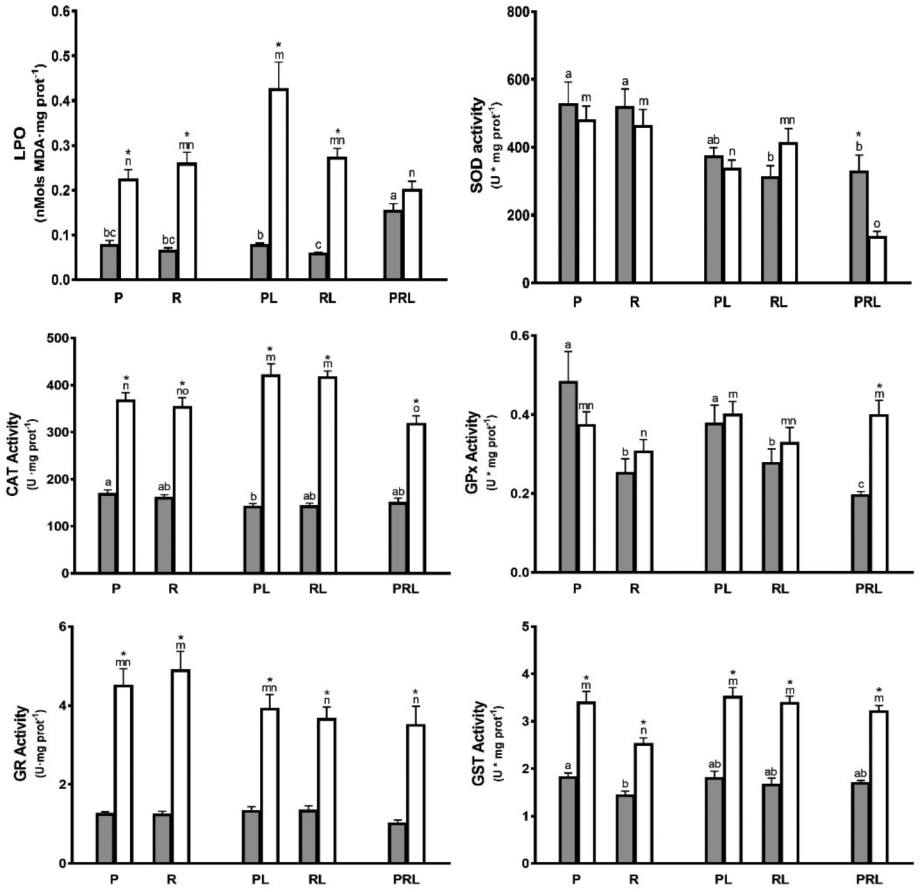


Figure 1. Lipid peroxidation (LPO) levels and SOD**,** CAT, GPx, GR and GST activities at 24h post-feeding in pyloric caeca (PC, grey bars) and proximal intestine (PI, white bars) of gilthead sea bream fed experimental diets. Values are presented as mean ± SEM (n=6 for P and R fed fish; n=9 for PL, RL and PLR fed fish). Significant differences between experimental conditions are shown by letters (a-c for PC and m-o for PI), and between intestinal regions with *.

Discussion and conclusion

LPO levels were higher both in proximal intestine of gilthead sea bream fed PL diet and in pyloric caeca of fish fed PRL diet compared to those fish fed with P diet, probably due to the increase of dietary unsaturated fatty acids content, in agreement with other studies. Despite this, no increase in peroxidation levels was found in RL fed gilthead sea bream in comparison to the R fed group.

In conclusion, data showed that dietary LO inclusion triggered LPO production when it was blended with palm oil, and diminished SOD activity regardless of the oil blend added. Although a similar up-regulation in CAT activity was found in both PL and RL fed fish, in the former, levels of LPO were higher, suggesting that those fish could had more problems to deal with oxidative stress.

EFFECTS OF AN ACUTE HYPOXIC STRESS ON PHYSIOLOGICAL RESPONSE AND SKIN IMMUNITY GENE EXPRESSION IN RAINBOW TROUT *Oncorhynchus mykiss*

I. García-Meilán*[1,2], A. R. Khansari [1], L. Tort [1]

[1] Department of Cell Biology, Physiology and immunology, Faculty of Biosciences, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain
[2] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 643, 08028, Barcelona, Spain
E-mail: irene.garcia@ub.edu

Introduction
In aquatic ecosystems, oxygen fluctuations can lead to temporary hypoxia, being an abiotic stressor for fish. The effects of such stressor depend on the length of time spent under hypoxia, and whether it is an acute stimulus (short-term, high intensity) or a chronic one (long-term, low intensity although persistent). The overcome of the stress situation depends on the ability to modulate physiological and biochemical systems, like changes in behavior, ventilation, etc., to maintain metabolic functions and maximize O_2 extraction from water. In this sense, stressors activate the hypothalamic-pituitary-interrenal axis (HPI) resulting in the release of cortisol that triggers secondary and tertiary responses such as immunomodulatory processes. It is assumed that these responses are usually dual, first stimulatory, and later inhibitory, depending on the stressor and its persistence. The aim of the study was to determine whether and how relationships between the central systemic response and peripheral skin response are taking place in trout subjected to hypoxic stress.

Materials and methods
Rainbow trout juveniles were acclimated to AQUAB fish facilities (Universitat Autònoma de Barcelona, UAB), subjected to a 12L:12D photoperiod and 18ºC in a closed recirculation system. Fish were fed ad libitum with a commercial diet (Skretting). After the acclimation period, trout with a final body weight of 80.8 ± 3.5g, were subjected to an acute hypoxia stress challenge, for one hour, performed by reducing the oxygen levels in water from 8-9 mg/L oxygen to 2 mg/L (by removing the aeration pumps and bubbling N2 into the system). Water oxygen levels were continuously monitored during the experiment. After the challenge, 8 fish per treatment, and time-point (control: without stress, 1h, 6h and 24h post-stress) were euthanized with phenoxyethanol. Samples of blood, skin mucus and tissues were collected for further analysis. Plasma was isolated by centrifugation (5 min at 5,000g) and stored at –20°C until analysis. Plasma and skin mucus cortisol, plasma glucose and lactate and gene expression in skin were analyzed by real time quantitative PCR (RT-qPCR).

Results
Figure 1 shows selected physiological and molecular responses after the stress. All of them were significantly affected by the hypoxic stress and their interaction with time after the challenge was significant. In this sense, rainbow trout presented a significant increase in hematocrit 1 and 6 hours after suffering hypoxic acute stress (Figure 1A). Moreover, a significant rise 1 hour post-stress of plasma and skin mucus cortisol (207.5% and 786.3%, respectively) was observed. Both parameters presented a similar decrease over time, reaching control values 6h after stress (Figure 1B).

Figure 1B also shows how stress triggers glucose release from tissues. Accordingly, glucose levels in fish one hour after the hypoxic treatment were significantly higher than in control ones (67.4%). However, 6 hours after the stressor, glucose levels achieved control values. The same pattern was found for plasmatic lactate where an increase by 51.8% was detected one hour after the stress (Figure 1C).

In skin pro-inflammatory (*ilβ1 and il6)*, anti-inflammatory (*il10* and *tfg*β1), innate immunity genes (*lysozyme, caspase 3*) and stress proteins (hsp70), were analyzed by qPCR. One hour after the hypoxic challenge, gene expression of *ilβ1, il10, caspase 3* and *hsp70* showed a rising trend. In this sense, one and six hours post-hypoxia *hsp70* showed 10.7 and 5.1 times higher relative gene expression than control rainbow trout (Figure 1D). Regarding time, *il10* and *caspase 3* gene expression significantly diminished 24h post-stress versus 1h, showing *caspase 3* even less expression than in control group. No clear trends were found for *il6, tfg*β1 and *lysozyme* gene expression.

Discussion and conclusion
Data shows that one hour acute hypoxia triggers the typical physiological stress responses as expected. Moreover, our results also show that skin gene expression was also activated, directly after stress, such as for the increase of *hsp70*, and inflammatory interleukins (*ilβ1, il10)* activation. Therefore, it is suggested that the stress response appears to be triggered simultaneously at both central and peripheral levels rather than sequentially after neuroendocrine axis activation.

CRYOPRESERVATION PROTOCOLS FOR ELASMOBRANCH SPERM CRYOBANKING: NEW TOOLS FOR SHARKS AND RAYS CONSERVATION

P. García-Salinas[a,b,*], V. Gallego[a], J.F. Asturiano[a]

[a] Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain
jfastu@dca.upv.es
[b] Associació LAMNA per a l'estudi dels elasmobranquis a la Comunitat Valenciana, Valencia, Spain

Introduction

Elasmobranchs are an ecologically diverse vertebrate group that plays a key role in the regulation of the ecosystems they inhabit (Stevens, 2000). But the life histories of sharks and rays make this group extremely sensitive to elevated mortality from fishing habitat destruction (Dulvy et al., 2014). Given this situation, the use of *ex situ* conservation breeding techniques, including artificial insemination, could be a strategy worthy of consideration in elasmobranch conservation plans. However, to perform this technique a reliable sperm supply is needed, and despite cryobanking is the most common way to guarantee the availability of viable sperm (Asturiano et al., 2017), there are not widespread protocols for elasmobranch sperm cryopreservation. In fact, information on elasmobranch sperm cryopreservation is limited to just two scientific publications, with information regarding two ray species and one shark (Daly et al., 2011; Daly and Jones, 2017). While the authors reported successful results in the cryopreservation of both rays no conclusive information was given about the shark besides the toxic effect of the cryoprotectant used on the sperm. To date there is no reported information on successful cryopreservation of shark sperm.

Material and Methods

Sperm from two species, the small-spotted catshark (*Scyliorhinus canicula*) and the rough skate (*Raja radula*) was obtained from alive animals kept in a public aquaria and from animals obtained from commercial fisheries.

An artificial elasmobranch seminal plasma extender (EE) was formulated to be similar in composition (solutes, pH, and osmolality) to the inner fluids of marine elasmobranchs: in mM; 433 Urea, 376 NaCl, 120 Trimethylamine N-oxide (TMAO), 8.4 KCl, 50 Glucose, 7 $CaCl_2$-$2H_2O$, 3.5 $NaHCO_3$, 0.08 Na_2SO_4, 1.4 $MgSO_4$; pH 6.5; Osmolality 1000 mOsm/kg). Short-term preservation of the sperm was tested by diluting (dilution ratio 1:9; sperm:EE) the small-spotted catshark sperm in EE and using sea water as a control. Samples were kept at 4 ºC. Sperm motility was assessed for a period 36 days. Only sperm samples with an initial motility higher than 60% were considered for the trials.

The cryopreservation protocols were performed by adding to the previous mixture of sperm and extender (EE) a series of different cryoprotectants: 10% methanol (MET) or 10% dimethyl sulfoxide (DMSO) or 20% fresh egg yolk. The combination of the cryoprotectants was also tested: 10% MET plus 10% fresh egg yolk, or 10% DMSO plus fresh egg yolk, or 5% MET plus 5% DMSO plus 10% fresh egg yolk. The cryopreservation mixture was used to fill 1.5 ml cryotubes and left for an equilibration period of 15 min at 4 °C to ensure the correct functioning of the cryoprotectants. Samples were frozen inside a styrofoam box partially filled with liquid nitrogen (LN). After the equilibration process, cryotubes were placed over a net metal platform floating 1 cm over the LN, for a period of 15 min. Cryotubes were completely submerged into the LN after that time, remaining there for 5-60 min. For the thawing process, cryotubes were extracted from the LN and submerged for 75 s in a water bath at 70 °C. Samples post-thawing motility was assessed using a microscope and video recording, and cells membrane integrity was checked using a fluorescence LIVE/DEAD Sperm Viability Kit with SYBR-14, which stains intact cells green, and propidium iodide (PI) that stains damaged cells red.

Results

Short-term storage trial. Sperm motility in seawater showed a significant decrease after 5 days, reaching values close to 30%, and close to 0 on day 19. In contrast, diluted samples in EE showed a significant decrease in motility values with respect to day 0 (85-90%) after the first days of storage (days 5 and 12), showing approximately 60-65% of motile cells, followed by a second significant motility decline on day 19, reaching values of 45-50%, and a progressive reduction until day 36 (<10% motility).

(Continued on next page)

Cryopreservation trials. In the rough skate the use of 10% DMSO or 10% MET rendered post-thawing motility values higher than 40%. However, the combination of 5% DMSO plus 5% MET caused a significantly lower result than the other protocols. The small volume of rays sperm samples avoided the test of egg yolk.

In the case of the shark sperm samples the best post-thawing motility values were obtained with a combination of 5% DMSO, 5% MET and 10% egg yolk, which induced mean values close to 35%. Overall, the addition of egg yolk increased the post-thawing motility values, by up to 42.1% in samples with initial motility values of 70%.

Discussion

We have formulated a specific sperm extender capable of maintaining spermatozoa motility capacity for several weeks in different elasmobranch species. Moreover, we achieved the cryopreservation of sperm supplementing the extender (EE) with different combinations of cryoprotectants. Best results were obtained with a combination of DMSO, methanol and egg yolk. Despite the setting up process was developed using sperm samples from small-spotted catshark (*Scyliorhinus canicula*) and rough skate (*Raja radula*), additional cryopreservation attempts have been carried out using other 8 species, including sharks and rays classified as Critically Endangered, such as the blue shark (*Prionace glauca*) and the bull ray (*Aetomylaeus bovinus*). In the end, the sperm of a total of 10 species of elasmobranchs have been cryopreserved for the first time, including sharks, whose cryopreservation had not previously been achieved.

These results can allow the creation of cryobanks for elasmobranch sperm, becoming new tools for their conservation, complementing *ex situ* conservation efforts developed by public aquaria worldwide. Besides gene conservation and reproductive research projects, a regular supply of frozen sperm will reduce the problems that result from the long-distance transport of specimens, inbreeding or the lack of synchronized reproductive cycles in captivity.

Bibliography

Asturiano, J.F., Cabrita, E., Horváth, Á. (2017). Progress, challenges and perspectives on fish gamete cryopreservation: A mini-review. *Gen. Comp. Endocrinol*. 245, 69–76.

Daly, J., Holland, M., Galloway, D. (2011) Preliminary investigations on sperm cryopreservation of a stingray, the sparsely spotted stingaree, in: Cryopreservation in Aquatic Species, 2nd Edition, eds. T. R. Tiersch and C. C. Green (World Aquaculture Society) 337–344.

Daly, J., Jones, R. (2017). The use of reproductive technologies in breeding programs for elasmobranchs in aquaria, in: Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, eds. M. Smith, D. Warmolts, D. Thorney, R. Heuter (Columbus, O: Special Publication of the Ohio Biological Survey), 363–374.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., et al. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife* 3, 1–34.

Stevens, J. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES J. Mar. Sci*. 57, 476–494.

COMPARATIVE ANATOMY OF THE REPRODUCTIVE STRUCTURES OF CHONDRICHTHYANS: SHARKS AND CHIMAERAS

P. García-Salinas[a,b,*], V. Gallego[a], J.F. Asturiano[a]

[a] Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain
jfastu@dca.upv.es
[b] Associació LAMNA per a l'estudi dels elasmobranquis a la Comunitat Valenciana, Valencia, Spain

Introduction

The Chondrichthyes are an ecologically diverse group of vertebrates, which appeared more than 400 million years ago (Compagno, 1990). But their complex life histories make this group extremely sensitive to elevated mortality from fishing and habitat destruction, being is one of the most threatened groups of vertebrates on the planet (Dulvy et al., 2014). Given this situation, the use of *ex situ* breeding programs could be a strategy worthy of consideration for their conservation. The reproduction in captivity of some species of elasmobranchs and chimaeras has been reported, but these events have traditionally relied on natural mating instead of the use of reproductive techniques (Daly and Jones, 2017). A potentially useful technique in breeding programs is the artificial insemination of females, but to do it a reliable supply of sperm is required. Traditionally, cannulation and abdominal massage are the most common procedures to obtain it (Penfold and Wyffels, 2019). However, all these reproductive techniques should consider the morphology and location of the different reproductive structures to be truly effective because the wide diversity of morphologies in these species can hamper the processes of sperm obtention and artificial insemination.

Material and methods

Males and females of 8 chondrichthyan species (7 sharks and one chimaera) belonging to the orders Carcharhiniformes (n=35), Hexanchiformes (n=1), Squaliformes (n=5) and Chimaeriformes (n=4) were studied to observe their reproductive systems and to obtain viable sperm. On dead specimens a dissection was performed to specifically gain access to the reproductive structures, as a previous step for sperm extraction. The focus on each dissection was on i) determine how to gain easy access to the urogenital papilla, ii) observe the number and disposition of urogenital pores, iii) observe urogenital sinus morphology, iv) access to seminal vesicle/uterus and v) sperm obtention. Detailed photographs were taken with a macro lens camera throughout the dissection procedure of every species and illustrated notes were taken.

Sperm obtention procedures were tested in dead and alive males, as well as from the oviducal glands of females. On dead males the extraction was performed using abdominal massage on the ventral region, or around the urogenital papilla in the cloacal cavity. Sperm was also obtained by introducing a polyurethane cat catheter, or nasogastric tube through the appropriate pore on the urogenital papilla, with the help of a sterile lubricating jelly. Last, sperm was extracted after dissection, by stripping directly on the seminal vesicle and urogenital sinus. To obtain sperm from live animals tonic immobility was induced to minimize struggling and reduce the stress before sperm extraction through abdominal massage. On dead females, their oviducal glands were removed and split along its longitudnial axis to expose its lumen. A pearly mucus was collected over the edge of a scalpel blade after scraping over its luminal epithelium, diluted in artificial elasmobranch extender (García-Salinas et al. 2021) and observed under the microscope.

Results

The overall structure of the female reproductive system is well preserved among the different species studied. The system is composed of the ovaries (one or two depending on the species), two paired oviducts with oviducal glands, two uterus and a series of sphincters (or isthmus) that isolate the different parts of the reproductive tract. The greatest differences observed (shape and size of the cervixes, uterus and oviducal glands) are related to the different modes of reproduction.

In males, the overall structure of the reproductive system in both sharks and chimaeras is similar, although chimaeras show unique secondary sexual characters of their group, such as the presence of a frontal tenaculum and prepelvic claspers. The reproductive system is formed by the testes (one or two depending on the species) epididymis, vas deferens, Leydig gland, seminal vesicle, urogenital papilla and claspers with siphonal sacs. There are some differences between the different morphologies of the seminal vesicles and the urogenital papillae, which can be important during the processes of cannulation to extract sperm.

(Continued on next page)

All techniques used to extract sperm offered positive results. The technique that allows the obtention of a greater volume of sperm is the direct pressure on the seminal vesicle during dissection. Cannulation is a technique that causes less damage to reproductive structures and allows sperm to be obtained in a cleaner way. Samples of motile sperm has been extracted from the oviducal gland of females of different species.

Discussion

Chondrichthyan fishes have a higher intrinsic risk of extinction compared to other fish and, nowadays, are one of the most threatened groups of the planet. Public aquaria play an important role in the conservation of these animals, through *ex situ* conservation programs. However, real sustainability of these programs is still far, especially when threatened species are involved. Nowadays the maintenance of zoological collections of public aquaria still rely on captures in the wild or in the spontaneous reproduction of the animals under their care. Breeding programs using reproductive technologies, such as sperm extraction and artificial insemination, could allow the advance of public aquaria towards sustainability and can be the key to reintroduction programs in the wild for threatened species. Some shark species have never been able to reproduce in captivity or have done it anecdotally. Although more research must be done on chondrichthyan reproduction, the goal of this study was to offer an anatomical guide that could be used as a tool for the conservation of these species. Although abdominal massage is the simplest technique for obtaining sperm, it is not the most effective in animals not fully mature or if clean samples are required. Cannulation is a more complex technique, but it allows to obtain sperm in a more precise and clean way if the anatomy of the animal is known. Finally, obtaining active sperm from the oviducal gland in females opens new research opportunities that should be exploited in the future, such is the obtention of viable gametes from by-catch. Much work remains to be done on the development and application of reproductive techniques in chondrichthyans, but these first steps can be crucial for the future conservation of these animals.

Bibliography

Daly, J., Jones, R. (2017). The use of reproductive technologies in breeding programs for elasmobranchs in aquaria, in: Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, eds. M. Smith, D. Warmolts, D. Thorney, R. Heuter (Columbus, O: Special Publication of the Ohio Biological Survey), 363–374.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., et al. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife* 3, 1–34. doi: 10.7554/eLife.00590.

García-Salinas, P., Gallego, V., Asturiano, J.F. (2021). Development of Sperm Cryopreservation Protocols for Sharks and Rays: New Tools for Elasmobranch Conservation. *Front. Mar. Sci*. 8:689089. doi: 10.3389/fmars.2021.689089

Penfold, L.M., Wyffels, J.T. (2019). Reproductive science in sharks and rays. *Adv. Exp. Med. Biol*. 1200, 465–488 doi:10.1007/978-3-030-23633-5_15.

COMPARATIVE ANATOMY OF THE REPRODUCTIVE STRUCTURES OF CHONDRICHTHYANS: RAYS AND SKATES

P. García-Salinas[a,b,*], V. Gallego[a], J.F. Asturiano[a]

[a] Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain
jfastu@dca.upv.es
[b] Associació LAMNA per a l'estudi dels elasmobranquis a la Comunitat Valenciana, Valencia, Spain

Introduction

Appearing almost 400 million years ago, the chondrichthyans are an old and ecologically diverse group of vertebrates with a key role in the regulation of the ecosystems they inhabit (Compagno, 1990). The class Chondrichthyes is classically divided into the Holocephalans (chimaeras), and the Elasmobranchs, commonly named sharks and rays. This last group, the rays, and their relatives (skates, guitarfishes, saw-fishes and alike), is the most diverse group among chondrichthyan fishes. As happen with the rest of chondrichthyans, rays possess life histories that make them sensitive to elevated anthropic pressure, and nowadays are one of the most threatened vertebrated groups on the planet (Dulvy et al., 2014). The use of *ex situ* breeding programs could be a strategy worthy of consideration for their conservation. Although the reproduction in captivity of rays has been reported for some species, these events have traditionally relied on natural mating instead in the use of reproductive techniques (Daly and Jones, 2017). Artificial insemination and sperm extraction are reproductive techniques commonly used for other species, that could be useful in their breeding programs (Penfold and Wyffels, 2019). However, all these reproductive techniques should consider the morphology and location of the reproductive structures to be truly effective. The lack of knowledge on that wide diversity of morphologies of the reproductive system can hamper the processes of sperm obtention and artificial insemination.

Material and methods

Males and females belonging to 11 batoids species of the order Rajiformes (n=20), Myliobatiformes (n=14) and Torpediniformes (n=4) were studied to observe their reproductive systems and to perform sperm extractions. On dead specimens a necropsy was performed to gain access to the reproductive structures, as a previous step for sperm extraction. The focus on each necropsy was on i) determine how to gain easy access to the urogenital papilla, ii) observe the number and disposition of urogenital pores, iii) observe urogenital sinus morphology, iv) access to seminal vesicle/uterus and v) sperm obtention. Detailed photographs were taken with a macro lens camera throughout the necropsy of every species and illustrated notes were taken.

Sperm extraction was tested in dead and alive males, as well as from the oviductal glands of dead females. On dead males the extraction was performed using abdominal massage on the ventral region or around the urogenital papilla or pressing on the seminal vesicle after necropsy. Sperm was obtained also after introducing a polyurethane cat catheter or nasogastric tube through the appropriate pore on the urogenital papilla with the help of a sterile lubricating jelly. In the case of live animals tonic immobility was induced to minimize struggling and reduce the stress before sperm extraction through abdominal massage. On dead females, their oviducal glands were removed and split along its anterior-posterior axis to expose its lumen. A pearly mucus was collected over the edge of a scalpel blade after scraping over its luminal epithelium, diluted in artificial elasmobranch extender (García-Salinas et al. 2021) and observed under the microscope.

Results

The overall structure of the female reproductive system is similar among the different species studied. The reproductive tract is composed of the ovaries (one or two depending on the species), two oviducts with oviducal glands, two uterus and a series of sphincters (or isthmus) that isolate the different parts of the reproductive tract. The greatest differences observed (shape and size of the cervix, uterus and nidamental glands) are related to the different modes of reproduction among oviparous, viviparous, and ovoviviparous animals.

In males, the general structure of the reproductive system is similar in all the species observed: the testes (one or two depending on the species) epididymis, vas deferens, Leydig gland, seminal vesicle, alkaline gland, urogenital papilla, and claspers with clasper glands. Some differences can be observed in the number, shape and position of the urogenital pores leading to the reproductive ducts, as well as on the morphologies of the seminal vesicles, which can be important during the processes of cannulation. The technique that allowed the obtention of a greater volume of sperm is the direct pressure on the seminal vesicle during necropsy. Cannulation is a technique that causes less damage to reproductive structures and allows sperm to be obtained in a cleaner way. Samples of motile sperm were extracted from the oviducal gland of females of different species.

(Continued on next page)

Discussion

Among the group formed by the rays and their relatives (skates, guitarfishes, saw-fishes and alike) are some of the most endangered marine animals in the planet. Public aquaria play an important role in the conservation of these animals, through *ex situ* conservation programs, but real sustainability of these programs is still far. Nowadays the maintenance of their zoological collections still rely on captures in the wild or in the spontaneous reproduction of the animals under their care. Breeding programs using reproductive technologies, such as sperm extraction and artificial insemination, could allow the advance of public aquaria towards sustainability and can be the key to develop reintroduction programs in the wild for threatened species. Although more research must be done on the reproduction of these animals, the goal of this study was to offer an anatomical guide to perform artificial reproduction and sperm extraction as steps towards the conservation of these species. Although abdominal massage is the simplest technique for obtaining sperm, the shape and position of pectoral fins can hamper the sperm extraction procedure, as there is not an easy access to the seminal vesicles. Thus, it is not the most effective in animals with a scarce amount of sperm or if clean samples are required. Cannulation is a more complex technique, but it allows to obtain sperm in a more precise and clean way if the anatomy of the animal is known. Finally, obtaining active sperm from the oviducal gland in females opens new research opportunities that should be exploited in the future, such is the obtention of viable gametes from by-catch. Much work remains to be done on the development and application of reproductive techniques in rays and their relatives, but these first steps can be crucial for the future conservation of these animals.

Bibliography

Compagno, L.J.V. (1990). Alternative life-history styles of cartilaginous fishes in time and space. *Environ. Biol. Fishes* 28, 33–75. doi:10.1007/BF00751027.

Daly, J., Jones, R. (2017). The use of reproductive technologies in breeding programs for elasmobranchs in aquaria, in: Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, eds. M. Smith, D. Warmolts, D. Thorney, R. Heuter (Columbus, O: Special Publication of the Ohio Biological Survey), 363–374.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., et al. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife* 3, 1–34. doi: 10.7554/eLife.00590.

García-Salinas, P., Gallego, V., Asturiano, J.F. (2021). Development of Sperm Cryopreservation Protocols for Sharks and Rays: New Tools for Elasmobranch Conservation. *Front. Mar. Sci.* 8:689089. doi: 10.3389/fmars.2021.689089

Penfold, L.M., Wyffels, J.T. (2019). Reproductive science in sharks and rays. *Adv. Exp. Med. Biol.* 1200, 465–488, doi:10.1007/978-3-030-23633-5_15.

INCLUSION OF *Dunaliella salina* IN COLD EXTRUDED DIETS RESULTS IN SEA URCHIN GONADS WITH HIGH CONSUMER ACCEPTANCE

Inês Garrido[a,b *], Tiago Sá[b], Luís F. Baião[a,b,c], Helena M. Amaro[a], Tânia Tavares[e], F. Xavier Malcata[e,f], Isabel Costa[d], A. Catarina Guedes[a,d], Luísa M.P. Valente[a,b]

[a]CIIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environment Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[b]ICBAS, School of Medicine and Biomedical Sciences, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c]Sense Test, Rua Zeferino Costa, 341, 4400-345 Vila Nova de Gaia, Portugal
[d]ISS, Ínclita Seaweed Solutions, CIIMAR – Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[e]LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[f]FEUP – Faculty of Engineering of University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
E-mail: garridoines44@gmail.com

Introduction
Sea urchin gonads have been increasingly demanded and marketed in Asian countries and Europe mainly due to its organoleptic features. *Paracentrotus lividus* is the most abundant species in southern Europe. To avoid an overexploitation of wild stocks echinoculture has become a sustainable solution. Baião et al. (2019) reported high gonad yield by using a formulated extruded diet for *P. lividus*, but resulted in gonads with a pale-yellow colour. Colour is the first stimuli presented to a consumer and a major marketability influencing factor; consumers prefer yellow-orange colour gonads. Echinenone is the most abundant carotenoid in the gonads and depends on availability, uptake and bioconversion of β-carotene from dietary sources (Symonds et al. 2007). The present study aimed to investigate the effectiveness of a natural source of β-carotene in producing *P. lividus* gonads with acceptable colour.

Materials and methods
A control diet (CTRL) was formulated and compared to four isonitrogeneous and isoenergetic experimental diets containing two levels of *Dunaliella salina* (0.75% or 1.5%, in diets D1 and D2, respectively) as a natural rich source of β-carotene. In two of these *Dunaliella* supplemented diets, a commercially available macroalgae mix was totally replaced by *Porphyra* (D1P and D2P). Another diet was included by supplementing the CTRL diet with 1.2% of crystal glycine (GLY). All diets were cold extruded (<30 °C) and softly dried (<45ºC); diets were distributed every 48h, during 8 weeks, to quadruplicate groups of sea urchins, placed in plastic mesh cages in a saltwater recirculation aquaculture system (RAS) with a stocking density of 3.5 kg.m^{-2}, temperature 18 ºC, salinity 35‰, and a 10h L/14h D photoperiod. At the end of the trial all sea urchins were individually weighted and measured. Gonads of 8 animals per tank were sampled for chemical composition, carotenoid characterisation by high performance liquid chromatography (HPLC), and evaluation of colour by lightness, redness, yellowness, hue angle and chroma (L*, a*, b*, C* and h* respectively) and texture.

Results
All diets were well accepted by the sea urchins, resulting in a similar SGR (0.1) among treatments. Diets were able to enhance gonad yield, that increased from 6.4 and 8.9 to 15.8 and 17.0, in males and females, respectively. Gonad yield and gonadal somatic index (GSI) were similar among diets, but varied significantly between sexes; females had higher yield and GSI than males. Final gonad composition presented significant differences between sexes and diets. Females had higher dry matter and energy, but lower protein content than males. Sea urchins fed diet D1 resulted in the highest gonadal protein content, whilst diet D2 presented the highest energy, irrespectively of the sex. Diets have significantly affected all free amino acids; urchins fed with the experimental diets presented significantly higher concentrations of arginine, valine, methionine and glycine in gonads than the urchins fed with the CTRL diet.

(Continued on next page)

The content of carotenoids in gonads did not vary significantly among dietary treatments, but significant differences were observed between sexes: males containing higher concentrations of total carotenoids, echinenone (most abundant pigment: 12.4 – 17.5 $\mu g.g^{-1}$ WW), α-carotene and β-carotene than females, but practically undetected lutein and zeaxantin. Female's most abundant pigments were lutein (3.7 – 5.3 $\mu g.g^{-1}$ WW) and echinenone (3.3. – 5.9 $\mu g.g^{-1}$ WW), followed by zeaxantin (1.9 – 3.2 $\mu g.g^{-1}$ WW). Gonad colour presented significant differences between sexes and diets. Gonads of males had higher L* and h*, but lower a*, b* and C* values than females. Sea urchins fed *Dunaliella*-diets produced gonads with lower L* and h* values, and higher a* value compared to those fed with CTRL diet. Sea urchins fed with diet D2 had firmer gonads than those fed diet D1P, but did not differ from the CTRL. L* was positively related with echinenone (0.7) and a* and b* positively correlated with lutein (0.8) and zeaxantin (0.8).

Conclusions

All tested diets were able to enhance gonad yield, with females having larger gonads than males, but without differences between diets. All *Dunaliella*-diets were able to produce redder and less luminous gonads than the CTRL diet. This indicates an improvement in gonad colour since consumers prefer gonads with lower values of L* and higher values of a* (Baião et al. 2020). Overall, diets supplemented with *Dunaliella* were able to improve the colour of sea urchin gonads in relation to the CTRL group, resulting in medium to bright orange/red gonads with high acceptance for consumers.

Acknowledgments

Work supported by Project CAVIAR - Market valorisation of sea urchin gonads through dietary modulation (FA_05_2017_015), financed through programme Fundo Azul.

References

Baião, L.F., Rocha, C., Lima, R.C., Marques, A., Valente, L.M.P., Cunha, L.M., 2020. Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications. Food Research International.

Baião, L.F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture. 506, 127-138.

Symonds, R.C., Kelly, M.S., Caris-Veyrat, C., Young, A.J., 2007. Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9'-cis-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 148, 432-444.

PROTECTIVE EFFECTS OF ANTIOXIDANTS TO PREVENT DOXORUBICIN-INDUCED SKELETAL DEFORMITIES IN FISH LARVAE

Sunil Poudel* [1,2,3], Gil Martins[1,2,3], Marisol Izquierdo[4], M. Leonor Cancela[1,2,5], Paulo J. Gavaia[1,2]

[1]Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2]Faculty of Medicine and Biomedical Sciences (FMCB)
[3] PhD Program in Biomedical Sciences, FMCB, University of Algarve, Faro, Portugal
[4] Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria
[5]Algarve Biomedical Center, University of Algarve, Faro, Portugal
Email: pgavaia@ualg.pt

Introduction

Oxidative stress has been related to various skeletal pathologies altering the activity of osteoclasts and osteoblasts, affecting bone remodelling. Reactive oxygen species are key components to increase oxidative stress, increase bone resorption and inhibit bone formation. A large number of studies suggests the importance of the antioxidant system to reduce the effect of bone pathologies and promote better skeletogenesis in aquaculture produced fish.

Materials and Methods

Microdiets were prepared manually by mixing squid powder first with water-soluble components, then with fat and lipid-soluble vitamins, and finally with gelation dissolved warm water. Resveratrol (34mg/kg) and Doxorubicin (30mg/kg) were dissolved on polar molecules whereas MitoTempo was dissolved in water. *S. aurata* larvae at 30 days were randomly stocked into 18 experimental tanks at a density of 2100 larvae/tank. The larvae were fed experimental microdiets with added antioxidants and pro-oxidants, alone or in combination. The antioxidant microdiets were fed every hour from 8:00 to 20:00, whereas the pro-oxidant (doxorubicin) diet was fed only once a week and was continued with a respective combination of control or antioxidant diets. Similarly, zebrafish (5 dpf) were randomly stocked into 24 experimental tanks at a density of 100 larvae/tank for 30 days. The larvae were fed with experimental microdiets with antioxidant and pro-oxidants alone or in combination. The antioxidant supplemented microdiets were fed by standardized spatula 15mg/day and increased by 5mg/day every week. Antioxidant supplemented microdiets were fed 4 times a day, whereas the pro-oxidant diet was fed only once a week. The microdiet combinations were Control, Resveratrol (RES), MitoTempo (MT), Doxorubicin (DOX), Doxorubicin + Resveratrol and Doxorubicin + MitoTempo. The effect of antioxidant and pro-oxidant supplemented diet on skeletal formation, the incidence and distribution of skeletal deformities and the stages of bone mineralization, mineral content and molecular markers of bone were determined.

Results

At 45 dah, *S. aurata* fed microdiets supplemented with pro-oxidants increased the incidence of bone deformities, deformities charge and decreased bone mineralization. These effects were significantly reversed while co-feeding with microdiets supplemented with antioxidants. Doxorubicin significantly reduced the total length of larvae which was significantly reversed with the co-treatment with Resveratrol. Deformities charge was significantly higher on the Doxorubicin supplemented group and were significantly reduced on the groups co-feeding with antioxidants. Cluster analysis showed a distinct difference between the groups on the distribution of skeleton anomalies. The mineralization of the skeleton elements was significantly affected by Doxorubicin. However, co-feeding with Resveratrol the effect was rescued. The differences were also observed on Meristic count between the groups supplemented with antioxidants and pro-oxidants supplemented microdiets. Calcium and Phosphorus content were also decreased on Doxorubicin supplemented groups. Similarly, on the zebrafish trial, the effect of microdiets on standard length was compared with the standard Zebrafeed diet (Sparos Lda, Olhão, Portugal). Resveratrol supplementation significantly increased the length as compared to Zebrafeed and control microdiet. Survival of zebrafish larvae was significantly decreased under doxorubicin supplementation but significantly reversed upon co-feeding with antioxidants. Doxorubicin supplementation also delayed larvae development and significantly decreased total length however, this effect was significantly reversed by co-treatment with antioxidants. Calcium, Phosphorus, Sodium, Potassium and Magnesium contents were also decreased on Doxorubicin supplemented groups whereas, while in co-feeding with antioxidants, the loss of mineral content was significantly rescued.

(Continued on next page)

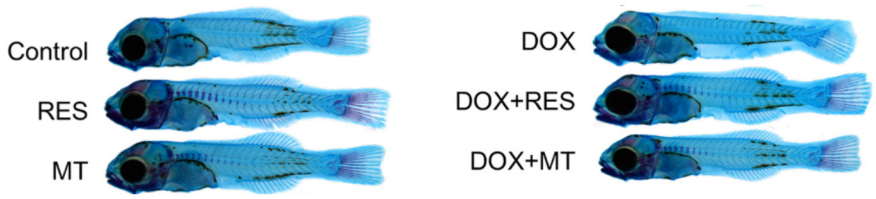


Figure: *S. aurata* larvae at 45 dah fed with microdiets supplemented with antioxidants and pro-oxidants stained with an Acid-free double staining protocol for bone and cartilage.

Conclusion

Our results showed that antioxidant supplements effectively prevent the incidence of bone anomalies and mineralization defects induced by pro-oxidants on both models. Resveratrol and MitoTempo supplementation can reverse pro-oxidant induced effects on bone anomalies, mineralization and oxidative stress. The use of antioxidants in fish diets can be beneficial for improving the health and quality of aquaculture produced fish.

Acknowledgements: 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, and by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020.

SKELETAL DEFORMITIES IN AQUACULTURE PRODUCED GREATER AMBERJACK *Seriola dumerili*

Paulo J. Gavaia[1,2*], Marisa Barata[3], Catarina Oliveira[1], Ana Candeias Mendes[3], Florbela Soares[3], Pedro Pousão-Ferreira[3], Elsa Cabrita[1]

[1] Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
[3] Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO), Olhão, Portugal
*pgavaia@ualg.pt

Introduction

The greater amberjack (*Seriola dumerili*, Risso 1810) is a species recently introduced in the Portuguese aquaculture and it is considered crucial for diversification of production. *Seriola Dumerili* represents a very interesting species due to its high added value and great export potential, since it is highly appreciated in international markets (like Japan). This species has a very high growth rate reaching up to 6 kg in 2.5 years (Mylonas et al 2016) and have a FCR close to 1 at 26ºC (Fernandez-Montero et al 2018). Despite the fast growth in captivity, there is very few data available about the nutritional requirements for this species regarding macronutrients, vitamins, essential fatty acids and minerals (Kotzamanis et al 2021). It has been shown that the levels of DHA and EPA must be strictly controlled, with imbalances in the optimal ratio of 1.6 leading to development of skeletal anomalies, particularly in the skull (Roo et al 2019). Also the dietary regimes followed during larval rearing can significantly influence the development of skeletal anomalies (Djellata et al 2021). In this study we have characterized the main typologies of skeletal anomalies observed in young adults of greater amberjack.

Methods

The experimental rearing was conducted in the IPMA-EPPO aquaculture Facilities (Olhão, Portugal) with the juvenile *Seriola* dumerili being placed in 18m^3 tanks with a constant supply of filtered seawater (temperature: 23-25ºC; salinity 36ppt; natural photoperiod; dissolved oxygen above 90% saturation level). A greater amberjack stock (N= 50; weighing on average 1171.79 ± 250.54 g wet weight) were maintained for 9 months and fed with a commercial diet specifically developed for *Seriola dumerili* (Sparos, Lda, Olhão, Portugal). Fish were sampled for weight and length and all the specimens presenting externally visible skeletal anomalies were euthanized with 500ppm phenoxyethanol. A total of 30 fish were examined and the typologies of skeletal anomalies analysed by digital radiography using a Kodac DSX 4000 pro soft X-Ray (Carestream).

Results and discussion

The most prevalent deformities found in the deformed group of *Seriola dumerili* were affecting the cranial structures particularly the skull bones and upper jaw (87%; Figure 1A) and the lower jaw (70%). Three of the specimens presented also deformities in the hyoid arch, significantly altering the morphology of the mouth apparatus, or even provoking a double mouth phenotype. The axial deformities were mostly present in the abdominal vertebrae (Figure 1B) with 33 % of the specimens showing deformed or fused vertebrae that in 13% of the cases caused lordosis.

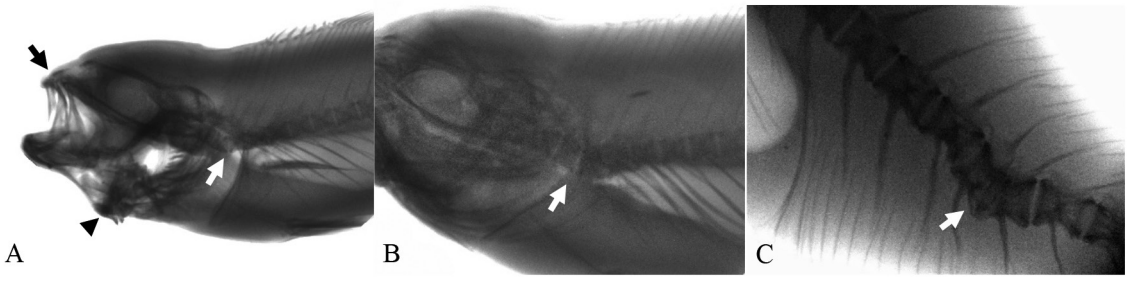


Figure 1. Deformities observed in *Seriola dumerili*. A) Cranial deformity by alteration in the skull bones (black arrow) and hyoid arch (arrowhead) affecting the branchial arches. Abdominal lordosis (white arrow). B) Detail of abdominal lordosis with deformed vertebra. C) Caudal lordosis

(Continued on next page)

Deformities in the caudal vertebrae and arches affected 20% of the specimens, including vertebral fusions and deformed vertebrae involved in vertebral curvatures like lordosis with incidence of 10%. The caudal fin vertebrae region was the less affected with only 2 specimens (7%) presenting mild deformities in the vertebral bodies.

Overall our results show that cranial deformities are the most prevalent type of skeletal anomaly present, significantly altering the phenotype of the specimens. This type of deformities has been associated to inadequate dietary levels of DHA and EPA during early stages (Roo et al 2019), but also to walling behaviour induced by positive phototaxis that leads juveniles to collide with white tank walls. This walling behaviour can be partially prevented by rearing fish in low brightness tanks (Sawada et al 2020). The skeletal anomalies observed are rarely observed in nature, but in aquaculture fish they produce negative impacts by reducing product value, marketing image, but also affecting the biological behaviour and well-being of the cultured animals (Boglione et al 2012). The successful implementation of the greater amberjack as a valuable species in Europe requires research efforts towards reducing the incidence of deformities, that is crucial for producing high quality fish for the markets.

Acknowledgments
This study was funded by the European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 (project SERINOVA MAR-16-02-01-FMP-0064), project DIVERSIAQUA II (MAR2020-P02M01-0656P) and Portuguese national funds (FCT-Foundation for Science and Technology) through project UIDB/04326/2020.

References

Boglione C, Gavaia P, Koumoundouros G, Gisbert E, Moren M, Fontagne S, Witten PE (2013). Skeletal anomalies in reared European fish larvae and juveniles. Part 1: Normal and anomalous skeletogenic processes. Reviews in Aquaculture 5: S99–S120.

Djellata A, Sarih S, Hernández-Cruz CM, Martínez-Rodríguez G, Gilannejad N, & Roo J (2021). The effect of different co-feeding protocols on greater amberjack (*Seriola dumerili*, Risso 1810) larvae. Aquaculture Nutrition 00: 1– 16. https://doi.org/10.1111/anu.13313

Fernández-Montero A, Caballero MJ, Torrecillas S, Tuset VM, Lombarte A, Ginés RR, Izquierdo M, Robaina L, Montero D (2018). Effect of temperature on growth performance of greater amberjack (*Seriola dumerili*) juveniles Aquaculture Research 49: 908-918

Kotzamanis Y Fawole FJ, Brezas A, Kumar V, Fontanillas R, Antonopoulou E, Kouroupakis E, Ilia EV (2021). Dietary lysine requirement of greater amberjack juvenile (*Seriola dumerili*, Risso, 1810). Aquaculture Nutrition, 00, 1– 12. https://doi.org/10.1111/anu.13344

Mylonas CC, Katharios P, Grigorakis K, Papandroulakis N et al 2016. Advances in greater amberjack (Seriola dumerili) research: the DIVERSIFY project. Aquaculture Europe 41: 12-19

Roo J, Hernández-Cruz CM, Mesa-Rodríguez A, Fernández-Palacios H, Izquierdo MS (2019). Effect of increasing n-3 HUFA content in enriched *Artemia* on growth, survival and skeleton anomalies occurrence of greater amberjack *Seriola dumerili* larvae. Aquaculture 500: 651– 659

Sawada Y, Sasaki T, Nishio K, Kurata M, Honryo T, Agawa Y. 2020. Positive phototaxis as the cause of jaw malformations in larval greater amberjack, *Seriola dumerili* (Risso, 1810): mitigation by rearing in tanks with low-brightness walls. Aquaculture Research 51(6):2261–2274

LONG LASTING EFFECTS OF EARLY TEMPERATURE EXPOSURE ON THE OTOLITH ASYMMETRY OF GILTHEAD SEABREAM LARVAE AND JUVENILES

G. Geladakis[1]*, S. Somarakis[2] and G.Koumoundouros[1]

[1] Biology Department, University of Crete, Heraklion, Greece
[2] Institute of Marine Biological Resources and Inland Waters (IMBRIW), Hellenic Centre for Marine Research (HCMR), HCMR, Heraklion, Crete, Greece
Email: georgiosgeladakis90@gmail.com

Introduction

Water temperature during early developmental stages often induces long lasting phenotypic plasticity in marine fish, with implications for the survival, growth and population structure of the wild stocks (Vagner *et al*. 2019, Kourkouta *et al*. 2021). Maladaptive plastic responses can arise as functional costs induced by developmental asymmetries (Gagliano et al. 2008). Otolith morphology is a very sensitive indicator of developmental errors because it is a reliable and permanent record of past growth events and is determined by the genetic and environmental interactions (Gagliano *et al*. 2008; Vignon & Morta 2010). In the present study we examined the effect of temperature during the short embryonic and yolk-sac larval period, on the otolith asymmetry levels of Gilthead seabream (*Sparus aurata*) at metamorphosis (57 days post hatching, dph) and early juvenile stage (94 dph).

Material and Methods

Nine groups of fish were subjected in triplicate to 17, 20 or 23°C, from the stage of epiboly onset to the end of yolk-sac larval stage. Subsequently, all groups were kept under identical rearing conditions and temperature (20°C) up to the end of the trial. From experimental population, a random sample of 10 fish was taken at 57 and 94 dph (30 fish per thermal regime). From each specimen, the largest pair of otoliths (sagittae) was removed and individually photographed. All otolith images were analyzed using the "ShapeR" package (Libungan & Palsson 2015). Following the extraction of the otolith contours, otolith bilateral asymmetry was assessed for ten traits; four univariate morphometric descriptors (maximum length, O_L; maximum depth, O_D; surface, O_S; perimeter, O_P) and six high-amplitude harmonics (H_2-H_7) produced by a normalized elliptic Fourier technique. All traits were tested for the type of bilateral asymmetry, i.e. fluctuating asymmetry, directional asymmetry or antisymmetry (Palmer & Strobeck 1986). The variance of the bilateral difference [index A_1=var(R-L)] was estimated for each temperature group at both sampling ages. The significance of the difference in A_1 between groups was tested by means of Bartlett test.

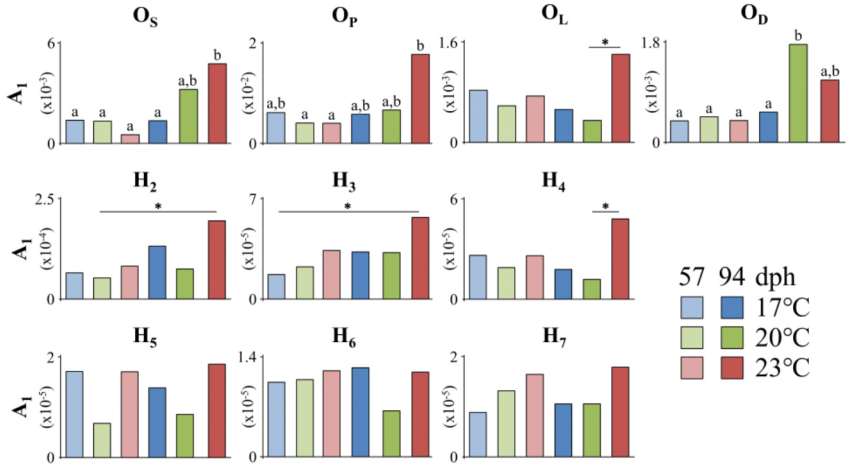


Fig. 1. Effect of developmental temperature (17, 20 and 23°C) on the otolith asymmetry (index A_1) of fish at 57 and 94 days post hatching (dph). O_L: max otolith length, O_D: max otolith depth, O_S: otolith surface area, O_P: otolith perimeter, H_2-H_7: harmonics two to seven. Values without a letter in common are statistically different ($p<0.05$). *($p<0.05$)

(Continued on next page)

Results& Discussion

Developmental temperature (DT) had a significant effect on the otolith bilateral asymmetry of Gilthead seabream. For all the univariate morphometric descriptors (O_L, O_D, O_S, O_P) and the harmonics H_2-H_4, the A_1 asymmetry index was significantly higher in the juveniles initially reared at 23°C DT (3.1±0.3 cm standard length, SL) than those reared at 17 (3.2±0.3 cm SL) and 20°C (3.2±0.3 cm SL) DT (Fig.1). Bilateral differences concerned antisymmetry for the descriptors O_S and O_P, directional asymmetry for O_L, H_3-H_4 and H_6-H_7, and fluctuating asymmetry for the O_D and H_2.

The programming of otolith asymmetry by the temperature experienced during the relatively short embryonic and yolk-sac period in fish, is supported by the findings of Lychakov *et al*. (2006) who suggested that the level of otolith asymmetry is established at the very onset of otolith growth (i.e. at the embryonic stage). Following Gagliano *et al*. (2008), individuals with higher levels of otolith asymmetry could encounter more difficulties in habitat detection during the critical settlement phase. In Gilthead seabream, in addition to its negative effects on otolith asymmetry (present study), elevated DT has been shown to induce a substantial decrease in aerobic swimming performance during the metamorphosis period (Kourkouta *et al*. 2021).

References

Kourkouta C, Printzi A, Geladakis G, Mitrizakis N, Papandroulakis N, Koumoundouros G (2021) Long lasting effects of early temperature exposure on the swimming performance and skeleton development of metamorphosing Gilthead seabream (*Sparus aurata* L.) larvae. Scientific Reports 11: 8787.

Gagliano M, Depczynski M, Simpson SD, Moore JAY (2008) Dispersal without errors: symmetrical ears tune into the right frequency for survival. Proceedings of the Royal Society B-Biological Sciences 275: 527-534.

Libungan LA, Palsson S (2015) ShapeR: An R package to study otolith shape variation among fish populations. Plos One 10: e0121102.

Lychakov DV, Rebane YT, Lombarte A, Fuiman LA, Takabayashi A (2006) Fish otolith asymmetry: Morphometry and modeling. Hearing Research 219: 1-11.

Palmer AR, Strobeck C (1986) Fluctuating asymmetry - Measurement, analysis, patterns. Annual Review of Ecology and Systematics 17: 391-421.

Vagner M, Zambonino-Infante J-L, Mazurais D (2019) Fish facing global change: are early stages the lifeline? Marine Environmental Research 147: 159-178.

Vignon M, Morat F (2010) Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Marine Ecology Progress Series 411: 231-241.

HOW DOES TAG IMPLANTATION AFFECT THE BEHAVIOUR OF EUROPEAN SEABASS *Dicentrarchus labrax?*

Dimitra G. Georgopoulou[1] *, Orestis Stavrakidis-Zachou[1,2], Nikos Mitrizakis[1], Nikos Papandroulakis[1]

1. Institute of Aquaculture, Hellenic Centre for Marine Research, AquaLabs, 71500, Gournes, Heraklion, Greece
2. Department of Biology, University of Crete, 71003 Heraklion, Greece
E-mail: d.georgopoulou@hcmr.gr

Introduction

The usefulness of ultrasonic telemetry on behavioral studies has been proven by a large number of field and laboratory studies (i.e. Abecasis et al, 2018; Schwinghamer, 2019). However, telemetry tags may have an effect on the physiological, behavioral and the performance attributes of the fish (Frank et al, 2009, McKenna et al, 2021) and could be permanent or temporal. These potential negative e□ects are still unknown for a number of species and tagging methods. Recent studies exist and focus mainly on the physiological and anatomical consequences of tag-implantation (Tsitrin, 2020; Justin et al, 2021). It is, thus, crucial to determine the behavioral attributes that are affected by the tag implantation, and the time needed for recovery. Here, we investigated the e□ect of body-implanted tags on the swimming behavior of adult European seabass (*Dicentrarchus labrax*). Three main behavioral aspects were studied, including the synchronization/polarized motion, the group cohesion and the group exploratory behavior.

Materials and methods

45 individuals were captured and transferred from sea cages to a rectangular tank (3.0x3.0x1.2m) 30 days prior to the experiment to allow for adaptation. Polyacetal (POM) cylinders (similar in size and shape with telemetry tags) were implanted into the peritoneal cavity of 20 fish following an experimental protocol that was approved by the ethical committee (Ref: 32257 09-02-2021). The remained fish were used as the control group. After the surgery, fish returned to the tanks and were being recorded from above using fixed IP cameras (HIKVISION DS-2CD1623G0-IZS) for a total period of 15 days. During this period feeding was realized once per day at 12:00. The treated fish returned to normal appetite levels four days after the surgery.

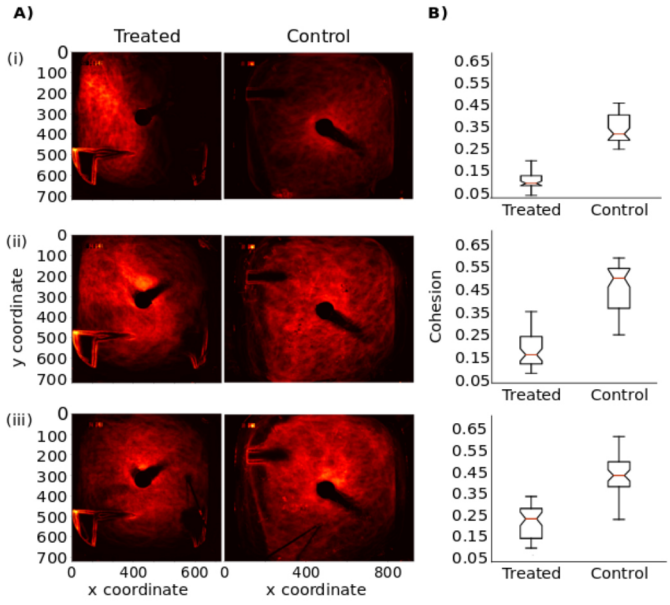


Figure 1. A) Heatmaps of the tanks showing the frequency of the exploration of the tank sites for both, the treated and the control group and for the first (i), the fifth (ii) and the last (iii) day of the experiment. The intensity of the red color indicates higher exploration of the respective tank site. B) Boxplots showing the group cohesion, expressed as the percentage of the area covered by the group, for both, the treated and the control groups and for the first (i), the fifth (ii) and the last (iii) day of the experiment. Red lines indicate median values. Lower values indicate higher cohesion.

(Continued on next page)

From the video recordings, four main variables were extracted using custom-made computer vision routines based on Python/OpenCV. Group directionality and speed were extracted using optical flow analysis. Group cohesion was determined as the normalized tank area covered by the group at each time frame. Last, the exploratory tendency showed the spatial preference of the group and was expressed as the frequency each tank site was covered by the fish.

Results

Preliminary results indicate that E. seabass exploratory behavior is temporally affected by the tag implantation but achieves recovery 10 days after surgery (figure 1A). In contrast, the group cohesion of the treated group remains high during the experiment as the group never covers more than the 40% of the tank area (figure 1B). Although the treated group becomes less cohesive as time progresses, the significant difference between the two groups remains, even 15 days after surgery. Further investigation is required to determine the time needed for recovery of this behavioural atrribute.

References

Abecasis, D., Steckenreuter, A., Reubens, J. et al (2018) A review of acoustic telemetry in Europe and the need for a regional aquatic telemetry network. Anim Biotelemetry 6, 12. https://doi.org/10.1186/s40317-018-0156-0

Frank H.J., Mather M.E., Smith J.M. (2009) What is "fallback"?: metrics needed to assess telemetry tag effects on anadromous fish behavior. Hydrobiologia 635, 237–249. https://doi.org/10.1007/s10750-009-9917-3

James E. McKenna, Suresh A. Sethi, Grant M. Scholten, Jeremy Kraus, Marc Chalupnicki (2021) Acoustic tag retention and tagging mortality of juvenile cisco Coregonus artedi. Journal of Great Lakes Research, https://doi.org/10.1016/j.jglr.2021.03.020.

Justin A.G. Hubbard, Brendan E. Hickie, Jeff Bowman, Lee E. Hrenchuk, Paul J. Blanchfield, and Michael D. Rennie (2021) No long-term effect of intracoelomic acoustic transmitter implantation on survival, growth, and body condition of a long-lived stenotherm in the wild. Canadian Journal of Fisheries and Aquatic Sciences. 78(2): 173-183. https://doi.org/10.1139/cjfas-2020-0106

Tsitrin E, McLean MF, Gibson AJF, Hardie DC, Stokesbury MJW (2020) Feasibility of using surgical implantation methods for acoustically tagging alewife (Alosa pseudoharengus) with V5 acoustic transmitters. PLOS ONE 15(11): e0241118. https://doi.org/10.1371/journal.pone.0241118

Schwinghamer, C.W., Tripp, S. and Phelps, Q.E. (2019) Using Ultrasonic Telemetry to Evaluate Paddlefish Spawning Behavior in Harry S. Truman Reservoir, Missouri. North Am J Fish Manage, 39: 231-239. https://doi.org/10.1002/nafm.10263

DOES STRUCTURAL ENRICHMENT SUPPORT THE WELFARE OF FARMED RAINBOW TROUT JUVENILES IN INDOOR FACILITIES?

Manuel Gesto* and Alfred Jokumsen

Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, DK-9850, Hirtshals, Denmark.
E-mail: mges@aqua.dtu.dk

Introduction
Many fish species show some kind of sheltering behavior. Most fishes seek for shelter, if available, as a physical protection against potential attackers or other threats and many species use shelters for other purposes, too (Kerry and Bellwood, 2017). Most fish-rearing facilities in aquaculture and fish-based research consist of barren tanks where environmental complexity is very low. This has raised concerns about the welfare of fish in captivity and different kinds of environmental enrichment have been tested as potential strategies to promote the welfare of captive fish, with different degrees of success depending on the species, enrichment type and type of welfare indicators considered (Näslund and Johnsson, 2016). In a series of experiments, we have focused on the effects of structural enrichment, in the form of simple PVC-screen shelters, on rainbow trout juveniles. In brief, growth, condition, external damage and stress resilience were monitored in the presence and absence (controls) of shelters. Furthermore, the potential effects of early-life exposure to shelters on the future performance and physiology of the fish were also evaluated.

Methods
In a first experiment, the immediate effects of exposing fish juveniles to the PVC shelters were tested. Juveniles (mean individual mass: 15.0 g; SD: 4.0 g) were reared in the presence or absence of shelters for up to nine weeks, in triplicated groups. Growth performance, condition and external damage in fins, skin, eyes, operculum and snout were monitored. Basal stress levels and the physiological responses to standardized acute and repeated stressors were also evaluated.

In a second experiment, fish were introduced to the shelters at an earlier stage (mean individual mass: 1.5 g; SE: 0.1 g) and fish groups were reared in the presence or absence of shelters for four months. The effects of shelter presence on growth, condition, external damage and stress resilience were monitored as in the previous experiment. In addition, shelters were then removed and, after some weeks of acclimation, fish juveniles grown in the presence or absence of shelters were allocated together after being PIT-tagged. The performance of the fish during co-habitation was monitored, both in normal rearing conditions and in conditions promoting competition (low density, low specific feeding rates), to evaluate the effects of the early exposure to the shelters on fish performance and coping/competitive ability.

Results and discussion
In this study, the design of the shelters was intentionally kept as simple as possible, since one of the main aims was to test the feasibility of using the shelters in the farm environment. In the presence of shelters, fish showed very marked sheltering behavior when submitted to disturbance, demonstrating a clear behavioral preference for shelter use when threatened. However, there were no major differences between sheltered and control (barren) tanks in terms of growth performance, condition factor, external tissue damage, or stress resilience, at least when fish were reared in normal conditions in terms of stocking density and feeding rations. Competitive tests showed that fish grown in the presence of shelters had an inferior ability for competition/growth when the stocking density was kept very low and feeding rations were restricted. It is at present unclear whether such an effect on competitive ability could have been prevented by increasing the time for acclimation of shelter-grown fish to the absence of shelters.

In view of the obtained results, the answer to the question posed in the title of this communication is complex. Fish had no apparent physiological- or performance-related benefits when giving the opportunity to live in a structurally enriched environment; however, they showed a clear behavioral preference for the shelters, particularly when disturbed. Regarding animal welfare, the importance of having access to preferred/wanted items and/or situations differs among the different approaches to the concept of Welfare (Maia and Volpato, 2016). Therefore, the relevance of the shelters for fish welfare in this case could be open to debate since it would depend on the considered welfare approach. It remains to be investigated whether using similar shelters in outdoor facilities, where the chances for external interference/stressors are much higher, would have stronger effects on fish performance and/or welfare.

(Continued on next page)

Figure 1: Picture showing the PVC shelters used during the experiments.

References

Kerry, J.T., Bellwood, D.R., 2017. Environmental drivers of sheltering behaviour in large reef fishes. Marine Pollution Bulletin 125, 254-259.

Maia, C.M., Volpato, G.L., 2016. A history-based method to estimate animal preference. Scientific Reports 6, 28328, 1-8.

Näslund, J., Johnsson, J.I., 2016. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish and Fisheries, 17, 1-30.

THE EU4OCEAN COALITION FOR OCEAN LITERACY AND SUSTAINABLE FOOD FROM THE OCEAN

Iwona Gin*

Nausicaá, Centre National de la Mer, Boulevard Sainte Beuve, 62200 Boulogne sur Mer, France
iwona.gin@nausicaa.fr

Why the EU4Ocean Coalition for Ocean Literacy?
The ocean is a source of life for human beings. It gives us food, oxygen and energy. It is home to many species and acts as climate regulator. Understanding how we influence the ocean and how the ocean influences us is at the core of ocean literacy. This understanding allows us to make responsible consumer choices to better protect our ocean and to use the opportunities it offers in a sustainable manner.

The European Ocean Coalition (EU4Ocean) connects diverse organisations and people that contribute to ocean literacy and the sustainable management of the ocean. Supported by the Directorate General for Maritime Affairs and Fisheries of the European Commission, this bottom-up inclusive initiative offers a dynamic topic-oriented working environment that stimulates collaboration, exchange of practices and dialogue across the many different target groups leading to the creation of new ocean literacy partnerships and innovative actions, co-designed by organisations and the youth.

The coalition is made up of three components: an EU4Ocean Platform for organisations and individuals engaged in ocean literacy initiatives, a Youth4Ocean Forum and a Network of European Blue Schools.

They focus on particular topics: Climate and Ocean, Food from the Ocean and Healthy and Clean Ocean.

Why the Ocean Literacy about Food from the ocean
The topic of Food from the Ocean is very close to each individual because it is linked to food and nutrition, consumer choices, sustainability labels and food waste. Raising awareness of what seafood we eat, from where it comes from and what impact unsustainable fisheries have on the ocean, can bring significant results in terms of behavioural change in the times when the consumer interest in the impact of food on their health and on the planet is growing, especially among young generations.

According to the FAO, the total food demand is projected to increase by 60% by 2050 given current trends. Seafood is a crucial and growing source of nutrition for billions of people around the world. However, about 30% of fish population is overfished or even exhausted because not sustainably exploited.

Aquaculture can be a possible solution as long as it is conducted sustainably. The sector has experienced a real boom over the past years and some achievements have been made in Europe to reach sustainable methods of production. It is important now to raise awareness about these types of seafood and the importance of the sustainable aquaculture production. However, the topic is complex, as it includes cultural, economic and environmental considerations and lacks public trust in the aquaculture products. Therefore, the EU4Ocean Coalition members have started to exchange and map existing activities, stakeholders and resources for joint ocean literacy activities in relation to the topic with the aim of developing new partnerships and actions, including scaling up activities and advocacy campaigns.

How to involve younger generations of consumers?
The EU4Ocean Coalition engages younger generations in the sustainable consumption of food from the ocean through the Youth4Ocean Forum and the Network of European Blue Schools.

The Youth4Ocean Forum is a free platform for young ocean changemakers between 16 and 30 years old. Their common goal is to tackle the climate crisis, fight marine pollution, ensure food security and shape their future with a healthy ocean. Therefore, the Youth4Ocean Forum provides young people with the opportunities to speak up for their generation in front of European institutions, share their ideas and connect with like-minded young people and experts all over Europe. It empowers the youth to solve challenges facing the ocean such as e.g. food security by helping them to develop and promote their individual projects and obtain accreditation of EU Young Ocean Advocates.

(Continued on next page)

The Network of European Blue Schools brings the ocean into the classroom. The programme challenges schools to “Find the Blue,” i.e. to develop a community project that addresses a marine and societal challenge. The project consists of several complementary activities under the umbrella of one central topic. It enables pupils to gain knowledge and skills by working for an extended period of time to explore and investigate an ocean topic, question or problem and explore their connection to the ocean while creating a network across Europe. On their journey to becoming a European Blue School, teachers and pupils improve their understanding of the ocean and develop a sense of responsibility towards it. This feeling of responsibility for the ocean encourages the pupils to make ocean friendly, sustainable consumer choices and become citizens of the global ocean.

PORCINE PROTEIN HYDROLYSATES PROMOTE GROWTH AND ENHANCES SYSTEMIC IMMUNITY IN GILTHEAD SEABREAM FED LOW FISHMEAL DIETS

Enric Gisbert[1], Antoni Ibarz[2], Joana P. Firmino[1], Laura Fernández-Alacid[2], Eva Vallejos-Vidal[3], Ricardo H. Salomón[1], Javier Polo[4], Ignasi Sanahuja[2], Lluis Tort[5], Felipe E. Reyes-López[6], Karl B. Andree[1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
[2] Dept. Cell Biol., Physiol. and Immunology, Faculty of Biology, University of Barcelona, Spain.
[3] Centro de Biotecnología Acuícola, Universidad de Santiago de Chile, Chile
[4] APC Europe SL, Granollers, Spain
[5] Dept. Cell Biol., Physiol. and Immunology, Universitat Autònoma de Barcelona, Spain
[6] Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Chile
* Email: enric.gisbert@irta.cat

Introduction

Protein hydrolysates are reputed in aquafeeds for their antimicrobial, antioxidant and immunomodulatory properties beyond their nutritional value. Although fish protein hydrolysates have been extensively studied (Siddik et al., 2021), the potential use of other sources of animal and plant protein hydrolysates have not extensively evaluated in fish. Among different sources of raw materials to be used for producing protein hydrolysates, rendering by-products have been reported to have relevant nutritional and functional roles in fish nutrition. In particular, the most common evaluated blood by-product in aquafeeds is the porcine spray-dried plasma, although blood protein hydrolysates may be an untapped safe source of animal protein hydrolysates for aquafeeds. The use of rendering blood by-products is an untapped source of highly quality ingredients for aquafeeds, regardless of the change in legislation that allows the use of animal proteins of porcine and avian origin in aquafeeds.

In the present study, authors aimed to evaluate the effects of porcine protein hydrolysates (PPH) on most common key performance indicators like growth and feed efficiency parameters, as well as on its influence on gilthead seabream immunity. Particularly, the immunomodulatory effects of the dietary administration of PPH to a bacterial challenge were tested at two levels: i) by evaluating gene expression level in splenocytes exposed to a short-term *ex vivo* stimulation with lipopolysaccharide (LPS) and ii) by measuring the antibacterial activity of skin mucus when incubated with different bacterial strains.

Materials and Methods

A control diet was formulated with low levels of fishmeal (FM) (7% FM) to contain 48% crude protein, 17% crude fat, and 22 MJ kg^{-1} gross energy. Based on this basal formulation, an experimental diet was formulated, in which porcine protein hydrolysate (PPH) (PEPTEIVA®; APC Europe, SA, Spain) was incorporated at 5% at the expense of FM (final FM levels = 2%). The PPH is a hydrolysate of porcine plasma containing 76% protein (>85% of protein in form of peptides smaller than 10KDa), 2.6% crude fat and 14.5% ash. Both diets were isonitrogenous, isolipidic, and isoenergetic.

Fish were fed two experimental diets (4 replicates per diet) for 92 days under the following environmental conditions: water temperature values ranging from 22 to 27 °C, 6.1 ± 0.2 mg L^{-1} of dissolved oxygen and natural photoperiod. Feeds were distributed four times per day by automatic feeders (feeding rate of 3.3% of the stocked biomass). At the end of the trial, an *ex vivo* assay with splenocytes from both groups was performed as described in Salomón et al. (2020), and the bactericidal properties of the skin mucus were assayed against *Escherichia coli* (DSMZ423), and two pathogenic bacteria, *Vibrio anguillarum* (CECT522T) and *Pseudomonas anguilliseptica* (CECT899T) that were co-cultured with skin mucus during a 24 h-period (Sanahuja et al., 2019).

Results and Discussion

Fish fed the PPH diet were 4.6% heavier than their congeners fed the control diet with low levels of FM. No differences in length nor condition factor were found. In addition, FCR values were lower in fish fed the PPH diet (1.08 ± 0.06) in comparison to those of the control group (1.23 ± 0.04). Similar results were observed in gilthead seabream fed diets containing high FM levels (46%) (Gisbert et al., 2015). Results from the *in vitro* assay with splenocytes of fish from both dietary groups revealed that PPH enhanced the systemic immune response of gilthead seabream as gene expression markers indicated. In particular, we found an up-regulation of gene markers involved in the humoral innate response (*igM*), as well as pro- (*il-1β*, *tnfα*) and anti-inflammatory cytokines (*il-10*, *tgfβ1*), whereas no significant changes in gene expression were

found in terms of *mn-sod*, *cat* and *lys*. Considering that fish skin mucus provides a stable physical, biological, and chemical barrier against invading pathogens, knowledge of its antibacterial capacity when exposed to a pathogenic organism is of relevance. In this sense, the mucus from gilthead seabream fed the PPH showed a higher bactericidal activity than the control group along the 24 h of skin mucus co-culture with the three bacterial species considered. In this sense, skin mucus inhibited the growth of *V. anguillarum* and *P. anguilliseptica*; thus, potentially protecting fish from vibriosis and pastelleurosis when fed diets containing low levels of FM. The immunomodulatory properties of the PPH diet may be attributed to the content of plasma in immunoglobulins, albumins, growth factors and biologically active peptides, which may mediate anti-inflammatory and immunomodulatory effects (Pérez-Bosque et al., 2016).

Conclusions

This study showed that porcine protein hydrolysate obtained from blood plasma is a safe and functional ingredient, especially in those formulated with low fishmeal levels. In particular, PPH promotes somatic growth and improves feed performance, whereas at the same time it enhances the immune response of animals. Considering these beneficial properties, this ingredient can be useful for its incorporation in aquafeeds.

Acknowledgments: This work has been financially supported by the project "Nutritional strategies for the improvement of productive performance: the use of functional feeds and health diets in aquaculture (DIETAplus)", funded by JACUMAR (Ministry of Agriculture, Fisheries and Environment of Spain, MAPAMA) and FEMP (EU), as well as by the ERC (ERC) by the MedAID project (Grant Agreement Nb. 727315).

References

Gisbert, E., Skalli, A., Campbell, J., Solovyev, M.M., Rodríguez, C., Dias, J., Polo, J., 2015. Spray-dried plasma promotes growth, modulates the activity of antioxidant defenses, and enhances the immune status of gilthead sea bream (Sparus aurata) fingerlings. *Journal of Animal Science* 93, 278-286.

Pérez-Bosque, A., Polo, J., Torrellardona, D., 2016. Spray dried plasma as an alternative to antibiotics in piglet feeds, mode of action and biosafety. *Porcine Health Management* 2, 15.

Sanahuja, I., Fernández-Alacid, L., Ordóñez-Grande, B., Sánchez-Nuño, S., Ramos, A., Araujo, R.M., Ibarz, A., 2019. Comparison of several non-specific skin mucus immune defences in three piscine species of aquaculture interest. *Fish & Shellfish Immunology* 89, 428-436.

Salomón, R., Firmino, J.P., Reyes-López, F.E., Andree, K.B., González-Silvera, D., Esteban, M.A., Gisbert, E., 2020. The growth promoting and immunomodulatory effects of a medicinal plant leaf extract obtained from Salvia officinalis and Lippia citriodora in gilthead seabream (*Sparus aurata*). *Aquaculture* 524, 735291.

Siddik, M.A.B., Howieson, J., Fotedar, R., Partridge, G.J., 2021. Enzymatic fish protein hydrolysates in finfish aquaculture: a review. *Reviews in Aquaculture*, in press.

FORECASTING WATER TEMPERATURE IN NORTHERN ADRIATIC LAGOONS: A FUNCTIONAL DATA APPROACH

Daniel Glaser[1], Camilla Bertolini[1], Carlo Gaetan[1] and Roberto Pastres[1*]

(1) DAIS, Ca' Foscari University of Venice, 30173 Venezia, Italy
Email: pastres@unive.it

Introduction

Large-scale food production in coastal and off-shore areas represents one of the most promising ways to tackle future food security issues. Traditionally the lagoons in the Northern Adriatic have been a highly important source of fish, shellfish and game. In the last decades, shellfish farming, in particular that of the allochthonous clam *Ruditapes philippinarum*, has become the most relevant halieutic resource in that region but, in perspective, extensive fish farming in the "Valli da pesca" may also once again become profitable, as a source of sustainable, high quality fish. However, these farming activities are facing some challenges, due to the increasing climate variability related to climate change.

To avoid (sub-)lethal stress to the farmed species it is important to understand the environmental conditions necessary for successful farming, as well as being able to predict the trend and short-term fluctuations of water quality variables, such as the water temperature, dissolved oxygen and salinity. The focus of this work is to develop an innovative data driven model approach, based on functional data analysis, for 1-2 day ahead forecasting of these variables in a lagoon. Thanks to near real-time processing of the data, this tool could help farmers in mitigating the consequences of heat waves and other extreme events related to a changing climate .

Methodology

Being one of the most relevant water quality variables for aquaculture, water temperature is here used to develop and demonstrate this methodology. In order to forecast its daily pattern, an additive model was employed:

$$W_n(t) = T_n + S_n(t) + \varepsilon(t) \qquad (1)$$

in which $W_n(t)$ is the water temperature on day n, T_n represents a trend component and $S_n(t)$ a seasonal one, associated with daily oscillations. This component was modelled using a functional data analysis (FDA) approach, which can be applied to observed data which can be characterised by smooth curves. In particular, a functional autoregressive model with external predictors (FARX) was identified and estimated. In FDA, a set of observations, in this case the detrended temperature observations pertaining to one day, are fitted using a smooth function, obtained by a linear combination of suitable simple basis functions. Subsequently, standard time series analysis methodologies, i.e. ARIMA, can be applied to the weight of the basis functions, rather than to the actual values in the time domain.

In this case, a set of orthonormal functions were used as basis: 5 functions allowed us to obtain a very good fit of the original 48 daily observations. In contrast to a Fourier expansion the functional principal components are not necessarily periodic functions, which facilitates the application to signals that have little or no periodicity.

Based on the shape of the curve of the water temperature observed in previous days and that of the forecast air temperature, the FARX model predicts a curve for the water temperature development of the next day. This curve is continuous: accordingly the output forecasting frequency is adjustable at will.

For fitting the FARX model we use the R package **refund** that provides several functions for creating, handling and analysing functional data, especially for computing regression for functional data.

This methodology was applied to two Northern Adriatic lagoons, the Venice Lagoon (VL) and the Marinetta Lagoon (ML) in the Po delta, to test the model performance on real data. Water quality data was provided by the "Venezia 2021" project (VL) and the Environmental Agency of Veneto (ML), the latter also providing data on the air temperature in both locations.

For each lagoon three different time windows were selected in order to cover various seasons and extreme weather events. The included times were: July 2015 (heat wave), November 2018 (storm "Adrian") and April 2017 (as control group).

(Continued on next page)

The parameter estimation was carried out using the observations from 10 consecutive days. As external input for both modelling stages, the corresponding air temperature and salinity were regarded and their contribution to improving the model quantified using the Bayesian Information Criterion (BIC).

Results & Discussion

With this methodology we were able to successfully forecast the next-day dynamic in a detailed manner (half-hourly prediction steps) for all six cases, with overall performance being better in the more stable sets of spring and summer. Figure 1 below shows the well fitting forecast of the seasonal and combined forecast to the observed curves by the example of the July 2015 forecast in the Venice Lagoon.

Despite having different physical compositions (e.g. depth, size, openness to sea) there was no perceivable difference in the model performance between the two examined lagoons. While air temperature was identified as the most relevant external input in 5 cases out of six, salinity could be used for improving the trend forecast. This could be an indicator that tidal patterns (for which salinity is a likely marker) have a slower, more diffused effect on the water temperature than air temperature does.

Conclusion

With little computational effort, this method is able to create significant results which are also ecologically interpretable, making this a strong alternative for forecasting high resolving time series in near real-time. It can easily be adjusted to model other environmental parameters such as dissolved oxygen or salinity. While the model performance is better when the input time series is smooth, there is no restriction to use only periodic functions.

This tool can help aquaculture farmers to obtain short-term forecasts of adverse extreme events and undertake mitigation action, for example by regulating water flows in the adriatic “Valli di pesca”.

A major restriction for application is still the limited forecasting horizon (here one day), which should be extended to several days after some supplementary analysis.

BIOINDICATORS FOR THE EVALUATION OF FISH FARMING, HEALTH AND PRODUCT QUALITY IN AQUACULTURE (BIOFIA)

Tom Goldammer[1,2*], Alexander Rebl[1], Ronald M. Brunner[1], Doret van Muilekom[1], Svenja Starke[3], Dominik Ewald[4], Annika Kübler[4], Jan Apel[4], Joachim Molkentin[5], Ute Ostermeyer[5], Aaron Knappe[5], Eva Spieck[6], Sabine Keuter[6], Marcel Malinowski[6], Julia Fuentes[7], Patrick Unger[7], Michael Schlachter[8], Jonas Müller[8,9], Henrike Seibel[8,9], Carsten Schulz[8,9]

[1] Leibniz-Institut für Nutztierbiologie (FBN), Institut für Genombiologie, Abt. Fischgenetik, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[2] Universität Rostock, Agrar- und Umweltwissenschaftliche Fakultät, Molekularbiologie und Fischgenetik, Justus-von-Liebig-Weg 2, 18059, Rostock, Germany
[3] Microganic GmbH, Betonstraße 19a, 49324 Melle, Germany
[4] MonitorFish GmbH, Hönower Straße 34, 10318, Berlin, Germany
[5] Max-Rubner-Institut (MRI), Bundesforschungsinstitut für Ernährung und Lebensmittel, Institut für Sicherheit und Qualität bei Milch und Fisch, Hermann-Weigmann-Straße 1, 24103 Kiel, Germany
[6] Universität Hamburg, Abteilung für Mikrobiologie & Biotechnologie, Ohnhorststraße 18, 22609 Hamburg, Germany
[7] Universität Rostock, Agrar- und Umweltwissenschaftliche Fakultät, Aquakultur- und Sea-Ranching, Justus-von-Liebig-Weg 2, 18059, Rostock, Germany
[8] Gesellschaft für Marine Aquakultur (GMA) mbH, Hafentörn 3, 25761 Büsum, Germany
[9] Christian-Albrechts-Universität zu Kiel, Institut für Tierzucht und Tierhaltung, Olshausenstr. 40, 24098 Kiel, Germany
*E-mail: tomgoldammer@fbn-dummerstorf.de

Introduction

Optimal husbandry conditions, healthy animals and innovative raw materials are prerequisites for sustainable aquaculture of animal organisms, the production of quality products and profitable sales. However, although fish grow up successfully in aquaculture from a human point of view, knowledge about animal welfare itself is rudimentary and, as a result, production success is reduced. With integrated monitoring systems, the animal welfare of aquatic organisms can be measured at various stages of production from hatching to harvest weight of the animals. Furthermore, it can be used to certify rearing conditions and aquaculture facilities and thus optimize fish production processes up to the slaughter process. The partners in the project BioFiA developing integrated testing and monitoring mechanisms for fish welfare for the juvenile life stages of salmon. For this purpose, a comprehensive molecular monitoring of the fish on the level of gene expression will be performed (Rebl et al., 2020). This will be supported by blood and stress hormone analyses (Seibel et al., 2021). In parallel, rearing conditions of the fish will be recorded using physicochemical and image sensors (MonitorFish GmbH). The analyses also include detection of bacteria and parasites in the fish and in the farming system (Unger & Palm, 2016, Spieck et al., 2020). AI algorithms are used to perform Big Data analyses of the monitored data. The comprehensive sensory analytics should enable early assessment and even prediction of animal welfare in real time. Currently, the project is looking at the impact of microalgae as innovative probiotic diets (Michl et al., 2019; Microganic GmbH). In addition, an examination of the product quality as food for humans is carried out (Molkentin et al., 2015).

(Continued on next page)

Fig. 1 Illustration of the comprehensive monitoring approach of the project. Sampling of salmon after feeding with microalgae is carried out by PhD students of the FBN and Kiel University at GMA Büsum. Figures surrounding the center image provide examples of the technologies included in the project.

References

Molkentin J, Lehmann I, Ostermeyer U, Rehbein H. Traceability of organic fish - Authenticating the production origin of salmonids by chemical and isotopic analyses. Food Control. 2015; 53:55-66. doi.org/10.1016/j.foodcont.2015.01.003

Michl SC, Beyer M, Ratten JM, Hasler M, LaRoche J, Schulz C. A diet-change modulates the previously established bacterial gut community in juvenile brown trout (*Salmo trutta*). Sci Rep. 2019; 9(1):2339. doi: 10.1038/s41598-019-38800-7.

Seibel H, Baßmann B, Rebl A. Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. Front Vet Sci. 2021; 8: 616955. doi: 10.3389/fvets.2021.616955

Rebl A, Korytář T, Borchel A, Bochert R, Strzelczyk JE, Goldammer T, Verleih M. The synergistic interaction of thermal stress coupled with overstocking strongly modulates the transcriptomic activity and immune capacity of rainbow trout (Oncorhynchus mykiss). Sci Rep. 2020;10(1):14913. doi: 10.1038/s41598-020-71852-8.

Spieck E, Sass K, Keuter S, Hirschmann S, Spohn M, Indenbirken D, Kop LFM, Lücker S, Giaveno A. Defining Culture Conditions for the Hidden Nitrite-Oxidizing Bacterium Nitrolancea. Front Microbiol. 2020; 11:1522. doi: 10.3389/fmicb.2020.01522.

Unger P, Palm HW. Parasitation of sea trout (*Salmo trutta trutta L.*) from the spawning ground and German coastal waters off Mecklenburg-Western Pomerania, Baltic Sea. Parasitol Res. 2016; 115(1):165-74. doi: 10.1007/s00436-015-4732-4.

PILOT CAGE FOR OFFSHORE FISH FARMING

Carla Gomes[a,*]; José Sardinha[b,c]; Barbara Marques[d]; Emanuel Fernandes[d]; Abílio Ferreira[c], Miguel Caetano[e]; Torres Marques[a]; João Bordado[c]

[a]Instituto de Ciencias e Inovação em Engenharia Mecanica e Engenharia Indústria (INEGI), Faculdade de Engenharia da Universidade do Porto (FEUP), Rua Dr. Roberto Frias, 400, 4200-465 Porto, Portugal
[b]Departamento de Engenharia e Ciencias Nucleares (DECN), Instituto Superior Tecnico (IST), Campus Tecnlógico e Nuclear, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Lisboa, Portugal
[c]Centro Recursos Naturais e Ambiente (CERENA), Instituto Superior Tecnico, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal
[d]Estaleiros Navais de Peniche, Porto de Peniche, Peniche, Portugal
[e]Instituto Portugues do Mar e da Atmosfera (IPMA), Rua Alfredo Magalhães Ramalho, 6, 1495-165 Algés, Portugal

*cgomes@inegi.up.pt;

Introduction

The necessity for aquaculture to sustainably feed the world's growing population with a healthy protein, in a responsible manner, is well accepted and understood in government and academic circles. The Portuguese offshore has particularities that have to be taken into account and technically resolved, namely with regard to the vigorous marine swell on the West Portuguese Atlantic coast. Significant technological advances are needed in order to develop sustainable technical solutions from a structural point of view, capable of providing the national aquaculture industry with oceanic cages that allow the establishment of a stable and competitive production base on a global level.

This work intends to contribute to this effort by providing a cage prototype that, after further improvements, is expected to boost the Portuguese aquaculture sector. Many different cage designs and models have been developed and a few are commercially available. Various parameters, like site location/exposure, mooring system, environmental conditions, affect the well-being of culture fish and farm performance. Nevertheless, geometry/shape, size, construction materials and proper design are critical for cage performance, especially on rough sea conditions. High-density polyethylene (HDPE) has been widely used because of its versatility, resistance and low cost. In this work, HDPE tube was also used as main structural material, covered by a nylon net for fish stabling, and a central tube aiming to control the up and down movement of the cage on the water column.

Materials & Methods

The effect of different forces on the performance of the developed cage was assessed by using models for dimensioning the cage considering two main types of forces: static forces as self-weight and hydrostatic pressure and hydrodynamic forces as drag, current and wave forces. Wind forces were not taken into consideration since the cage is submerged.[1,2] The sizing model considers its position relative to water level, up to 30 m depth. These models were developed according with DNV[3]. The dimensioning was performed on Abaqus software.

Cage structure was manufactured using HDPE tube (200 mm of diameter) connected to a central tube made of fiberglass (500 mm of diameter) that ensures the movement of descending and ascending the cage. HDPE tubes were assembled using a standard welding processes, forming a tubular structure that is connected by steel cables to the central fiberglass tube.

Results & Discussion

The cage concept (fig. 1) was designed and developed to meet challenges identified by aquaculture, such as autonomy, maintenance and robustness. The cage incorporates a up and down movement system that allows it to be placed safely, if rough wave conditions arise, for its structural resistance and for the healthy development of the fish. The cage is characterised by an external tubular structure where the net that allows the stabling of the fish is fixed and connected to a central tube. Details regarding geometry, materials and construction methodologies used are provided.

(Continued on next page)

The external tubular structure is circular in its water plane and rounded in depth, allowing greater efficiency in the space for fish to swim in circular patterns[4]. Other reason for this type of geometry was the better resistance to stresses when installed in different locations, as often occur at Peniche (wave height of 7 m and average period of 11 sec*: IPMA, 2018*). The structure was manufactured with HDPE tubes, a material with good energy absorption capacity that makes it interesting for applications subject to fatigue conditions.

The central tube is expected to accommodate different cage functional systems for autonomous operation, such as the automatic feeder, monitoring system, batteries and the up and down movement system of the cage. This component was designed in composite material to incorporate systems that require greater robustness.

This work was supported by Fundo Europeu dos Assuntos Marítimos e das Pescas (FEAMP) through the Operational Programme Mar2020. The authors acknowledge Mar2020 for financial support (grant number: 16-02-01-FMP-0041). Abilio Ferreira gratefully acknowledges support from Mar2020 for a research fellowship. The authors have no competing interests to declare.

1. Ágústsson G. Design considerations and loads on open ocean fish cages south of Iceland. *Univ Icel*. 2004;(June).
2. Moe H, Fredheim A, Hopperstad OS. Structural analysis of aquaculture net cages in current. *J Fluids Struct*. 2010;26(3):503-516. doi:10.1016/j.jfluidstructs.2010.01.007
3. Kovacs G. Environmental conditions and environmental testing. *INTELEC, Int Telecommun Energy Conf*. 1993;2(October):92-99. doi:10.1109/INTLEC.1993.388591
4. Kumar V. Engineering consideration for cage aquaculture. *IOSR J Eng*. 2014;4(6):11-18. doi:10.9790/3021-04661118

EUROPEAN SEABASS (*Dicentrarchus labrax*) GUT MICROBIOTA MODULATION BY DIETS WITH DEFATTED *Tenebrio molitor* LARVAE MEAL AS MAIN PROTEIN SOURCE

Ana Basto[1,2], Ana Teresa Gonçalves[3]*, Mariana Ferreira[1,2], Luísa M.P. Valente[1,2]

[1]CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[2]ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3]GreenCoLab, Associação Oceano Verde, University of Algarve, Campus Gambelas, H8, 8005-139 Faro, Portugal

*Presenting author: anagoncalves@greencolab.com

Introduction
Insect protein is a sustainable alternative to animal and plant protein and has been introduced as a core ingredient in aquafeeds. In addition, insects have a good essential amino acid (EAA) profile and are rich in vitamins, minerals, and functional compounds such as chitin, antioxidant and antimicrobial peptides. The defatted *Tenebrio molitor* larvae meal (*d*TM) is a highly digestible protein, able to meet European seabass (*Dicentrarchus labrax*) EEA requirements (Basto et al., 2020). The aim of this work was to assess the effects of partial and total replacement of fish meal (FM) by *d*TM meal in seabass gut microbiota community structure, functionality and interactions.

Material and Methods
Juvenile European seabass (55 ± 2g) were distributed over 12 tanks and were fed with practical extruded diets formulated with 45% FM (CTRL) that was gradually replaced by *d*TM at 40% (TM40), 80% (TM80) and 100% (TM100). Fish were fed for 10 weeks and starved for 24 hours prior being sacrificed. Intestines from 5 fish per tank were collected under aseptic conditions, flash frozen in liquid nitrogen and subsequently processed for microbiota analysis. DNA was extracted from intestinal mucus with the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) following manufacturer's instructions, and the 16S rRNA gene was amplified and sequenced in a MiSeq Illumina platform. Raw data were processed for filtering and quality control with QIIME2, taxonomic assignment was performed, and alpha and beta diversity were calculated. The modulation of the functional profile of the microbiome was predicted with PICRUST2. Correlations between taxa was assessed by SparCC and networks were built to further understand taxa interactions.

Results and Discussion
Gut microbiota community richness (Chao1 index) and diversity (Shannon index) were not affected by the FM replacement by TM. In terms of beta diversity, diets had a limited effect on the communities' composition. European seabass gut communities were composed mainly by Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. Genera *Flavobacterium*, *Acinetobacter* and *Micrococcus* were the most abundant, regardless dietary treatment. Interestingly, a random forest analysis indicated that the most relevant taxonomical groups belonged to genera *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Burkholderia* and *Acidovorax*, and their abundances were modulated by *d*TM inclusion, but not in a dose dependent manner. As an example, *Flavobacterium* and *Acidovorax* increased abundance in TM40 and TM80, but these same genera were significantly reduced in TM100; whilst the abundance of *Acinetobacter* was reduced in TM40 but increased in TM80 and TM100.
Microbiome functional prediction showed some upregulated pathways in TM40 microbial community when compared with CTRL. Those were mostly related with energy production and peptidoglycan biosynthesis. On TM80 community very few pathways were putatively modulated. When comparing TM40 with TM100, a major downregulation in microbiome pathways related with energy production was observed.

Correlation analysis was performed to identify potential interactions between microorganisms that hold potentially commensal, mutualistic, or competitive relationships. The genus *Acidovorax* was highly correlated with several less abundant taxa with potential beneficial effects for the host, such as *Lactobacillus*, *Burkholderia*, among others. This was not observed in all diets, since in TM100 the *Acidovorax* correlation cluster included other taxa such as *Acinetobacter* and *Limnohabitans*. Previously, Basto et al. (2021) reported the effects of FM replacement by *d*TM in European seabass and demonstrated that up to 80% substitution no negative effect on fish performance were observed. Interestingly, microbiome overall analysis indicates no major changes up to 80% FM replacement, but the total replacement by *d*TM evidenced slightly different effects on microbial community composition and function.

(Continued on next page)

Conclusion

This study provides insights on the effects of defatted *T. molitor* larvae meal as main protein source in European seabass gut microbiota and brings light to taxa interactions and functional profiles that can explain the physiological output observed in fish.

References

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 521, 735085.

Basto, A., Calduch, J., Oliveira, B., Petit, L., Sá, T., Maia, M.R.G., Cabral-Fonseca, S., Matos, E., Pérez-Sánchez, J., Valente, L.M.P., 2021. The use of defatted *Tenebrio molitor* larvae meal as a main protein source is supported in European sea bass (*Dicentrarchus labrax*) by data on growth performance, lipid metabolism and flesh quality. Front Physiol. 12, 659567.

Aknowledgements

This study was supported by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF) and project ANIMAL4AQUA, funded by Portugal2020, financed by ERDF through the Operational Competitiveness Program (COMPETE) - POCI-01-0247-FEDER – 017610. A. Basto and M. Ferreira acknowledge Fundação para a Ciência e a Tecnologia (FCT) for grants SFRH/BD/138593/2018 and SFRH/BD/144843/2019 (FCT/FSE), respectively.

EUROPEAN SEABASS *(Dicentrarchus labrax)* GUT MICROBIOTA MODULATION BY DIETS WITH *Gracilaria gracilis* BIOMASS INCLUSION

Ana Teresa Gonçalves1*, Marco Simões2, Ricardo Passos2, Pedro Pires2, Damiana Pires2, Beatriz do Carmo2, Teresa Baptista2,3

1GreenCoLab – Associação Oceano Verde, University of Algarve, Campus Gambelas H8, 8005-139 Faro, Portugal
2MARE – Marine and Environmental Sciences Centre, ESTM, Politécnico de Leiria, Av Porto de Pesca, 2520-620 Peniche, Portugal
3School of Tourism and Maritime Technology, Polytechnic of Leiria, Campus 4 – Rua do Conhecimento nº 4, 2520-614 Peniche, Portugal
Email: teresa.baptista@ipleiria.pt

Introduction

Seaweeds are an important source of nutrients and have been part of human nutrition worldwide. Their composition includes a large set of bioactive compounds that confer the algae antioxidant, immunostimulant, antibacterial and antiviral properties among others. In aquafeeds, algae inclusion as a main ingredient has been studied mainly as a fish meal replacement protein source, but more recently other properties have been highlighted pushing their usage as fish health boosters. The macroalgae *Gracilaria gracilis* has an interesting composition that includes biologically active phytochemicals and others that confer functional properties to be used in fish nutrition. Peixoto et al (2019) showed that a 5% inclusion of *Gracilaria* sp. biomass improved seabass resistance to a pathogen due to its immunostimulatory and antioxidant capacity. However, evidence have shown that algae can act as prebiotics, modulating gut microbial communities, stimulating the production of functional metabolites in the gut, and this might be one of the origins of the observed better physiological performances in fish.

The aim of this work was to evaluate the modulation of European seabass gut microbiota when fed with diets with *Gracilaria gracilis* biomass inclusion or its extract.

Material and Methods

Experimental diets were formulated to include seaweed dry powder at 2.5% and 5%, or 0.35% of the algae extract. The control diet was the base for all the diets, and these were provided to juvenile seabass (*Dicentrarchus labrax*) (17.49 ± 6.07 g; mean ± SD) *ad libitum* for 47 days.

By the end of the feeding trial, intestines were aseptically removed and a portion of anterior and another of posterior intestine were collected and stored at -80 °C until DNA extraction. DNA was extracted from the intestine mucosa and mucus and the V3 and V4 hypervariable region of the 16S rRNA gene was sequenced in a Miseq Illumina platform. Raw reads were processed in QIIME2, and as a first step, they were filtered for quality, merged and chimeras were removed with DADA2. Rarefaction was applied for alpha and beta diversity analysis. Taxonomical assignment of the obtained ASVs (amplicon sequence variant) was performed based on greengenes database and microbial community composition in both intestinal compartments of fish fed the four experimental diets was evaluated up to genus level (or species when available). Random forest analysis was performed to highlight the most important genera in the communities, and microbiome functionality was predicted with PICRUST2.

Results and Discussion

The community richness (Chao1 index) and diversity (Shannon index) were not altered by the diets, however, diets with algae biomass (both 2.5% and 5%) showed a tendency for higher richness and diversity. Both Weighted and Unweighted unifrac distances analysis indicated differences between anterior and posterior intestinal sections, however, the same analysis did not show differences in the microbial communities of each experimental group. The most abundant phyla were Proteobacteria, followed by Actinobacteria and Firmicutes, and in the anterior intestine groups fed with algae the last two phyla had higher abundance, whereas in posterior intestine abundances were more similar. Algae inclusion in diets significantly reduced the abundance of members of genus *Photobacterium* in both intestine sections, and inclusion of 2.5% algae biomass reduced the abundance of *Staphylococcus* genus' members.

(Continued on next page)

Based in a random forest analysis, the most important genera within the communities according to abundance shifts with diet were *Photobacterium*, *Staphylococcus*, *Acinetobacter*, *Micrococcus* and *Sphingomonas,* and their abundances were reduced when fish were fed diets with algae, but not always with algae extract.

Functional prediction of the intestinal microbiomes showed that in anterior intestine several pathways are modulated whereas in posterior modulation occurred in only few. These pathways were mainly related with metabolism and biosynthesis of protective compounds such as ectoine and were upregulated in fish fed diets supplemented with algae.

Conclusions

This study highlights that feeding fish with diets with *Gracilaria gracilis* biomass up to 5% did not change the gut microbial community structure. However, it produced an effect on specific taxa that might be related to disease states, and the reduction of abundance of these microorganisms in algae groups encourages to consider a possible association with reduced mortalities observed by Passos et al (2021), after a challenge with *Photobacterium damselae* subsp. *piscicida*. Despite the few changes in community composition, function of the microbiome was putatively modulated in a beneficial manner. This study brings light to the effects of algae as functional ingredient in fish gut microbiota and will serve as base for future studies.

References

Peixoto, M.J., Ferraz, R., Magnoni, L.J., Pereira, R., Gonçalves, J.F., Calduch-Giner, J., Pérez-Sánchez, J., Ozório, R.O.A., 2019b. Protective effects of seaweed supplemented diet on antioxidant and immune responses in European seabass (*Dicentrarchus labrax*) subjected to bacterial infection. Sci Rep 9. https://doi.org/10.1038/s41598-019- 52693-6.

Passos, R.; Correia, A.P.; Ferreira, I.; Pires, P.; Pires, D.; Gomes, E.; Carmo, B.; Santos, P.; Simões, M.; Afonso, C.; Baptista, T. (2021) Effect on health status and pathogen resistance of gilthead seabream (*Sparus aurata*) fed diets supplemented with *Gracilaria gracilis*. Aquaculture. 531:735888. https://doi.org/10.1016/j.aquaculture.2020.735888.

Aknowledgements

This study had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UIDB/04292/2020 granted to MARE, and the project MAR-02.01.01-FEAMP-0084- SeaWeedFeeds.

IMMUNOMODULATION OF SENEGALESE SOLE *(Solea senegalensis)* POST-LARVAE BY ALGAE BLENDS AS FUNCTIONAL INGREDIENTS IN MICRODIETS

Ana T. Gonçalves[a]*, Wilson Pinto[b], Diogo Peixoto[c], Daniel Afonso[b], Benjamin Costas[c], Maria Morais[b], Helena Abreu[d], Joana Silva[e], João Navalho[f], Ana Mendes[g], Sara Castanho[g], Cátia Marques[g], Rita Colen[h], Pedro Pousão-Ferreira[g], Sofia Engrola[h], Jorge Dias[b], Luís Conceição[b]

[a] GreenCoLAB, Faro, Portugal
[b] SPAROS Lda, Olhão, Portugal
[c] CIIMAR, Matosinhos, Portugal
[d] AlgaPlus, Ilhavo, Portugal
[e] Allmicroalgae, Pataias, Portugal
[f] Necton S.A., Olhão, Portugal
[g] IPMA-EPPO, Olhão, Portugal
[h] CCMAR, Faro, Portugal

*E-mail: anagoncalves@sparos.pt

Introduction

Algae, either micro- or macro-algae, are sustainable natural ingredients that have been used in human nutrition due to their rich composition in high valued nutrients such as proteins and long-chain polyunsaturated fatty acids (LC-PUFAs). More recently, they have been acknowledged for their potential as a nutraceutical resource due to their enriched composition in pigments, minerals, vitamins and other bioactive compounds. In early life stages, fish are immature in their responses to the environment and their health and development can easily be compromised. Microalgae have been used to promote larvae health in larviculture, however the study of their effects in feeds as a health promoter is recent. Peixoto et al (2021) showed the potential of microalgae as larvae robustness enhancer in Senegalese sole (*Solea senegalensis*), however, considering the contents of bioactive compounds in macroalgae, their synergetic application may be a cost-efficient strategy to potentiate algae as functional ingredient in aquaculture (Batista et al 2020). The aim of this study was to evaluate the immune response of *S. senegalensis* postlarvae when fed microdiets with algae blends (*Nannochloropsis* sp. and *Gracilaria gracilis*) included as functional ingredient to boost immunocompetence and overall performance.

Material and methods

Senegalese sole post-larvae were fed the CTRL diet, or the blend at 3% or 6% inclusion (BLEND3 and BLEND6 in equal proportions of micro- and macroalgae). The trial was conducted from 34 to 63 days after hatching (DAH) and molecular and physiological immune response was evaluated at 49 and 63 DAH (12 and 29 days of feeding). Growth performance and whole-body immune response were assessed, as well as the expression of a panel of genes related with immune response. Here, the modulation of the expression of hepcidine (*HAMP*), *complement C3*, g type lysozyme (*gLys*) the cytokines *IL1b* and *IL10* and the toll like receptors *TLR1* and *TRL5* was evaluated and integrated with the observed physiological output. Data analysis was performed at univariate level and data were further integrated in a canonical discriminant analysis to infer different performances. A follow-up trial was performed to evaluate the capacity of post-larvae to cope with a *Tenacibacullum maritimum* infection inflicted by bath exposure.

Results and Discussion

Overall growth performance was enhanced in fish fed diets with algae blends, mainly BLEND3. Lysozyme and peroxidase activity was higher in both blend groups but was more evident in BLEND6 group. Interestingly, molecular analysis revealed lower expression of *gLys* in fish fed the blends at 49DAH. Expression of *HAMP*, *IL10* and *TLR5* was modulated in BLEND6 at 63DAH, and were slightly higher than in BLEND3. Multivariate analysis showed that at 49DAH groups are not separated based on the evaluated parameters, however at 63DAH both groups with blend inclusion, BLEND3 and BLEND6 were significantly discriminated from CTRL group. When fish were challenged with *T. maritimum* fish responded with a 20% increase in survival when compared with CTRL group, validating that algae blends inclusion in post-larvae microdiets improve fish robustness and resistance to diseases.

(Continued on next page)

Conclusions

Micro- and macro-algae blends inclusion in microdiets have a great potential to promote health and resistance in Senegalese sole post-larvae. Algae blend is a sustainable ingredient and supports a cost-efficient strategy for larviculture since the inclusion of macroalgae mitigate the high production costs of microalgae and provides a more diverse bioactive compounds composition to the mixture. Here we observed that overall, the BLEND3 produced similar results than BLEND6 therefore the former is a more cost-effective inclusion rate.

References

Peixoto, D.; Pinto, W.; Gonçalves, A.T.; Machado, M.; Reis, B.; Silva, J.Navalho, J.; Dias, J.; Conceição, L.; Costas, B. (2021) Microalgal biomasses have potential as ingredientes in microdiets for Senegalese sole (Solea senegalensis) post-larvae. Journal of Applied Physology. DOI: 10.1007/s10811-021-02431-1

Batista, S.; Pereira, R.; Oliveira, B.; Baião, L.F.; Jessen, F.; Tulli, F.; Messina, M.; Silva, J.L.; Abreu, H.; Valente, L.M.P (2020) Exploring the potential of seaweed Gracilaria gracilis and microalga Nannochloropsis oceanica, single or blended, as natural dietary ingredients for European seabass Dicentrarchus labrax. Journal of Applied Phycology 32, 2041–2059.

Acknowledgements

This work was funded by project 24517_VALORMAR supported by Compete 2020, Lisboa 2020, CRESC Algarve 2020, Portugal 2020 and the European Union through FEDER/ERDF.

ALGAE AS A NUTRACEUTICAL INGREDIENT IN AQUACULTURE: UNLEASHING THEIR POTENTIAL AS GUT HEALTH MODULATORS

Ana T. Gonçalves[a]*, Rita Jacinto[a], Hugo Pereira[a], Helena Abreu[b], Joana Silva[c], João Navalho[d], Jorge Dias[e], Luís Conceição[e]

[a] GreenCoLAB, Faro, Portugal; [b]AlgaPlus, Ilhavo, Portugal; [c]Allmicroalgae, Pataias, Portugal; [d] Necton S.A, Olhão, Portugal; [e]SPAROS Lda, Olhão, Portugal

*E-mail: anagoncalves@greencolab.com

Introduction

Fish health management in aquaculture is a fundamental axis for the sector's performance and sustainability. Fish are frequently exposed to aggressions inherent to the culture conditions jeopardizing their homeostasis reducing growth and defensive performance. The gut and associated microbiota comprise a pivotal organ with key role in digestion, nutrient acquisition, metabolism, immune defense and also endocrine and neuronal regulation that are highly responsive to environmental changes. These changes often result in higher epithelial permeability, inflammatory responses and dysbiosis associated with reduction of functionality. Thus, gut health management is crucial for fish health and nutraceutical strategies can be applied to support gut health and function.

Algae are one of the most abundant marine resources and their nutritional properties have made them highly valued ingredients for human and animal nutrition. In addition, their diverse composition in bioactive substances such as polysaccharides (e.g. fucoidans, alginic acid, ulvans), polyphenols (e.g. phlorotannins) and pigments (e.g. fucoxanthin, zeoxanthin) makes them targets for human medicine and cosmetics due to their anti-inflammatory, antioxidant, antimicrobial properties, and their ability to stimulate cell and tissue repair. In aquaculture their potential has been highlighted as alternative protein and fatty acids source, however their prebiotic and functional properties indicate a possible relevant application in fish nutraceuticals for gut health management and performance.

The aim of this study was to assess the potential of algae biomass as gut health promoters for gilthead seabream (*Sparus aurata*), using a gut explant model.

Material and methods

Gilthead seabream (210±10 g) were used to obtain the explants cultures. Intestines were dissected in aseptic conditions, separated in anterior and posterior sections, and were cut in small portions. These small sections were open to expose the lumen and were placed in plastic plates with lumen facing up. Medium was added to the wells and tissues were rested for a short period to acclimate. Several algae biomasses were tested, and these were the macroalgae *Gracilaria gracilis*, *Fucus* sp., and *Ulva* sp., and the microalgae *Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Skeletonema* sp., *Isochrysis galbana* and *Tetraselmis* sp..

Algae were used in their commercial form in dried biomass, and their protective and therapeutic effects were tested in the explants by application of suspensions in culture medium. Gut explants were stimulated with a commercial lipopolysaccharide (LPS) to promote an inflammatory response, and algae protective effect was tested with previous incubation of the tissue with an algae suspension (6h), whereas the therapeutic potential included incubation of the tissue only after LPS exposure (2h). Explant's stability was assessed by measuring the lactate dehydrogenase activity in culture medium, and tissue response was assessed by measuring the expression of a panel of genes. These included genes related with inflammatory response (e.g. *IL1b*, *COX2*), adaptive immune response (e.g. *MHCI*, *mIgM*), epithelium integrity (e.g. *OCL*, *CLD*) and antioxidant response (e.g. *CAT*, *GPx*). Multivariate integrative statistical approach was used to discriminate and rank responses to infer the potential of the different biomasses.

(Continued on next page)

Results and Discussion

Seabream explants were responsive to commercial LPS, and after 6h an engaged inflammatory response was observed with alterations in tissue epithelial integrity at molecular level. When gut tissues were incubated with algae suspensions a modest immune modulation was observed with some species (mostly microalgae). The protective effect of algae was reflected in an overall attenuated response to the LPS exposure, although not always significant. Algae suspensions homogeneity are relevant for tissue stimulation, and especially in the case of macroalgae, the biomass fragments size variability might influence gut explant response. However, when applied as therapeutic strategy after inflammatory cascade was triggered, gut response was soothed indicating a potential regulatory action exerted by algae.

This model allows a fast screening and ranking algae potential as gut performance enhancers, reducing the number of animals used and still accounting for the complexity of the intestinal multiple cell/bacteria interactions and arrangements. Algae biomass have shown a strong potential as functional ingredients for fish gut health management, and it is relevant to further explore the optimization of their functional properties by, for instance, increasing the availability of their bioactive compounds with cell disruption or other cost-efficient methods.

GENOMIC IMPUTATION BETWEEN HIGH DENSITY AFFYMETRIX ARRAYS USING HISTORIC GENOMIC DATA FROM A COMMERCIAL NORTHAMERICAN ATLANTIC SALMON (*Salmo salar*) BREEDING PROGRAM

L. Gonzalez[1]*, J.A.K. Elliott[2], F. Powell[2], M. Herlin[1]

[1] Culmarex, C/ Don Carnal, 13, P.I. El Labradorcico 30889 Águilas, Murcia, Spain
2 Kelly Cove Salmon Ltd., Cooke Aquaculture Inc., Saint John E2L 3H3, Canada
Email: lgonzalez@cookeaqua.com

Introduction
Since 2014, the Canadian company CookeAqua Inc. (CAI) has invested in the development of high-density genomic tools to increase the selection precision in its breeding program for the North American strain of Atlantic Salmon (*Salmo salar*). Over the past seven years, two 50K and 70K Affymetrix arrays (Thermofisher Scientific Inc.) were used to genotype broodstock candidates belonging to both the nucleus and multiplier families units. With the aim to unify and improve the quality of the company's historical genomic data, the present study explored the possibility of applying genotype imputation techniques.

Materials and Methods
Real historic genomic data from CAI breeding program was used for the present imputation study. The working dataset consisted of genotypes from a group of 332 animals analyzed on both NASsa50k and SsaCooke arrays.

A total of 66,204 SNP markers, with known physical positions on the North American Atlantic salmon reference genome (S. Lien, T.M. Knutsen and F. Grammes, personal communication,) were selected based on several quality parameters including minor allele frequency (MAF), observed heterozygosity, expected heterozygosity and Hardy Weinberg equilibrium test.

To test imputation accuracy, a range of reference sample sizes (i.e. proportion of animals with complete genotypes included in the input dataset) was considered. A total of ten replicate datasets were created and subsequently imputed for each reference sample size (ranging from 30% to 90%).

Genotype accuracy was evaluated using two indicators: 1/ the concordance rate (i.e. the percentage of imputed genotypes which are matching true genotypes) and 2/ the imputation quality score (IQS) -as defined by Lin et al. in 2010- which adjusts for "chance" agreement.

Missing genotypes were imputed using Beagle v5.2 software program (Browning et al., 2018).

Results
Genotype imputation results are presented below.

It was possible to impute, with high confidance (>80%), between 10,902 and 18,995 SNP markers for each animal. A greater precision was achieved when imputing SNP markers from the NASsa50K array. In addition, the results showed that, as the SNP MAF values increased, the IQS values tended to decrease. Finally, this study permitted to define a cost-effective protocol to apply genomic imputation in the context of a commercial Atlantic salmon breeding program. For both Affymetrix arrays used in CAI program, a good precision for the imputed haplotypes was obtained when the reference sample population size was equal to 75%.

(Continued on next page)

Table 1. Description of the genotypic datasets used in the imputation study.

N of animals	HD array	N SNP markers	N shared markers	% No Call	N of valid SNPs for imputation study
332	NASsa50K	56,067	32,889	7.3%	54,608 (97.4%)
	SsaCooke	72,213		24.7%	57,205 (79.2%)

Table 2. Genotype imputation accuracy according to the reference sample size and the imputed array.

Reference sample size (%)	NASsa50K-SsaCooke NASsa50K imputed N imputed SNPs =20,000		NASsa50K-SsaCooke SsaCooke imputed N imputed SNPs = 15,000	
	Concordance rate	IQS	Concordance rate	IQS
30	0.77±0.21	0.38±0.25	0.71±0.26	0.31±0.40
45	0.88±0.19	0.73±0.25	0.78±0.25	0.43±0.39
60	0.95±0.18	0.91±0.20	0.86±0.23	0.66±0.37
75	0.96±0.18	0.94±0.19	0.90±0.21	0.79±0.32
90	0.96±0.18	0.98±0.08	0.92±0.20	0.85±0.28

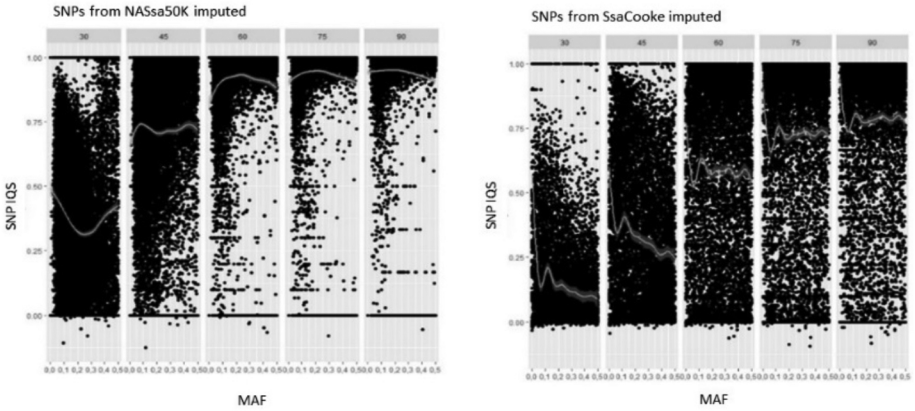


Figure 1. Genotype imputation accuracy according to the reference sample size and SNP markers' minor allele frequency.

Acknowledgements

The authors thank Thomas Moen (AquaGen SA), Tim Martin Knutsen (AquaGen SA), Fabian Grammes (AquaGen SA), Sigbjørn Lien (Norwegian University of Life Sciences), Jesús Fernández (INIA), Paulino Martinez (USC) and Elizabeth G. Boulding (University of Guelph) for their technical assistance. They also thank Genome Canada for funding the "Salmon and chips" GAPP project which enabled the development of the NASsa50K array.

References

Browning, B.L., Zhou, Y. and Browning, S.R. (2018). A one-penny imputed genome from next-generation reference panels. The American Journal of Human Genetics, 103(3), 338–348.

Lin, P., Hartz, S. M., Zhang, Z., Saccone, S. F., Wang, J., Tischfield, J. A., and others (2010). A new statistic to evaluate imputation reliability. PloS one, 5(3), e9697.

INVESTIGATING THE IMPACT OF WATER TEMPERATURE ON SEXUAL MATURATION IN ATLANTIC SALMON POST-SMOLTS

Christopher Good[1*], Curtis Crouse[1], Travis May[1], John Davidson[1], Tom Ole Nilsen[2], Åsa Maria Espmark[3]

[1]The Conservation Fund Freshwater Institute, Shepherdstown, WV 25443, USA
[2]Department of Biological Sciences, University of Bergen, 5020 Bergen, Norway
[3]Research Station for Sustainable Aquaculture, Nofima, 6600 Sunndalsøra, Norway
Email: cgood@conservationfund.org

Introduction

The onset of sexual maturation in Atlantic salmon is a highly flexible process, with numerous environmental factors capable of influencing this process. Mature salmon are undesirable for aquaculture producers for several reasons, including the reduced value of the consequently downgraded product. Recirculating aquaculture systems (RAS) are increasingly being utilized to produce smolt, post-smolt, and even harvest-sized Atlantic salmon; however, RAS environments have been associated with increased prevalence of precocious maturation in Atlantic salmon populations. Among other things, it has been speculated that the relatively higher water temperatures of RAS could provide the necessary environmental cue to induce sexual maturation. We sought to investigate this area through two experiments, (i) assessing the prevalence of maturation in post-smolt salmon raised in replicated freshwater RAS at either 12 °C or 14 °C, and (ii) determining whether there is a size threshold at which post-smolts exposed to an increase in water temperature from 12 °C or 14 °C will exhibit increased levels of sexual maturation. This presentation will discuss the findings of these studies and their ramifications for RAS production of post-smolt Atlantic salmon.

Materials and methods

Study 1. Mixed-sex diploid Atlantic salmon (approximately 50 g in weight, 243 days post-hatch in age) were stocked into six replicated RAS, three operated at 12 °C and three at 14 °C; salmon were raised at these temperatures under controlled conditions until final sampling at 489 days post-hatch (mean weight 1,323 g), at which time all fish were assessed for maturation via typical external signs, and gonadosomatic indices. Study 2. Post-smolt Atlantic salmon raised at 12 °C were stocked into 12 replicated tanks in a flow-through system, with three random tanks receiving an elevation in temperature from 12 °C to 14 °C at time points corresponding to source population mean weight reaching 100 g, 150 g, 250 g, and 350 g. All salmon were subsequently raised to a mean weight of approximately 600 g, after which maturation was assessed via gonadosomatic indices.

Preliminary Results

At the time of abstract submission (July 2021), Study 1 data are still being analysed, while Study 2 is ongoing and scheduled to conclude in August 2021. All final results will be presented and discussed in depth at Aquaculture Europe 2021. Preliminary results from Study 1 suggest that maturation was significantly ($p<0.05$) reduced in RAS operated at 12 °C vs. 14 °C, although maturation was still relatively prevalent (approximately 20% of the population) in the 12 °C treatment group. External evaluations of typical maturation signs (kype, color, ovipositor, etc.) correctly predicted maturation status, as assessed through gonadosomatic indices, >94% of the time.

EXTRUSION PROCESSING OF FISH FEED PELLETS: INFLUENCE OF SILICA AND OIL CONTENT ON THE PRODUCT PROPERTIES

M. Gräfenhahn[1*], J. Wiertz[1], C. Schillinger[2], C. Bader[3], V. Stohl[3], I. Huismann[3]

[1]Laboratory for Food & Feed Applications; Brabender GmbH & Co. KG, Duisburg (Germany)
[2]Silica Applied Technology Consumer, Health and Nutrition; Evonik Operations GmbH, Hanau (Germany)
[3]Fulda University of Applied Sciences, Department of Food Engineering, Fulda (Germany)
*Email: maria.graefenhahn@brabender.com

Over the last several decades, aquaculture has gained importance since wild capture can no longer meet the global demand for fish. Therefore, this market has grown exponentially, and with it, the demand for fish feed. In particular feed with high amounts of fat has gained relevance, since it allows for many species (e.g., salmon, trout) the most efficient growth and, thus, farming.

Extrusion processing is often used to produce fish feed pellets. Within the heated barrel containing rotating screws, the raw materials are mixed, heated, and sheared along the extruder. The combined thermal and mechanical stresses applied to the material lead to its plasticization and cooking. Due to the temperature and pressure gradients between the melt and the room conditions, the material can expand after it leaves the die and is pelletized through a rotating knife. Although addition of oil is desired, it has a negative influence on the cooking process during extrusion, as its lubricating properties reduce the specific thermal and mechanical energy input and, consequently, the degree of cook. In general, a lower degree of cook has a negative influence on the product quality of the feed pellets, i.e., decreased water stability and hardness. Therefore, to cook the material sufficiently and to achieve the aimed product properties, manufacturers avoid adding high oil levels during extrusion. Instead, manufacturers add oil in a separate coating step post-extrusion. This additional step makes the production of aquatic feed more complicated and expensive. Additionally, if fat does not bind properly to the pellets fat leakage can occur, whereby the fat drains out of the pellet, consequently, leading to increased fat oxidation. The addition of additives, especially silica, would resolve these issues while achieving high fat levels in high quality fish feed pellets without a coating step. Since the increase of fat leads to a decrease in the matrix viscosity, the mechanical energy input is expected to decrease leading to a lower degree of cook. As silica is known for its water- and oil-binding capacity as well as for its ability to control rheology and therefore to increase viscosity, its addition is expected to counteract this effect leading to an enhanced gelatinization during the extrusion process. This strategy will be introduced and the relationship between process conditions, addition of different oil and silica concentrations and resulting product properties (among others pellet hardness, pellet abrasion and fat content) will be evaluated.

THE EFFECTS OF OXYGEN SUPPLEMENTATION ON FARMED ATLANTIC SALMON (*Salmo salar)* BEHAVIOR USING ACOUSTIC TELEMETRY

J. Grant[a*], C. L. Stockwell[a], R. Filgueira[b]

[a] Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2
[b] Marine Affairs Program, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2
Email: jon.grant@dal.ca

Introduction

The solubility of oxygen in seawater is dependent on pressure, salinity, and temperature, where warmer waters decrease the solubility of oxygen. Temperature and dissolved oxygen (DO) together play a crucial role in the growth and health of farmed fish (Elliott & Elliott, 2010). When DO falls below the optimal range (~6.0 mgL^{-1} at 15°C) fish stress and mortality levels increase. In the past few years, examples of mortality event have been documented worldwide, associated with hypoxic water events interacting with stress from factors such as disease (Galea *et al*., 2018; Evans, 2020). In order to counteract the negative effects of low DO on fish health, farms have initiated oxygen supplementation including pumping of oxygen nanobubbles via diffusers placed inside sea cages. The response of fish to changes in oxygen as well as the presence of bubblers was investigated on a commercial Atlantic salmon (*Salmo salar*) farm using acoustic tags to monitor 3D fish position.

Materials and Methods

The farm study site is located in St. Margarets Bay, Nova Scotia, Canada with 6 net pens measuring 48m diameter with maximum depth of 11 m and arranged in one column running southwest to northeast. Fifteen adult salmon (60-72 cm and 4.5 kg) were surgically implanted with VEMCO acoustic tags (model V9P-180). An acoustic array was installed consisting of 8 HR2 receivers at 2 and 7m depth, used to triangulate horizontal position of each fish. Tags were programmed to transmit resulting data on 3D position every 3 seconds.

An oxygen supplementation trial was conducted in autumn 2019, during which compressed oxygen was continuously supplied to all cages on site. The study cage was supplied with 4 ring-shaped diffusers placed at 7m depth. No control cage was available due to the risk of fish mortality during low DO months. Temperature and DO data were collected using AquaMeasure wireless sensors in cage center at 2m and 7m depth.

Three fish variables were calculated using positioning data: velocity [ms^{-1}], distance from cage center [m], and turning angle [°], and depth [m]. The study was split into two periods 1) during the oxygen trial (2 Oct 2019 – 7 Nov 2019) and 2) after the trial (8-25 Nov 2019).

Results

Temperature ranged from ~8-14°C during the study, with similar values between 2 and 7m depths during the entire time series. There was a gradual temperature decrease through the autumn, then declining more steeply at the water column overturn in early November. Oxygen was always slightly higher at 2m than 7m, fluctuating over a range of 7-8.3 mg l^{-1} until the end of October when it rapidly declined to a low of 6.0 mg l^{-1}. From that minimum, oxygen showed a continual increase throughout November to 9 mg l^{-1} during the fall overturn.

Swimming velocity of the tagged population averaged 0.72 ± 0.39 ms^{-1} with higher velocities during the day than at night. Fish depth showed a daily cycle with deeper values during day than at night. The tagged population averaged 14.5 ± 5.1 m from cage center swimming in a counterclockwise direction with an average turning angle of 53.2 ± 44.1°.

During the oxygen trial, all tagged individuals displayed a slower swimming speed, negatively related to water temperature (Fig. 1), contrary to literature. Furthermore, 77% of tagged individuals showed a change towards cage edge during the trial compared to after, with 85% of tagged fish displaying straighter turning angles during the trial. During the trial, 85% of the tagged fish swam significantly shallower than after the trial.

Climate change is triggering increased sea surface temperatures and decreased dissolved oxygen, leading to more sites requiring supplemental oxygen. Our work suggests behavioural changes associated with oxygen supplementation. However the implications of these changes for feeding, growth, and fish health are not yet clarified. Continuously developing farm sensing technology associated with intervention such as oxygenation provides further scope for management options leading to improved fish welfare and more sustainable aquaculture.

(Continued on next page)

References

Elliott, J. M., & Elliott, J. A. (2010). Temperature requirements of Atlantic salmon, brown trout and Arctic charr: predicting the impacts of climate change. *Journal of Fish Biology*.

Evans, O. (2020). *Authorities investigating Mowi 20,000 salmon mortality*. Retrieved from Salmon Business: https://salmonbusiness.com/authorties-investigating-mowi-20000-salmon-mortality/ . Accessed 21 March 2021.

Galea, S., Street, E., & Dunlevie, J. (29 May 2018). *Macquarie Harbour salmon: 1.35 million fish deaths prompt call to 'empty' waterway of farms*. Retreived from ABC News Australia: https://www.abc.net.au/news/2018-05-29/salmon-deaths-in-macquarie-harbour-top-one-million-epa-says/9810720 . Accessed on 1 April 2021.

OPTIMIZATION OF FLOCCULATION OF MICROALGA *Picochlorum* sp. USING CHITOSAN

Grubišić, M.*[a], Ivančić Šantek, M.[a], Šantek, B.[a], Čož-Rakovac[b]

[a]Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia, [b] BioProCro Centre of Excellence, Zagreb, Croatia
e-mail address: *mgrubisic@pbf.hr

Introduction

Harvesting of microalgae biomass represents a major technological and economic challenge in the overall microalgae production process (Kumaran et al., 2021; Augustine et al., 2019). Since traditional harvesting methods, such as centrifugation and filtration, significantly contribute to production costs, developing alternative methods with low energy consumption is needed to improve the economic feasibility of microalgal biomass production on an industrial scale (Perez et al., 2017; Lama et al., 2016). One low-cost strategy for addressing this challenge involves using flocculation as the initial dewatering and concentrating step (Morales et al., 1985; Matter et al., 2019). Chitosan, a natural cationic polyelectrolytic biopolymer composed of linear poly-amino-saccharide chains, is considered less toxic, biodegradable, and relatively inexpensive (Matter et al., 2019). The present work evaluated the potential of chitosan as a flocculation agent for the flocculation of the marine microalga *Picochlorum* sp. The effect of flocculant concentration and culture pH on cell flocculation were investigated.

Materials and methods

All experiments were conducted with the 26-day old culture of marine microalgae *Picochlorum* sp., which was previously isolated from the Adriatic Sea. Chitosan solution was prepared by dissolving 5 g L^{-1} in 1% acetic acid. The initial pH of the culture was adjusted to 8.0 with 1 mM sodium hydroxide. Experiments were performed in 100 mL Erlenmeyer flasks with 100 mL of culture suspension at room temperature using a magnetic stirrer. First, the effect of chitosan concentration and pH of the culture on cell flocculation was studied. Further, the minimal concentration of chitosan for cell flocculation was determined at optimal pH. Experiments were conducted as follows: (1) chitosan was added to the culture and culture was mixed at 450 rpm for 10 minutes, (2) optional step, the pH value of the culture was set to a chosen value, (3) the culture was mixed for another 10 minutes at 250 rpm and (4) flocculated cells were allowed to settle down for 30 min (without mixing). The flocculation efficiency was determined by measuring the optical density of microalgal culture before chitosan addition and after settling the flocculated cell. The optical density of the culture was measured at 540 nm. All experiments were done in triplicates.

Results

Optimal conditions for flocculation of microalga was conducted in three steps. First, the effect of chitosan dosage on biomass recovery from culture suspension was examined by applying chitosan concentrations in the range from 5 to 80 mg L^{-1}. The flocculation efficiency of 98.15% was achieved at 25 mg L^{-1} of chitosan. An increase of chitosan concentration above 25 mg L^{-1} did not significantly increase flocculation efficiency, so this concentration was applied in the following experiments. Since chitosan was dissolved in 1% acetic acid, the addition of flocculant solution decreased the pH of the microalgal culture suspension. Therefore, the effect of pH on flocculation efficiency was further investigated in the pH range between 7.5 and 9. Flocculation efficiency of the culture increased with the increase of alkalinity of the culture suspension. At pH 8.0, a high flocculation efficiency of 97.46 % was obtained with the smallest alkali volume used for the pH adjustment. Finally, the minimum concentration of chitosan required for efficient flocculation was determined at pH 8.0 in the concentration range between 12 and 25 mg L^{-1} (12,15,17,20,22 mg L^{-1}) of chitosan. Low flocculation efficiencies were obtained at chitosan concentrations below 25 mg L^{-1}, ranging from 13,54% at 15 mg L^{-1} to 19,8% at 22 mg L^{-1}, confirming the previously selected chitosan concentration of 25 mg L^{-1} as the lowest chitosan concentration needed for achieving flocculation efficiency above 95 %.

Conclusion

High flocculation efficiency of 97.46 % was obtained at chitosan concentrations above 25 mg L^{-1} in the pH range 8.0-9.0. The optimal chitosan concentration for efficient flocculation of microalga was 25 mg L^{-1} at pH 8.0, with minimal volume of alkali used for the culture pH adjustment.

(Continued on next page)

Literature

Kumaran, J., Bright Singh, I. S., Joseph, V. (2021) Effective biomass harvesting of marine diatom *Chaetoceros muelleri* by chitosan-induced flocculation, preservation of biomass, and recycling of culture medium for aquaculture feed application. *Journal of Applied Phycology*, **33**, 1605-1619.

Augustine, A., Tanwar, A., Tremblay, R., Kumar, S. (2019) Flocculation processes optimization for reuse of culture medium without pH neutralization. *Algal Research*, **39**, 101437.

Lama , S., Muylaert, K., Karki, T. B., Foubert, I., Henderson, R. K., Vandamme, D. (2016) Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation. *Bioresource Technology*, **220**, 464-470.

Perez, L., Salgueiro, J. L., Maceiras, R., Cancela, A., Sánchez, A. (2017) An effective method for harvesting of marine microalgae: pH induced flocculation. *Biomass and Bioenergy*, **97**, 20-26.

Morales, J., de la Noüe, J., Picard, G. (1985) Harvesting marine microalgae species by chitosan flocculation. *Aquaculture Engineering*, **4**, 257-270.

Matter, I. A., Bui, V. K. H., Jung, M., Seo, J. Y., Kim, Y-E., Lee, Y-C., Oh, Y-K. (2019) Flocculation harvesting techniques for microalgae: A review. *Applied Sciences*, **9**, 3069.

PRO- AND ANTI- INFLAMMATORY *IN VITRO* EFFECTS OF CANTHARIDIN IN GILTHEAD SEABREAM (*Sparus aurata*) HEAD-KIDNEY LEUCOCYTES

J.C. Campos-Sánchez, F.A. Guardiola, M.A. Esteban*

Immunobiology for Aquaculture Group, Department of Cell Biology and Histology. Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia (Spain)
josecarlos.campos@um.es.

Introduction

The inflammatory process has widely been studied in mammals, but we know very little about this mechanism in fish (Esteban, 2012). Cantharidin, a toxic vesicant terpene secreted by male blister beetles of *Meloidae* and *Oedemeridae* families has been used to study inflammation in mice and humans due to its properties to produce irritations on the dermis, rashes, and bladders (Ivetic Tkalcevic *et al*., 2012). In addition, low doses of cantharidin have been used in both folk and traditional Chinese medicine due to its anticancer, antibiotic, antiviral, and immune-regulating properties (Whitman *et al*., 2019). The main aim of the present study was to assess the related-inflammatory effects of cantharidin on gilthead seabream head-kidney leucocytes.

Material and methods

Ten specimens (403.6g ± 16.5 g, 27.3 ± 0.3cm) of gilthead seabream (*Sparus aurata*) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain), were anesthetized with clove oil (20 mg L^{-1}, Guinama®) and bled from the caudal vein. Head-kidney was dissected out by a ventral incision and leucocyte suspensions were obtained by forcing fragments of the organ through a nylon mesh (mesh size 100 μm). The cells were washed twice (400 x g 10 min) in sRPMI culture medium (Gibco), counted (Z2 Coulter Particle Counter) and adjusted to $2x10^7$ cells mL^{-1} in sRPMI (Esteban *et al*, 1998). Then, leucocytes were incubated with cantharidin at final concentrations of 5, 1.25, and 0 μg mL^{-1} (DMSO diluted in sRPMI; Control) for 3, 6 and 12 h. After incubation, samples were divided: one-half was processed according to Reynolds (1963) to determine possible morphologic alterations by transmission electron microscopy, whilst the other-half was used to analyse the gene expression of NF-κB transcription factors (*c-rel*, *nf-κb1*, *rela*, *relb* and *nf-κb2*), and inflammatory-related genes (*il-1β*, *tnf-α il-6*, *il-10* and *tgf-β*) by using the $2^{-\Delta Ct}$ method with some modifications (Cordero *et al*., 2015). Results were expressed as mean ± standard error of the mean (SEM) and data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups and each group respect to time, respectively. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

Ultrastructural results obtained in the head kidney leucocytes incubated with cantharidin varied depending on the cell type analysed. Apoptotic and necrotic acidophilic granulocytes were observed after their incubation with cantharidin at 3, 6 and 12 h. Besides this, at 12 h of incubation with cantharidin, macrophages changed their rounded general morphology to a more irregular one and exhibited a high cytoplasmic vacuolisation. However, the ultrastructure of the lymphocytes was not modified as consequence of the incubation with cantharidin.

Regarding the gene expression, *c-rel* was up-regulated in leucocytes incubated for 6 and 12 h with 1.25 and 5 μg mL^{-1} of cantharidin compared to control leucocytes. Contrarily, the gene expression of *nf-κb1* was down-regulated in a time and dose-dependent manner. Both, the proinflammatory cytokines *tnf-α* and *il-1β* were up-regulated in leucocytes incubated with 5 μg mL^{-1} of cantharidin at 3 h compared to control leucocytes and those incubated with 1.25 μg mL^{-1} of cantharidin also at 3 h. Besides, although *il-1β* gene expression was also up-regulated in leucocytes incubated for 6 h with 1.25 μg mL^{-1} of cantharidin in comparison leucocytes from the control group, but not in comparison to with these incubated with 5 μg mL^{-1} of cantharidin, the gene expression of this gene was down-regulated in leucocytes incubated for 12 h with 5 μg mL^{-1} of cantharidin, in comparison with control leucocytes.

Otherwise, not significant differences were detected in the gene expression of *rela*, *relb*, *nf-κb2, il-6, il-10* and *tgf-β* of leucocytes incubated with cantharidin. Therefore, it seems that cantharidin, as a selective inhibitor of serine/threonine protein phosphatases, is able to trigger the activation of proinflammatory genes in leucocytes of gilthead seabream incubated for few hours through the activation of NF-κB signalling pathway. On the contrary, prolonged exposures of leucocytes with cantharidin leads to death cell and morphological changes of some leucocyte populations (Huang *et al*., 2011; Zhou *et al*., 2018).

(Continued on next page)

Conclusion

Present results offer a new approach of the potential properties of cantharidin as inflammation trigger in gilthead seabream, as well as its possible therapeutical use due to the fact that incubation of cells with the substance for a long time produces apoptosis.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) co-funded with the European Regional Development Funds (ERDF/FEDER) (Grant no. AGL-2017-83370-C3-1-R) and *Fundación Séneca de la Región de Murcia* (*Grupo de Excelencia* 19883/GERM/15).

References

Cordero, H., Brinchmann, M.F., Cuesta, A., Meseguer, J., Esteban, M.A. Skin mucus proteome map of European sea bass (*Dicentrarchus labrax*), Proteomics. 15 (2015) 4007–4020. https://doi.org/10.1002/pmic.201500120

Esteban, M.A. An Overview of the Immunological Defences in Fish Skin, ISRN Immunol. 2012 (2012) 1–29.

Esteban, M.A., Mulero, V., Muñoz, J., Meseguer, J. Methodological aspects of assessing phagocytosis of *Vibrio anguillarum* by leucocytes of gilthead seabream (*Sparus aurata* L.) by flow cytometry and electron microscopy, Cell Tissue Res. 293 (1998) 133–141. https://doi.org/10.1007/s004410051105.

Huang, W.W., Ko, S.W., Tsai, H.Y., Chung, J.G., Chiang, J.H., Chen, K.T., Chen, Y.C., Chen, H.Y., Chen, Y.F., Yang, J.S. Cantharidin induces G2/M phase arrest and apoptosis in human colorectal cancer colo 205 cells through inhibition of CDK1 activity and caspase-dependent signalling pathways, Int. J. Oncol. 38 (2011) 1067–1073. https://doi.org/10.3892/ijo.2011.922.

Ivetic Tkalcevic, V., Hrvacic, B., Bosnar, M., Cuzic, S., Bosnjak, B., Erakovic Haber, V., Glojnaric, I. Cantharidin-induced inflammation in mouse ear model for translational research of novel anti-inflammatories, Transl. Res. 160 (2012) 137–145. https://doi.org/10.1016/j.trsl.2012.02.001.

Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy., J. Cell Biol. 17 (1963) 208–212. https://doi.org/10.1083/jcb.17.1.208.

Whitman, D.W., Fe Andrés, M., Martínez-Díaz, R.A., Ibáñez-Escribano, A., Sonia Olmeda, A., González-Coloma, A. Antiparasitic properties of cantharidin and the blister beetle *berberomeloe majalis* (Coleoptera: Meloidae), Toxins (Basel). 11 (2019) 16–21. https://doi.org/10.3390/toxins11040234.

Zhou, H., Xu, J., Wang, S., Peng, J. Role of Cantharidin in the activation of IKKα/IκBα/NF-κB pathway by inhibiting PP2A activity in cholangiocarcinoma cell lines, Mol. Med. Rep. 17 (2018) 7672–7682. https://doi.org/10.3892/mmr.2018.8860.

IN VITRO HEMOLYTIC, HEMAGGLUTINATING, CYTOTOXIC AND BACTERICIDAL EFFECTS OF λ-CARRAGEENIN

J.C. Campos-Sánchez, F.A. Guardiola, M.A. Esteban*

Immunobiology for Aquaculture Group, Department of Cell Biology and Histology. Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia (Spain)
josecarlos.campos@um.es.

Introduction

Biological activities of seaweed polysaccharides have attracted the attention of researchers due to their medical potential. Carrageenin is a mucopolysaccharide derived from the cell walls of red algae *Chondrus crispus* which has been used in a high-extended manner for decades as a model of acute inflammation in rats and rabbits (Winter *et al*., 1962). Nonetheless, although immunostimulant properties of carrageenin have been demonstrated in teleost fish, molecular and biological properties and its action mechanism has not been studied in fish (Silva *et al*., 2010). For this reason, the aim of the present study was to evaluate the possible haemolytic, hemagglutinating, cytotoxic and bactericidal, effects of λ-carrageenin.

Material and methods

In this study, ranging concentrations from 0, 0.1, 1, 10, 100 to 1000 μg mL^{-1} of λ-carrageenin were used in all the techniques carried out. Thus, erythrocytes from gilthead seabream (*Sparus aurata*) (284,53g ± 17,21g, 26,02cm ± 0,39) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain) were isolated according to Morcillo *et al*. (2016). The hemolysis of erythrocytes after incubation with λ-carrageenin in phosphate-buffered saline (PBS, containing 0.35 % sodium chloride) with 10 mM glucose was determined by the correlation of the cell viability and the liberation of the oxyhaemoglobin to the medium at 3 and 6 h (Morcillo *et al*., 2016). Hemagglutination of erythrocytes was visualized in a 96-well microtiter plate after 6 h of incubation with λ-carrageenin at room temperature (Li et al., 2008). Otherwise, λ-carrageenin in Eagle's Minimum Essential Medium (EMEM) were used to evaluate their cytotoxic activity at 3 and 6 h in the cell line PLHC1 (ATCC® CRL2406™), hepatocellular carcinoma from *Poeciliopsis lucida,* by using the MTT assay (Berridge *et al*., 1993; Denizot *et al*., 1986). Besides, four opportunist marine pathogenic bacteria (*Vibrio harveyi, Vibrio anguillarum, Photobacterium damselae,* and *Tenacibaculum maritimum*) were used to determine the bactericidal activity of λ-carrageenin in PBS (Stevens *et al*., 1991). The results were expressed as mean ± standard error of the mean (SEM) and data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

Results showed an increase in the haemolytic activity of erythrocytes at both 3 and 6 h after incubation with 1000 μg mL^{-1} of λ-carrageenin in comparison to control. The increases were also statistically significant in erythrocytes incubated with 100 μg mL^{-1} of λ-carrageenin at 6 h. In addition, λ-carrageenin showed hemagglutinating activity only at doses of 1000 μg mL^{-1} in comparison to the rest of the concentrations tested. Regarding the cytotoxic assay with the PLHC1 cell line, it was detected an increase of this activity only at 6 h of incubation with all assayed doses of λ-carrageenin in comparison to control. This activity was statistically higher with the highest concentration of λ-carrageenin (1000 μg mL^{-1}). On the other hand, only a dose-dependent increase of the bactericidal activity against *Photobacterium damselae* was found when the bacteria were incubated with the two highest doses of the polysaccharide (100 and 1000 μg mL^{-1}) in comparison to lower doses of λ-carrageenin. Therefore, a high concentration of λ-carrageenin seems to have hemolytic, hemagglutinating, cytotoxic and bactericidal, biological properties that could be derived from its high molecular weight and sulphate concentration, as well as other seaweed polysaccharides (Silva *et al*., 2010).

Conclusion

The present results offer a different approach of the effect of λ-carrageenin in fish, being able to extend its use not only as a model to reproduce inflammation, but also for therapeutical purposes in the aquaculture sector due to the properties here demonstrated.

(Continued on next page)

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) co-funded with the European Regional Development Funds (ERDF/FEDER) (Grant no. AGL-2017-83370-C3-1-R) and *Fundación Séneca de la Región de Murcia* (*Grupo de Excelencia* 19883/GERM/15).

References

Berridge, M.V., Tan, AS. Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction, Arch Biochem Biophys. 303 (1993) 474–82.

Denizot, F., Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, J. Immunol. Methods. 89 (1986) 271–277. https://doi.org/10.1016/0022-1759(86)90368-6.

Esteban, M.A., Mulero, V., Muñoz, J., Meseguer, J. Methodological aspects of assessing phagocytosis of *Vibrio anguillarum* by leucocytes of gilthead seabream (*Sparus aurata* L.) by flow cytometry and electron microscopy, Cell Tissue Res. 293 (1998) 133–141. https://doi.org/10.1007/s004410051105.

Li, Y.R., Liu, Q.H., Wang, H.X., Ng, T.B. A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*. Biochimica et Biophys Acta 1780 (2008) 51–57

Morcillo, P., Romero, D., Meseguer, J., Esteban, M.A., Cuesta, A. Cytotoxicity and alterations at transcriptional level caused by metals on fish erythrocytes in vitro. Environ Sci Pollut Res Int. 23 (2016) 12312–12322. doi: 10.1007/s11356-016-6445-3.

Silva, F.R.F., Dore, C.M.P.G., Marques, C.T., Nascimento, M.S., Benevides, N.M.B., Rocha, H.A.O., Chavante, S.F., Leite, E.L. Anticoagulant activity, paw edema and pleurisy induced carrageenan: Action of major types of commercial carrageenans, Carbohydrate Polymers. 79 (2010) 26-33. https://doi.org/10.1016/j.carbpol.2009.07.010.

Stevens, M.G., Kehrli, M.E., Canning, P.C. A colorimetric assay for quantitating bovine neutrophil bactericidal activity, Vet. Immunol. Immunopathol. 28 (1991) 45–56. https://doi.org/10.1016/0165-2427(91)90042-B.

Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proceedings of the society for experimental biology and medicine, 111(3), 544-547.

IN VITRO EFFECTS OF *Chiliadenus glutinosus* (L.) FOURR. AQUEOUS EXTRACT IN HEAD-KIDNEY LEUCOCYTES OF GILTHEAD SEABREAM (*Sparus aurata* L.)

J.C. Campos-Sánchez, F.A. Guardiola, M.A. Esteban*

Immunobiology for Aquaculture Group, Department of Cell Biology and Histology. Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia (Spain) josecarlos.campos@um.es.

Introduction

Nowadays, the use of extract from medicinal plants for control of fish diseases in aquaculture is a plausible alternative to drugs, antibiotics and chemicals due to their, immunostimulant and bactericidal properties (Van Hai, 2015). In this sense, *Chiliadenus glutinosus* (L.) Fourr. is an endemic species of plant used in the Spanish popular medicine as remedy to prevent and treat human diseases and to date, it has poorly been studied in fish (Las Heras Etayo *et al*., 2021). Thus, the aim of the present study was to evaluate the immunobiological effects of aqueous extracts of *C. glutinosus* on head-kidney leucocytes of gilthead seabream.

Material and methods

Six specimens (128.49g ± 3.84g, 18.11cm ± 0.25cm) of gilthead seabream (*Sparus aurata*) obtained from a local farm (Murcia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain), were anesthetized with clove oil (20 mg L^{-1}, Guinama®) and bled from the caudal vein. Head-kidney was dissected out by a ventral incision and leucocytes' suspensions were obtained by forcing fragments of the organ through a nylon mesh (mesh size 100 μm) and washed twice (400 x g 10 min) in sRPMI culture medium (Gibco). Leucocytes were counted (Z2 Coulter Particle Counter) and adjusted to $2x10^7$ cells mL^{-1} in sRPMI (Esteban, 1998). Then, leucocytes were incubated with ranging concentrations from 0 (PBS diluted in sRPMI; Control) to 1 mg mL^{-1} of an aqueous extract of *C. glutinosus* for 24h. After incubation, the following parameters were analysed: viability, respiratory burst, peroxidase and phagocytic activities. The results were expressed as mean ± standard error of the mean (SEM) and data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups and each group respect to time, respectively. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

Results obtained in the head kidney leucocytes showed a decrease in the viability and peroxidase activity of leucocytes incubated with 1 mg mL^{-1} of aqueous extract in comparison with leucocytes incubated with the rest of concentrations, although in the case of the peroxidase this difference was only significant compared with the doses of 0, 0.001 and 0.1 mg mL^{-1}. Respiratory burst activity was decreased in leucocytes incubated with 1 and 0.5 mg mL^{-1} of aqueous extract compared to those incubated with 0, 0.001 and 0.1 mg mL^{-1}. Otherwise, phagocytic capacity was decreased in leucocytes incubated with 0.125 mg mL^{-1} of aqueous extract in comparison with leucocytes incubated with lower doses. In addition, this capacity was decreased to a greater extent in leucocytes incubated with 1 mg mL^{-1} of aqueous extract compared to leucocytes incubated with 0.125 mg mL^{-1}. Phytochemical composition of aqueous extracts (polyphenolic compounds majorly, and terpenes) seems to be responsible for affecting the immune activities of head kidney leucocytes of gilthead seabream by modulating their redox metabolism (Amorati and Valgimigli, 2018; Rodríguez, 2003).

Conclusion

The present results offer a detailed view of the potential immunosuppressive effects of *C. glutinosus* due to soluble bioactive molecules present on this medicinal plant, from which therapeutic uses could be developed in fish of commercial interest.

(Continued on next page)

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) co-funded with the European Regional Development Funds (ERDF/FEDER) (Grant no. AGL-2017-83370-C3-1-R) and *Fundación Séneca de la Región de Murcia* (*Grupo de Excelencia* 19883/GERM/15).

References

Amorati, R., Valgimigli, L. Methods to Measure the Antioxidant Activity of Phytochemicals and Plant Extracts, J. Agric. Food Chem. 66 (2018) 3324–3329. https://doi.org/10.1021/acs.jafc.8b01079.

Esteban, M.A., Mulero, V., Muñoz, J., Meseguer, J. Methodological aspects of assessing phagocytosis of *Vibrio anguillarum* by leucocytes of gilthead seabream (*Sparus aurata* L.) by flow cytometry and electron microscopy, Cell Tissue Res. 293 (1998) 133–141. https://doi.org/10.1007/s004410051105.

Las Heras Etayo, N., Llamas, F., Acedo, C. Ethnobotanical Research and Compilation of the Medicinal Uses in Spain and the Active Principles of *Chiliadenus glutinosus* (L.) Fourr. for the Scientific Validation of Its Therapeutic Properties, Plants. 10 (2021) 1–21. https://doi.org/https://doi.org/10.3390/plants10030584.

Rodríguez, A., Esteban, M.A., Meseguer J. Phagocytosis and peroxidase release by seabream (*Sparus aurata* L.) leucocytes in response to yeast cells, Anat. Rec. - Part A Discov. Mol. Cell. Evol. Biol. 272 (2003) 415–423. https://doi.org/10.1002/ar.a.10048.

Van Hai, N. The use of medicinal plants as immunostimulants in aquaculture: A review, Aquaculture. 446 (2015) 88–96. https://doi.org/10.1016/j.aquaculture.2015.03.014.

EXPRESSION OF IMMUNE-RELATED GENES IN EUROPEAN EEL (*Anguilla anguilla*) AFTER BACTERIAL CHALLENGES

S. Vicente[1], A. Sridhar[2], J. Mayor-Lafuente[1], M. Cámara-Ruiz[1], M.A. Esteban[1], F.A. Guardiola[1]*

[1]Immunobiology for Aquaculture Group, Department of Cell Biology and Histology. Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia (Spain) *Email: faguardiola@um.es

[2]Laboratory of Aquabiotics/Nanoscience, Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu, India

Introduction

In the recent decades, the great expansion of all aquaculture types has contributed to emphasizing studies on the immune system and defence mechanism of fish against diseases associated with intensive farming. In European eel (*Anguilla anguilla*), some of those diseases are caused by viruses (*e.g.*, Alloherpesvirus-1 and *Herpesvirus anguillae*), nematodes as *Anguillicoides crassus* or some genus of bacteria as *Vibrio*, *Aeromonas* or *Tenacibaculum* which can infect in all life cycle stages (Chang *et al*., 2002). However, little is known about eel's immune system and its defense mechanisms against pathogenic microorganisms. Therefore, understanding them would be important to prevent and control infections (Raida & Buchmann, 2008), providing the possibility to develop various prophylactic and therapeutic methods trying to improve the critical situation of their populations. Taking these considerations, the aim of this study was to investigate the expression of several immune-related genes in head-kidney (HK), skin and gills of European eel bath challenged with *V. anguillarum* and *V. vulnificus* after 72 h post challenge.

Material y methods

Forty eight adults specimens (110.08 ± 18.65 g weight and 39.58 ± 2.19 cm length) of European eel obtained from commercial fish market (*Comunidad de Pescadores del Palmar*, El Palmar, Valencia, Spain), and maintained in the Marine Fish Facilities at the University of Murcia (Spain) were randomly distributed into three experimental groups by duplicate [6 tanks of 50 L filled with 20 L of water, 4 fish per tank, 8 fish per group] where the following groups were established: 1) Unchallenged (control group, immersion with sterile distilled water instead of bacteria); 2) Bath challenged with *V. anguillarum* (immersion with a sub-lethal bacteria concentration of 3.0×10^8 CFU L^{-1}); 3) Challenged with *V. vulnificus* (immersion with a sub-lethal bacteria concentration of 3.0×10^8 CFU L^{-1}). For the challenges, fish were exposed separately in other tanks (6 tanks of 50 L filled with 20 L of water) for 60 min with strong aeration and returned to their respective tanks. After 72 h, fish were sacrificed by using an overdose of MS-222 (Sandoz, 1,000 mg L^{-1} water), dissected and fragments of HK, skin and gills were collected and used to analyse the expression of several immune-related genes (*c3*, *c1*, *tnfα*, *il-1β*, *il-10*, *irf7*, *irf3*, *lysc*, *tlr2*, *mhc2*, *igm* and *cd3*) by real-time qPCR. The results were expressed as mean ± standard error of the mean (SEM). Data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups. The level of significance used was $p < 0.05$ for all statistical analysis.

Results and discussion

The expression of the immune-related genes measured in HK did not show any variations between unchallenged and challenged fish. In the case of skin, the expression of *igm*, *mhc2* and *il-1β* genes was up-regulated in fish challenged with *V. vulnificus* compared to unchallenged ones. In addition, our results showed a higher relative expression of *igm* and *mhc2* genes in fish challenged with *V. vulnificus* in comparison with the fish challenged with *V. anguillarum*. Contrarily, no significant variations were detected in the expression of *c3*, *c1*, *irf7*, *irf3* and *lysc* genes, although in the case of *lysc* gene a downward trend was observed from the highest value at unchallenged fish to lowest value at those fish challenged with *V. vulnificus*. The expression of *cd3*, *tlr2*, *tnfa* and *il-10* genes in skin was undetected in none of experimental groups. In gills, the expression of *igm* and *irf7* genes was up-regulated in challenged fish with *V. vulnificus* regarding unchallenged group. In the case of *mhc2* gene, the relative expression was up-regulated in fish challenged with *V. anguillarum* compared to unchallenged fish whilst not variations were found between challenged groups. Nevertheless, the expression of the other studied genes was not affected by challenge trial and the expression of *tnfα* gene was not detected. Interestingly, the expression of *cd3* gene was only detected in the gills although no variations were observed between experimental groups. As the principal lymphoid organ, HK is responsible for the cellular response and secretion of cytokines to the infection site as first steps of immune response (Olabuenaga, 2006). The no variations in the expression of immune-related genes in this organ may be due to early sampling time (72 h) because of the modulation of immune response has not been carried out. Contrarily, the up-regulation of some immune-related genes in skin (*igm*, *il-1β, mhc2*) and gills (*irf7*, *igm*, *mhc2*)

(Continued on next page)

after a bath challenge with *V. vulnificus* and *V. anguillarum* could be related with the important role of MALTs (Mucosal-Associated Lymphoid Tissue) as physical, biological, and chemical barrier against pathogens (Esteban, 2012) and the role of *il-1β* as the leader of the pro-inflammatory response (Adamek *et al*., 2013; Cecchini *et al*., 2013).

Conclusion

The presents results offer a new molecular view to enlarge the knowledge of the immune system of European eel in response to bath-challenge trial against two common pathogenic bacteria for this species, broadening the horizons of the knowledge on the first steps of the immune response against these pathogens. In fact, the information provided by this study could optimize culture conditions and disease preventions protocols.

Acknowledgements

This work was partly supported by the Spanish Ministry of Economy and Competitiveness (MINECO) co-funded by the European Regional Development Funds (ERDF/FEDER) (grant no. AGL2017-88370-C3-1-R) and *Fundación Séneca de la Región de Murcia* (*Grupo de Excelencia*, Grant no. 19883/GERM/15; Saavedra Fajardo Program, Grant no. 20407/SF/17).

References

Adamek, M., Syakuri, H., Harris, S., Rakus, K. Ł., Brogden, G., Matras, M., Irnazarow, I., & Steinhagen, D. (2013). Cyprinid herpesvirus 3 infection disrupts the skin barrier of common carp (*Cyprinus carpio* L.). *Veterinary Microbiology*, *162*(2-4), 456-470. https://doi.org/10.1016/j.vetmic.2012.10.033

Cecchini, S., Paciolla, M E., Biffali, M., Borra, Ursini, M.V., Lioi, M.B. (2013) Ontogenetic profile of innate immune related genes and their tissue-specific expression in brown trout, *Salmo trutta* (Linnaeus, 1758), *Fish Shellfish Immunol*. *35* 988–992, https://doi.org/10.1016/j.fsi.2013.05.026

Chang P.H., Pan Y.H., Wu C.M., Kuo S.T. & Chung H.Y. (2002) Isolation and molecular characterization of herpesvirus from cultured European eels *Anguilla anguilla* in Taiwan. Diseases of Aquatic Organisms 50, 111–118.doi: https://doi.org/10.3354/dao050111

Esteban, M.A. (2012). An overview of the immunological defenses in fish skin, Int. Sch. Res. Not. 2012 853470, https://doi.org/10.5402/2012/853470, 10. 5402/2012/853470.

Olabuenaga, S. E. (2000). Sistema Inmune en Peces. *Gayana (Concepción)*, *64*(2), 205-215. https://doi.org/10.4067/S0717-65382000000200010

Raida, M.K. Buchmann, K. (2008). Development of adaptive immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. *Fish Shellfish Immunol*. 25 533–541, https://doi.org/10.1016/j.fsi.2008.07.008.

STUDY OF THE EFFECTS OF STARVATION IN THE SKELETON OF GILTHEAD SEABREAM (*Sparus aurata*) BY COMPUTED TOMOGRAPHY

D. Ceballos-Francisco[1], F.A. Guardiola[1*], N. García-Carrillo[2], F.J. Pardo-Fernández[2], A. Cuesta[1], M.A. Esteban[1]

[1]Immunobiology for Aquaculture Group, Department of Cell Biology and Histology. Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia (Spain)
*Email: faguardiola@um.es
[2.] Preclinical Imaging Unit, Laboratory Animal Service, Core Facilities University of Murcia, 30120, Murcia, Spain

Introduction

Fish skeleton is a highly conserved organ system which provide support and protection of soft parts of the body, maintaining the movement by muscles attachment and acting as mineral reserve (Witten et al., 2017). Bone tissue takes part in the regulation of calcium-phosphate metabolism since it is the major mineral storage organ in the vertebrate body. Unlike terrestrial vertebrates, fish can absorb minerals from surrounding water across the skin, oral and branchial epithelium, as consequence stressors related to water and ion homeostasis, such as starvation, have greater physiological impact on fish (Leatherland and Woo, 2010; Suarez-Bregua et al., 2018). Many fish species are exposed to starvation or restricted food intake in particular phases of their life cycles and during this period, they have to direct energy reserves from growth to the support of vital processes, which triggers metabolic changes in many tissues including bones (Suarez-Bregua et al., 2018). Therefore, this study attempts to explore the effects of starvation in the skeleton of gilthead seabream (*Sparus aurata*) subjected to 60 days fasting.

Material and Methods

Ten juvenile specimens (5 months old) of gilthead seabream (*S. aurata*) (26 ± 3 g and 12 ± 2 cm) were obtained from a local fish farm (Murcia, Spain) and kept in re-circulating seawater aquaria (250 L). Fish were allowed to acclimatize for 4 weeks in the Marine Fish Facility at the University of Murcia and were fed a commercial diet (Skretting, Spain) based on fish meal (21%). The temperature and salinity of the water were 22 ± 2°C and 28‰, respectively. The artificial photoperiod was of 12 hour (h) light: 12 h dark. Fish specimens were randomly assigned into two groups (n = 5 each) to be studied under two different conditions (fed and starved). The control group (fed fish) was fed with commercial pellets (Skretting) at a rate of 1.5% body weight day-1 and the last group was starved, both groups were kept in these conditions for 60 days. Image analysis and measurements were performed using the Carestream Molecular Imaging Albira CT system in conjunction with Pmod and Amide packages. Boxes ROIs were drawn within the density range previously determined for fish bone (from 200 Hounsfield Units) in Amide software.

Results and discussion

At 60 days, the fed group gained in body weight [26.385 ± 1.950 (Mean ± SD) to 53 ± 6.931 g] and size (12.566 ± 0.625 to 16 ± 1.00 cm) while at 60 days starved group decreased in weight and size 19.66 ± 2.200 g and 12.416 ± 1.625 cm respectively. Intensive farming of certain commercial fish species uses starvation to control several parameters that can affect health quality and produce economic losses (Caruso et al., 2011). One of the proven effects of starvation on fish body is cessation of growth (Sakyi et al., 2020). Furthermore, when we visually analyse the CT image of the skeleton of fed fish at 60 days, the structure appears normal. However, in the image of the skeleton of the starved gilthead seabream some morphological differences were notice, the abdominal region seems compressed, the orbit wider and deeper. In this sense, deficiencies of minerals, vitamins and phospholipids that can be acquired through diet have been reported to cause skeletal disorders in fish species like Atlantic salmon (*Salmo salar*), common carp (*Cyprinus carpio*), haddock (*Melanogrammus aeglefinus*) or halibut (*Hippoglossus hippoglossus*) (reviewed by Leatherland and Woo, 2010). Moreover, the skeleton of the starved fish is observed radiologically denser and small gaps are visible in the vertebrae. During migration of Atlantic salmon from estuaries to spawning areas, the compacity of bony tissues significantly decreases owing to osteoclastic resorption, resulting in significant reduction of vertebral bone mass (Kacem et al., 1998). Moreover, scales, another part of the skeleton, demineralize during the up-stream migration where maturing Atlantic salmon stop feeding (Kacem and Gustafsson, 2000).

Conclusion

This study demonstrates that starvation affects not only muscle mass and fat but also bone structure and bone density. Computed tomography have probe to be a useful tool to explore aquaculture specimens under different conditions, allowing qualitative and quantitative information of bone structure in a non-destructive way.

(Continued on next page)

Acknowledgements

This work was supported by the MINECO co-funded by the European Regional Development Funds (ERDF/FEDER) (grant no. AGL2017-88370-C3-1-R) and Fundación Seneca de la Región de Murcia (Grupo de Excelencia grant no. 19883/GERM/15).

References

Witten, P.E., Harris, M.P., Huysseune, A., & Winkler, C. (2017). Small teleost fish provide new insights into human skeletal diseases. Methods in Cell Biology. 138, 321-346. https://doi.org/10.1016/bs.mcb.2016.09.001

Leatherland, J.F. and Woo, P. T. (2010). Fish Diseases and Disorders, Volume 2: Non-infectious Disorders, Second Edition (Vol. 2).

Suarez-Bregua, P., Guerreiro, P.M., & Rotllant, J. (2018). Stress, glucocorticoids and bone: a review from mammals and fish. Frontiers in endocrinology, 9, 526. https://doi.org/10.3389/fendo.2018.00526.

Caruso, G., Denaro, MG, Caruso, R., Genovese, L., Mancari, F. y Maricchiolo, G. (2012). Short fasting and refeeding in red porgy (*Pagrus pagrus*, Linnaeus 1758): Response of some haematological, biochemical and non specific immune parameters. Marine Environmental Research, 81, 18-25., 81, 18-25. https://doi.org/10.1016/j.marenvres.2012.07.003.

Sakyi, M.E., Cai, J., Tang, J., Xia, L., Li, P., Abarike, E.D., Kuebutornye, F.K.A., & Jian, J. (2020). Short term starvation and re-feeding in Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758): Growth measurements, and immune responses. Aquaculture Reports. https://doi.org/10.1016/j.aqrep.2019.100261.

Kacem, A., Meunier, F.J., & Bagliniere, J.L. (1998). A quantitative study of morphological and histological changes in the skeleton of *Salmo salar* during its anadromous migration. Journal of Fish Biology, 53, 1096-1109. https://doi.org/10.1111/j.1095-8649.1998.tb00466.x.

Kacem, A., & Gustafsson, S. (2000). Demineralization of the vertebral skeleton in Atlantic salmon *Salmo salar* L. during spawning migration. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 125, 479-484. https://doi.org/10.1016/S1095-6433(00)00174-4.

CULTURE OF *Catostylus tagi,* A NEW EDIBLE JELLYFISH

S.K.M. Gueroun[1,2,3*], T.M. Torres[4], A. dos Santos[5,7], N. Vasco-Rodrigues, R. Gouveia[6], J. Canning-Clode[1,8], C. Andrade[2,3,7]

[1]MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Edifício Madeira Tecnopolo, Piso 0, Caminho da Penteada, 9020-105, Funchal, Madeira, Portugal
[2] Mariculture Centre of Calheta, Madeira, Portugal
[3] Madeira Oceanic Observatory - ARDITI/OOM, Funchal, Madeira, Portugal
[4] *Universität Bremen*, Bremen, Germany
[5] Instituto Português do Mar e da Atmosfera (IPMA), Av. Alfredo Magalhães Ramalho, 6, 1495-165 Algés, Portugal
[6] Oceanário de Lisboa. Esplanada D. Carlos I, 1990-005 Lisbon, Portugal
[7] CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal
[8] Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA
Email: sgueroun@mare-centre.pt

Introduction

Jellyfish have been commonly considered a nuisance for human activities. However, some species bear potentials for food, cosmetic, pharmaceutical and biotechnology fields. *Catostylus tagi*, a jellyfish common in the western Atlantic and Portuguese coasts, holds several biochemical properties along to be edible for human consumption (Raposo *et al*., 2018; Amaral *et al*., 2018). Its collagen and antioxidant properties showed a potential new bio-resource for the cosmetics and food sector (Calejo *et al*., 2009; Morais *et al*., 2009). While local *C. tagi* fisheries might respond to the needs of the various sectors, the ability to control its life cycle and biomass production might be the key for future sustainability in this rising new product.

Materials and methods

Catostylus tagi specimens were samples and *in vitro* fertilisation performed in different temperature, salinity and light conditions. Once the planula obtained, different trials were performed investigating the optimum conditions (temperature, salinity and food type) for planula survival and settlement, polyps development, and asexual reproduction (podocyst, strobilation and ephyra production). The life cycle of the species was also documented from the planula to the juvenile stage.

Results

Catostylus tagi displays the typical Rhizostomida metagenetic life cycle. The results showed a high tolerance of the different life stage to the wide range of salinities and temperatures. The planula settlement was significant influenced by temperature ($P < 0.001$) and salinity ($P < 0.01$), but no interaction was detected ($P = 0.31$). Polyp development was enhanced at higher temperature with an optimum of 25 °C and all salinities. Among the asexual reproduction, only the strobilation processes were significantly influenced by temperature and food type ($P < 0.01$) (Figure 1).

Discussion and conclusion

C. tagi displays a typical meta-genetic life cycle observed in Rhizostomatidae: a benthic scyphistoma phase that reproduces asexually via strobilation releases ephyrae that grow into a pelagic medusa, which reproduce sexually. The trials showed *C. tagi* high tolerance to a wide range of salinities and temperature. This plasticity reflects the species adaptation to estuaries conditions known for their highly dynamic environments, subject to rapid changes in the *in situ* conditions associated with varying freshwater flows.

While *C. tagi* appears as an euryhaline and eurytherm species, each stage (planula, polyps) and process (planula settlement, polyps development and strobilation, ephyrae production) responded distinctively to the different conditions. While the specifications of each stage need to be fulfilled to reach optimum production, the environmental plasticity makes *C. tagi*, among other jellyfish species, a good candidate for aquaculture

(Continued on next page)

References

Amaral, L. *et al*. (2018) Jellyfish ingestion was safe for patients with crustaceans, cephalopods, and fish allergy. *Asia Pac*. *Allergy*, **8**, e3.

Calejo, M. T. *et al*. (2009) Isolation and biochemical characterisation of a novel collagen from *Catostylus tagi*. *J*. *Biomater. Sci*. *Polym*. *Ed*., **20**, 2073–2087.

Morais, Z. B. *et al*. (2009) Composition and in vitro antioxidant effects of jellyfish *Catostylus tagi* from Sado estuary (SW Portugal). *J*. *Aquat*. *Food Prod*. *Technol*., **18**, 90–107

Raposo, A. *et al*. (2018) Eating jellyfish: safety, chemical and sensory properties. *J*. *Sci*. *Food Agric*., **98**, 3973–3981

DYNAMIC BRAIN TRANSCRIPTOME ANALYSIS OF FLATFISH METAMORPHIC REMODELING PROCESS

L. Guerrero-Peña*[1], P. Suarez-Bregua[1], L. Méndez-Martínez[1], P. García-Fernández[2], R. Tur[2], J.A. Rubiolo[3], J.J. Tena[4] and J. Rotllant[1]

[1]Acuabiotec Lab, Department of Biotechnology & Aquaculture. Institute of Marine Research, (IIM-CSIC), 36208 Vigo, Spain
[2] Pescanova Biomarine Center, O Grove, Pontevedra, Spain
[3]Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo 27002, Spain
[4]Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas/Universidad Pablo de Olavide, Sevilla, Spain
email: lguerrero@iim.csic.es

Introduction

Metamorphosis is a fascinating process by which a larva completely changes its morphology in order to face the new challenges of adult life. In fish, including paradigmatic species with commercial value such as flatfish, this process initiated in the brain has traditionally been considered to be a critical rearing point and despite the pioneering molecular work carried out on the metamorphosis of the Japanese flounder, Atlantic Halibut and Sole the underlying molecular basis of flatfish metamorphosis is still relatively poorly characterized. In this study we performed a brain transcriptome profiling of three key stages of turbot metamorphic remodeling process (pre-metamorphic, climax of metamorphosis and post-metamorphic) using RNA sequencing (RNA-seq). A total of 1,570 genes were differentially expressed in the three developmental stages and we found a specific pattern of gene expression at each stage. Unexpectedly, at the climax stage of metamorphosis we found highly expressed genes related to the immune response, while biological pathway enrichment analysis in pre-metamorphic and post-metamorphic were related with cell differentiation and oxygen carrier activity, respectively. In addition, our results confirm the importance of TSH, increasing its expression during metamorphosis. Based in our results we assume that inflammation events during climax of metamorphosis stage could be related to processes of larval tissue resorption and replacement as occurs in other vertebrates.

Material y métodos

Newborn turbots (*Scophthalmus maximus*) were reared under a standard commercial production cycle and supplied by the company Insuiña SL, Grupo Nueva Pescanova (Pontevedra, Spain). Brains of the three key developmental stages in turbot (Al-Maghazachi and Gibson 1984) were extracted: pre-metamorphic (15dpf), climax of metamorphosis (30dpf) and post-metamorphic (57dpf). Fish brains were dissected and fixed in RNAlater (Thermo Fisher Scientific) for 24 hours at 4 °C and preserved at -80 °C until use. Brain samples were removed from RNAlater solution and homogenized in RLT buffer (RNeasy Mini Kit (Qiagen). Total RNA was extracted and purified using the RNeasy Mini Kit (Qiagen) with on-column DNase digestion (Qiagen) according to the manufacturer´s instructions. Approximately 1 μg of total RNA was initially used for BGISEQ-500 standard library construction at BGI (Beijing Genomics Institute, China). Prepared cDNA libraries were sequenced on a BGISEQ-500 platform and single-end reads of 50 base pairs (bp) length were generated per sample. A differential expression analysis and clustering was performed between the different stages to find genes involved in each stage of interest. In addition, an enrichment of the gene ontology (GO) was carried out to know the biological processes in which the genes are involved. The results of RNA-seq were confirmed by quantitative real-time polymerase chain reaction of five genes of different pathways.

Results and discussion

We sequenced the brain transcriptome in three key developmental stages across turbot metamorphosis (pre-metamorphic, climax and post-metamorphic stages). Nine cDNA libraries (three replicates per each metamorphic stage) were sequenced and more than 180 million 50 bp single-end reads were generated. After filtering the reads were mapped to the turbot reference genome obtaining average mapping rates of 88.98%, 88.78% and 89.23% for pre-metamorphic, climax and post-metamorphic stages, respectively. To identify DEGs in the turbot brain during metamorphosis, we performed pairwise comparisons among three postembryonic developmental stages. A total of 1,570 genes were differentially expressed in the three metamorphic developmental stages. A high proportion of DEGs were found when pre-metamorphic vs climax stages (338 up and 221 down-regulated) and pre-metamorphic vs post-metamorphic stages (415 up and 487 down-regulated) were compared (Fig.1).

(Continued on next page)

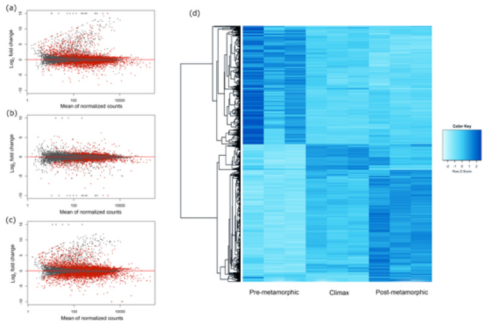


Figure 1. MA plot of all transcriptome genes from pairwise comparisons: (a) pre-metamorphic vs climax, (b) climax vs post-metamorphic and (c) pre-metamorphic vs post-metamorphic. The red dots plotted represent genes with an adjusted *p*-value < 0.1, while gray dots are those genes that do not show the established significance. (d) Heatmap displaying the genes hierarchically clustered according to the expression profiles throughout metamorphosis. Each column represents an individual triplicate from each metamorphic stage (pre-metamorphic, climax and post-metamorphic) and each row represents different genes. The colors from light blue to dark blue indicate gene expression from low to high, respectively.

However, a significantly lower number of DEGs (33 up and 76 down-regulated) were found after comparison between climax and post-metamorphic stages (Figure1). All DEGs from the three developmental stages were combined into a single set and hierarchically clustered within a heatmap in order to have an overview of the gene expression profiles across metamorphosis (Figure 1). In the heatmap, DEGs were clustered according to gene expression level. Overall, we can observe two major clusters. Most genes clustered on the top half of heatmap displayed an expression decrease throughout the metamorphosis process (Figure 1) while groups of genes on the bottom half showed an increased expression over time. Specifically, a set of tightly clustered DEGs showed a marked expression peak at the metamorphic climax while they exhibited a down-regulated gene expression at the pre- and post-metamorphic stages (Figure 1). In summary, our results show for the first time, a significant activation of the innate immune system during the metamorphosis process in flatfish, thus corroborating that metamorphosis is a time of dramatic changes in development that affects the entire organism.

Acknowledgment

This work was funded by the Spanish Economy and Competitiveness Ministry project AGL2017-89648P to J. Rotllant. L. Guerrero-Peña was supported by pre-doctoral fellowship of the Spanish Personnel Research Training Program funded by Spanish Economy and Competitiveness Ministry (PRE2018-085475). L. Méndez-Martínez, was supported by pre-doctoral fellowship of the Xunta de Galicia (IN606A-2020/006).

References

Al-Maghazachi, S.J., and R Gibson. 1984. “The Developmental Stages of Larval Turbot, Scophthalmus Maximus (L.).” *Journal of Experimental Marine Biology and Ecology* 82: 35–51.

PARTIALLY DEFATTED BLACK SOLDIER FLY MEAL INCLUSION IN JUVENILE PACIFIC WHITE SHRIMP DIETS: EFFECTS ON GROWTH PERFORMANCES

C. Guidou*[1]; C. Trespeuch[1]; E. de Swaef[2]; J. Dantas Lima[2]

1 MUTATEC – 1998, Chemin du Mitan – 84300 Cavaillon, France
c.guidou@mutatec.com
2 IMAQUA - KMO zone Lozen Boer - Ambachtenlaan 27A - B-9080 Lochristi, Belgium

Introduction

For several years, feed manufacturers are searching for new sources of proteins in order 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.

The objective of the study carried out in the facilities of IMAQUA (Merelbeke, Belgium) between March 2020 and April 2020 is to evaluate the zootechnical performances of juvenile shrimps (*Penaeus vannamei*) when a part of fishmeal is replaced by a partially defatted black soldier fly (BSF) meal at different inclusion levels in comparison to a conventional feed (CTRL).

Materials and methods

A BSF meal, produced by a French company (MUTATEC), is incorporated in pelleted feeds at different inclusion rates (6.4; 12.7 and 19.1%), as replacement material for fishmeal (respectively 33; 66 and 100% of replacement). These feeds have been used to feed 600 Pacific white shrimps from 0.24 grams to 2.74 grams (28 days of trial). The shrimps were divided per group and in triplicates (50 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, observed mortality and feed consumption per group.

Results

All results were positive. Although not significant, an increased final weight (and related weight gain) was observed in all treatments where fishmeal was replaced with insect meal. The feed conversion ratios were also better for the shrimps fed with insect meal than the CTRL group shrimps. The optimal inclusion level was 12.7% (66% of fishmeal replacement), the specific growth rate was significantly better than the CTRL for this group of shrimps. An increase in average final weight up till 16.8% for this group could be observed after 28 days of feeding. At the end of a complete culture period this would result in a substantial increase in productivity. Also, survival was slightly higher in all dietary treatments compared with the CTRL.

Conclusion

These results suggest a positive impact on growth performance in shrimp. It therefore seems possible to replace a significant portion (up to 100%) of fishmeal by BSF meal.

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 colour and firmness of the shrimp fed with different proportion of mealworm meal (Panini *et al.,* 2017b).

As BSF meal contains some antimicrobial peptides, some health effects are expected. This trial ended with a bacterial challenge which showed promising results that will be presented later.

(Continued on next page)

References

Cummins, Rawles, Thompson, Velasquez, Kobayashi, Hager, and Webster. 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 473. Pages 337-344.

Panini, Eduardo Lima Freitas, Guimaraes, Rios, Da Silva, Nascimento Vieira, Fracalossi, Ian Samuels, Schwinden Prudencio, Peres Silva and Amboni. 2017a. Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. Aquaculture 473. Pages 115-120.

Panini, Pinto, Nobrega, Vieira, Fracalossi, Samuels, Prudencio, Silva and Amboni. 2017b. Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp Litopenaeus vannamei. Food Research International 102. Pages 445-450.

PARTIALLY DEFATTED BLACK SOLDIER FLY MEAL INCLUSION IN JUVENILE RAINBOW TROUT DIETS: EFFECTS ON GROWTH PERFORMANCES

C. Guidou*[1]; C. Trespeuch[1]; J. Dias[2]

1 MUTATEC – 1998, Chemin du Mitan – 84300 Cavaillon, France
c.guidou@mutatec.com
2 SPAROS LDA, Área Empresarial de Marim, 8700-221 Olhão, Portugal

Introduction

For several years, feed manufacturers are searching for new sources of proteins in order 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.

The objective of the study carried out between December 2018 and May 2019 is to evaluate the zootechnical performances of juvenile trouts when a part of fishmeal is replaced by a partially defatted black soldier fly (BSF) meal at different inclusion levels in comparison to a conventional feed (CTRL) and a feed containing another insect, the mealworm (TM).

Materials and methods

A BSF meal, produced by a French company (MUTATEC), is incorporated in extruded feeds of different sizes (1.2 mm; 2 mm) and at different inclusion rates (5 to 25%), as replacement material for fishmeal. These feeds have been used to feed rainbow trout (Oncorhynchus mykiss) from 7 grams to 65 grams (86 days of trial).

Results

All results were positive. After 31 days of trial, fish fed with BSF meal show significantly better growth performances than the CTRL group fish. Trouts fed with feeds containing BSF meal showed faster growth and better feed conversion ratios than the CTRL group fish, which indicates an efficient assimilation of the feed. After 64 days of trial, growth results and feed conversion ratios of all BSF groups remain better (non-significant). At equivalent fishmeal replacement rate, results with BSF meal are superior (+7% mean body weight) than results with TM meal (non-significant). Also, survival was high and similar between treatments.

Conclusion

These results suggest a positive impact of BSF meal incorporation on growth performance in trouts. It therefore seems possible to replace a significant portion (up to 60%) of the fishmeal by BSF meal.

Other trials in experimental stations and in fish farms showed that the replacement of a part of the fishmeal by a partially defatted BSF meal improves the growth performance of the rainbow trout during the growing period (120g-350g).

References

Dumas, Raggi, Barkhouse, Lewis, and Weltzien. 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, Vol 492. Pages 24-34.

Rema, Saravanan, Armenjon, Motte and Dias. 2019. Graded incorporation of defatted yellow mealworm (*Tenebrio molitor*) in rainbow trout (*Oncorhynchus mykiss*) diet improves growth performance and nutrient retention. Animals, Vol 9. Pages 1-10.

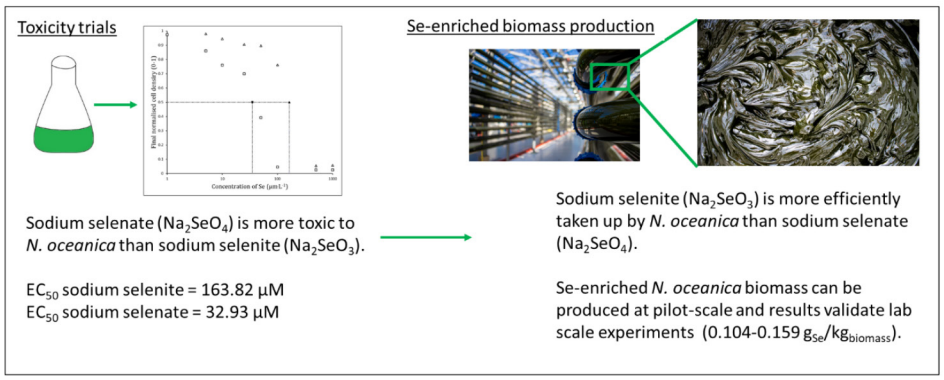
Renna, Schiavone, Gai, Dabbou, Lussiana, Malfatto, Prearo, Capucchio, Biasato, Biasibetti, De Marco, Brugiapaglia, Zoccarato and Gasco. 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens*) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss*) diets. Journal of Animal Science and Biotechnology, Vol 492. Pages 24-34.

SELENIUM ENRICHMENT IN THE MARINE MICROALGA *Nannochloropsis oceanica*

B. O. Guimarães, K. D. Boer, P. Gremmen, A. Drinkwaard, R. Wieggers, R. H. Wijffels, M. J. Barbosa, S. D'Adamo

University and Research (WUR), Bioprocess Engineering, AlgaePARC, P.O. Box 16, 6700 AA, Wageningen, Netherlands
Email: barbara.guimaraes@wur.nl

Se-enriched ingredients have recently gained interest in the aquaculture industry as feed supplements due to their positive effects on fish health, growth, and potential effects on animal welfare. This study aims to assess which inorganic selenium (Se) species is suitable to produce Se-enriched *Nannochloropsis oceanica* (*N. oceanica*) biomass for aquafeed applications. The effective concentration for 50% growth inhibition (EC_{50}) and Se bioaccumulation of the two inorganic forms of Se, sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4), were assessed at different concentrations after twelve days of cultivation. Toxicity results showed that selenate, EC_{50} = 32.93 µM, had a greater negative effect on cell growth than selenite, EC_{50} = 163.82 µM. Total intracellular Se was analysed by inductively coupled plasma - optical emission spectrometry (ICP-OES) and high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS), which revealed that selenite was better accumulated by *N. oceanica*. Further investigation at 30 µM of selenite in the growth medium resulted in Se bioaccumulation with a minor effect on cell growth and reached a Se intracellular content of 0.131 $g_{Se}/kg_{biomass}$ after 12 days. Thus, 30 µM of selenite was selected for batch pilot-scale cultivation in a 1500 L tubular photobioreactor. Total Se accumulated in the biomass at pilot-scale was in the same order of magnitude compared with flasks (0.104 - 0.159 $g_{Se}/kg_{biomass}$). The results from this pilot-scale study are fundamental for a proof of concept laboratory to pilot-scale production and are a critical bridging step for the potential use of Se-enriched *N. oceanica* for aquafeed.



DEVELOPMENT OF GENOMIC MARKERS ASSOCIATED TO PRODUCTION TRAITS IN LUMPFISH *(Cyclopterus lumpus)*

A. P. Gutierrez[1], M. Bekaert[1], H. Migaud[1], T. Cavrois-Rogacki[2], A. Davie[1]

[1] Institute of Aquaculture, University of Stirling, Stirling, UK.
[2] Otter Ferry Seafish Ltd., Tighnabruaich, Argyll, UK

*E-mail: alejandro.gutierrez@stir.ac.uk

Introduction

Biological control of sea lice infection in Atlantic salmon cages has become an important alternative to tackle one of the most important diseases affecting salmon aquaculture. A handful of cleaner fish species have gained great importance in the control of sea lice (Barrett et al. 2020), among them, the use of lumpfish (*Cyclopterus lumpus*) has shown great promise (Imsland et al. 2018). Lumpfish is a sub-Arctic species found on both sides of the North Atlantic and commonly found along the Icelandic, Norwegian and British coastlines as well as the East coast of North America (Davenport 1985). Current lumpfish production is reliant on wild caught broodstock to meet the increasing demand. Lumpfish life cycle has been closed and hatchery reproduction is now possible.

Genomic resources are called to play a fundamental role in the improvement of selective breeding practices of aquaculture species. The development of these resources has been scarce for emerging species such as lumpfish, therefore, there is an imperative need to obtain genomic information that will support the establishment of effective and sustainable selective breeding programs. The recent release of the lumpfish genome sequence will facilitate the identification of genomic markers and the development of genomic tools. The aim of our study was to develop genomic tools and identify genomic regions linked to commercially important traits in lumpfish including gender and growth.

Materials and Methods

Fish used in this study belong to a population obtained from the Otter Ferry SeaFish farm (OFS). Ten lumpfish families from wild origin were produced in spring 2019 at OFS facilities. Four of these families were maintained in separate tanks until they reached a mean weight of 3-9g. Fin clips were obtained from all parents and from the four families including the 50 bigger, 50 smaller and 25 random fish from each family.

Two ddRAD libraries were prepared, containing all 536 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 & Durbin 2009) and assembled using Stack v2.41 (Rochette et al. 2019). Identified SNPs were collected from the 536 samples, and filtered according to the presence of at least two alleles, MAF >0.10, present in 75% samples and expected mendel segregation.

Genome wide association analysis was performed using the package R/SNPassoc v1.9-2 (González et al. 2007) using the “log-additive” model (except for sex, where ”co-dominant” model was used) and R/qtl2 v0.20 (Broman et al. 2019), analysing four traits, including gender, weight, condition factor and standard length.

Results and Discussion

Approximatelly 3.2 billion paired end reads were obtained as raw data from sequencing, from these, 3.2 million unique loci were detected and mapped across the available lumpish genome. After SNP calling and quality control, 10,630 informative SNPs were identified.

Association analyses were able to identify many genomic regions linked to the analyzed traits. The analysis of gender showed the highest association, identifying a single major QTL located in chromosome 13 (Figure 1A). Markers located in this region were further analyzed and a set of 16 markers was capable of accurately predict sex in all samples. Analysis of growh traits showed a polymorphic behaviour as expected, identifying significant association in many chromosomes, showing evidence of overlap in the QTL regions identified for weight and length in chromosomes 7, 12, 13, 15, 17 & 21 (Figure 1B). Of particular importance was the association between growth and gender shown in a region of chromosome 13 which could have implications in selective breeding. Moving forward, markers located in these identified QTL regions are candidates for the development of low density SNP panels that will provide a low cost alternative for producers to use genomic information in the development of selective breeding programs and improve production traits.

(Continued on next page)

References

1. Barrett L.T., Overton K., Stien L.H., Oppedal F. & Dempster T. (2020) Effect of cleaner fish on sea lice in Norwegian salmon aquaculture: a national scale data analysis. International Journal for Parasitology 50, 787-96.
2. Broman K.W., Gatti D.M., Simecek P., Furlotte N.A., Prins P., Sen Ś., Yandell B.S. & Churchill G.A. (2019) R/qtl2: Software for Mapping Quantitative Trait Loci with High-Dimensional Data and Multiparent Populations. Genetics 211, 495-502.
3. Davenport J. (1985) *Synopsis of biological data on the lumpsucker, Cyclopterus lumpus (Linnaeus, 1758)*. Food & Agriculture Org.
4. González J.R., Armengol L., Solé X., Guinó E., Mercader J.M., Estivill X. & Moreno V. (2007) SNPassoc: an R package to perform whole genome association studies. Bioinformatics 23, 654-5.
5. Imsland A.K.D., Hanssen A., Nytrø A.V., Reynolds P., Jonassen T.M., Hangstad T.A., Elvegård T.A., Urskog T.C. & Mikalsen B. (2018) It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. Biology Open 7.
6. Li H. & Durbin R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25, 1754-60.
7. Rochette N.C., Rivera-Colón A.G. & Catchen J.M. (2019) Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28, 4737-54.

CAN JELLYFISH BE A FUTURE AQUA-FEED COMPONENT? A FIRST NUTRITIONAL INSIGHT

P. Guttuso[1,2] *, N. Nogueira[1,2,3], P. Canada[2,3], SKM. Gueroun[1,2,4], J. Canning-Clode[4,5], C.Andrade[1,2,3].

[1]CMC - Mariculture Center of Calheta, Directorate for the Sea, Av. D Manuel I, Nº7, 9370-133, Calheta, PT
[2]OOM - Oceanic Observatory of Madeira, ARDITI - Regional Agency for the Development of Research Technology and Innovation, Ed. Madeira Tecnopolo, 9020-105, Funchal, PT
[3]CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal4450-208, Matosinhos, PT
[4]MARE - Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI). Edifício Madeira Tecnopolo, Caminho da Penteada, 9020-105 Funchal, Madeira, Portugal
[5]Smithsonian Environmental Research Center, Edgewater, USA

*Email: guttusopaolo@gmail.com

Introduction

In the last decades, jellyfish seem to have increased globally, significantly impacting different human activities in coastal areas (Purcell et al., 2007). Besides food purposes, these organisms have been recently evaluated as a potential resource for other industries, such as agriculture (Emadodin et al. 2020) or pharmaceutics (Leone et al. 2015). Nevertheless, little is known about the usefulness of jellyfish for feed production, probably due to their intrinsic characteristics as the high moisture level and the presence of venom in certain species. Moreover, research on the nutritional benefits of using jellyfish in aqua-feeds as raw material has been relatively unexplored.

Materials and Methods

Based on a systematic review of the literature, following the methodology recommended by Moher et al. (2009), the jellyfish nutritional composition was characterized, and the existing gaps for accurate comparability were highlighted. Furthermore, the available nutritional characterization was compared with the most used aqua-feed ingredients, critically evaluating the possible benefit and limitation of usage in the aqua-feed formulation. Data were divided into four main categories: proximate composition (Pc), amino acid (AA) and fatty acid (FA) profile, mineral (Mi) content.

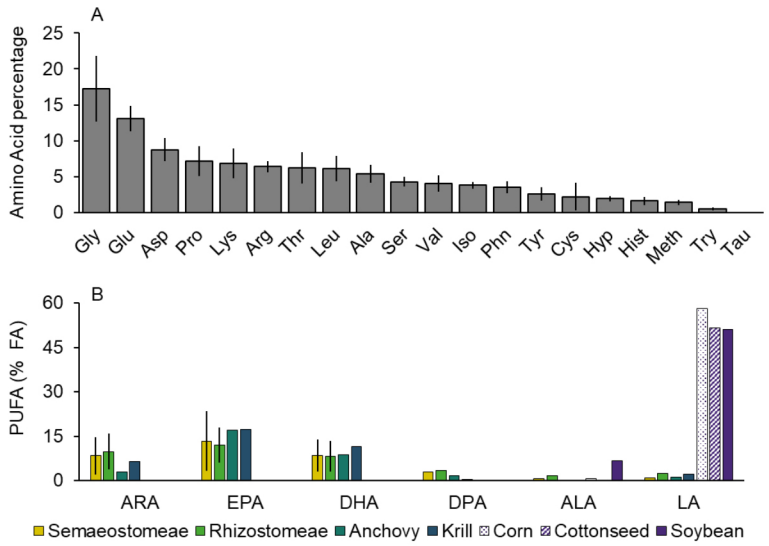


Fig. 1 Amino acid ranking in jellyfish (A) and most representative PUFA (% FA) in jellyfish and reference ingredient for aqua-feed production (B)

(Continued on next page)

Results

Of the 55 identified and validaded articles, proximate composition results showed that water ranged between 91.1 to 98 % wet weight; ash between 15.40 – 85.6 % dry weight (dw); proteins between 0.2 - 76.8% dw; lipids were 0.17 – 12.3 % dw and carbohydrates between 0.1-22.71% dw, with the proportion varying between body parts and species. Amino acid profiles were proportionally quite homogeneous and presented specific pattern; the most representative AAs were Glycine (16.6 ± 4.8 % AA), Glutamic acid (12.7 ± 1.8 % AA), Aspartic acid (8.6 ± 1.7 % AA) and Proline (7.7 ± 1.8 % AA) (Figure 1A). Although fatty acid variability was mainly associated with seasonal shift, SFAs and PUFAs were more abundant than MUFA. The most representative PUFAs were Arachidonic acid (ARA) (2.8-23.7 % FA), Eicosapentaenoic acid (EPA) (1.23-25.9% FA) and Docosahexaenoic acid (DHA) (0.8-25.9 % FA). Overall, jellyfish hold a qualitative FA profile closer to commonly used fish oil sources, such as anchovy and krill, than other oil sources currently used as fish oil replacement (soybean, corn and cottonseed) (Figure 1B). Macro elements are consistent in jellyfish and show a defined pattern for body composition. Microelements manifested more variability and were reported within the following range concentrations in mg/kg dry weight: Fe (0.59- 252) Cu (0.11 – 49.82), Zn (3.61 – 400), Si (13.01- 81.76), Mn (0.11 – 18.66).

Discussion and Conclusion

Despite quantitative differences in species and body parts, overall, the ratio between each nutritional compound was relatively homogeneous. However, the detected variability underlined in different references limited an accurate, comprehensive quantitative nutritional characterization. Factors such as seasonality, water quality, methods, and processing techniques should be further investigated for effective future jellyfish exploitation and further clarify the mechanism associated with jellyfish' apparent nutritional plasticity. With the lack of knowledge about local and temporal biomass availability, this aspect made not possible an accurate definition of jellyfish as an aqua-feed ingredient. Nevertheless, it was underlined the qualitative jellyfish raw biomass potentiality as Protein, PUFAs and Mineral sources that already at this stage can candidate them as a potential component for functional feed to be investigated.

Reference

Brotz L, Cheung WWL, Kleisner K, Pakhomov E, Pauly D (2012) Increasing jellyfish populations: Trends in Large Marine Ecosystems. Hydrobiologia 690:3–20.

Emadodin I, Reinsch T, Ockens R-R, Taube F (2020) Assessing the Potential of Jellyfish as an Organic Soil Amendment to Enhance Seed Germination and Seedling Establishment in Sand Dune Restoration. Agronomy 10:863.

Leone A, Lecci RM, Durante M, Meli F, Piraino S (2015) The bright side of gelatinous blooms: Nutraceutical value and antioxidant properties of three Mediterranean jellyfish (Scyphozoa). Mar Drugs 13:4654–4681.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Statement. 89:873–880.

Purcell JE, Uye SI, Lo WT (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. Mar Ecol Prog Ser 350:153–174.

AQUACULTURE OF THE SCIAENIDAE FAMILY: MAIN SPECIES CULTIVATED WORLDWIDE AND EMERGING SPECIES IN LATIN AMERICA

J. Chacón-Guzmán[1-2]*, R. Jiménez-Montealegre[1], E. Gisbert[3], S. Ramos-Júdez[3], J. W. Hong[4-5], J. Pérez-Urbiola[6], N. Duncan[3]

[1]Escuela de Ciencias Biológicas, Universidad Nacional (UNA), Heredia 40101, Costa Rica.
[2]Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica, Universidad Nacional, Universidad Estatal a Distancia, Costa Rica.
[3]IRTA, Centre de Sant Carles de la Ràpita, Aquaculture Program, Carretera de Poble Nou, km 5.5, E- 43540 Sant Carles de la Ràpita, Tarragona, Spain.
[4] State Key Laboratory of Large Yellow Croaker Breeding, Ningde 352103, China
[5] College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China.
[6]Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Baja California, México.

E-mail: jonathan.chacon.guzman@una.cr

Introduction

The deficit of fisheries resources, the increase in the world population and the growing per capita consumption of fish (20,5 kg 2020), are some of the factors that have led to greater investment in world marine fish farming. The statistics for 2019 presented a consolidated and growing Atlantic Salmon (*Salmo salar*) industry, with the highest world production (2.615.962 tons) of a species cultured in the sea. In addition to salmon, other marine fish present significant advances in technological development with productions greater than two hundred thousand tons, European seabass (*Dicentrarchus labrax*) 263.214, gilthead seabream (*Sparus aurata*) 258.753, and the large yellow croaker (*Larimichthys crocea*) 225.547 tons. These advances have encouraged interest in culturing new species in regions that have little tradition of marine aquaculture, such as Latin America. The Sciaenidae family made up of 289 species in 69 genera, presents in addition to the large yellow croaker, several species with important productions and other species with high potential for culture. This work compiles the main factors of success and problems generated in the aquaculture industry of Sciaenidae and also makes reference to the state of technological development and future perspectives of emerging species of the Sciaenidae family in Latin America.

World production

The family Sciaenidae represents one of the groups of marine fish (fully marine from egg through to adult) with the highest production in the world (340.273 tons - 2019) with a high market value of 839.569.920 U.S. dollars. Three species contribute 99,94% of the world aquaculture production of the Sciaenidae family, the large yellow croaker, *L. crocea*, cultured in China (225.549 tons), the red drum, *Sciaenops ocellatus* (77.008 tons) cultured in the USA, China, Guadeloupe, Israel, Martinique, Mauritius and Mayotte, and meagre, *Argyrosomus regius* (37.526 tons), cultured in Egypt, Spain, Greece, Turkey, Croatia, France, Saudi Arabia, Italy, Tunisia, Cyprus, Portugal, and Algeria. The remaining 0,06% (189,6 ton), was contributed by the Shi drum, *Umbrina Cirrosa* cultured in Greece and Turkey, the mi-iuy corvina, *Miichthys miiuy* cultured in the Republic of Korea, and the mulloway, *Argyrosomus japonicus* cultured in Mauritius.

Fig. 1. Species of the Sciaenidae family investigated for aquaculture in Latin America

(Continued on next page)

Success factors and problems

The three Sciaenidae species with the highest production in the world have shown high potential for aquaculture. They stand out for being euryhaline species (0-66 ppt), eurythermal (2-38 °C) and have high fecundity that allows them to produce large quantities of eggs per spawn (500-3.270.000). Red drum can spawn naturally, and reproductive dysfunctions have been overcome with hormonal therapies, for example in Red drum (GnRHa 100-160 µg + HCG 5 mg kg^{-1}), and with low doses in *L. croceus* (GnRHa 3-10 µg kg^{-1}) and in *A. regius* (GnRHa 15 µg kg^{-1}). Survival has been achieved in larval culture between 7,7 and 75% and growth rates in grow-out of 2,14 g/day^{-1}, with low feed conversion rates between 0,9 and 1,9. High larval survival rates have been correlated to the use of copepods for food in some species. Market values range between $ 7,14–14,28 U.S. dollar, which are attractive and have allowed the profitability of aquaculture production. The adaptation of Sciaenidae to technologies implemented in other species has been successful, for example, the production technologies of *A. regius* are based on knowledge and production technologies developed for the production of gilthead seabream and European seabass. A relevant aspect to consider is that these industries have developed in countries with availability of goods and services for aquaculture and where local and national governments have promoted industrial applied research, both scientifically and financially. A negative aspect to consider is the low resistance to handling, physical injuries are common after physical handling in Sciaenidae species. The little progress in the production of secondary species may be related to several factors. In the case of *U. cirrosa*, its industry is considered to be overshadowed by the good characteristics and success of the culture of *A. regius*. In *M. miiuy* and *A. japonicus*, technological problems are reported for the production of larvae and juveniles.

Emerging Species of Latin America

In Latin America there has been great interest in the development of aquaculture for the Scienidae family since the late 20th century. Research on eleven species are currently reported (Fig. 1). Two have greater technological development, the totoaba *Totoaba macdonaldi* and the corvina pampera *Cilus gilberti*. The other species are in the initial stages of technological development or have been studied sporadically.

Conclusions

The Sciaenidae family has the highest world aquaculture production of a marine species group, with biological characteristics that give high potential for aquaculture. The three consolidated species have in common that previously developed culture technologies were quickly and easily adapted for their production, allowing high larval survival rates and spawning of constant quality, using when needed established GnRHa hormonal therapies to overcome reproductive dysfunctions. Latin America has a high interest and potential to develop aquaculture with species of the Sciaenidae family. It is recommended for the Latin American region to analyse the technologies used for these established Sciaenidae to apply these technologies to native Latin American Sciaenidae to both utilise successful management aspects and avoid problems encountered.

CAN LIPIDOMICS HELP IDENTIFYING EGG QUALITY IN BALLAN WRASSE (*Labrus bergylta)*?

Andreas Hagemann*[1], Arne M. Malzahn[1], Antonio Sarno[1], Julia Farkas2, Luciana Alves Musialak[3], Elin Kjørsvik[3] and Bjørn Henrik Hansen[2]

1 Department of Fisheries and New Biomarine Industry, SINTEF Ocean, Trondheim, Norway
2 Department of Climate and Environment, SINTEF Ocean, Trondheim, Norway
3 Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
Email: andreas.hagemann@sintef.no

Introduction

What makes an egg a good egg is a long-discussed topic in many biological disciplines, but especially in aquaculture, as the fish farmer is betting that the eggs will result in a high value fish. Many different ideas have been postulated how to describe a good egg, and fertilization success, development into a normal embryo and high hatch rates are probably the most named descriptors. Brooks et al (1997) elegantly described a good egg as one that, "[…] contains all the necessary materials (both genetic and nutritive) to support the development of the subsequent embryo." This definition is certainly true and gives a good hint what to consider but does not supply a testable tool to describe egg quality.

Ballan wrasse (*Labrus bergylta*) is in the limelight of aquaculture research due to their role as cleaner fishes in salmon aquaculture. A major drawback is that the salmon industry still depends on wild caught fish as there are still many unsolved challenges in ballan wrasse aquaculture, amongst them the determination of egg quality. Eggs from wild fish are usually considered of better quality (Srivastava, Brown, 2011), and fertilization rates, hatch rates, survival and size at hatch are usually higher than in eggs produced in captivity.). To lay a knowledge foundation, we studied different egg characteristics with the potential to predict egg (and offspring) quality to provide insights into the interplay between lipidomics, fatty acids and various oocyte traits of ballan wrasse eggs.

Materials and methods

Eggs used in the current study were purchased from two commercial hatcheries in Norway. We studied 6 different egg batches, spawned and fertilized naturally on spawning mats placed in spawning tanks. One batch was spawned by an established broodstock, while five other batches were spawned by wild fish which has been in captivity 1 - 3 years. The eggs were scraped off the spawning mats and transported same day to SINTEF Sealab, Trondheim where the eggs were acclimatized and transferred into flow-through incubators. The eggs were sampled 14 times until 16 days post fertilization. We analysed several biometric measurements using microscopy and computer aided image analysis, respiration rates, egg dry weight, elemental analysis (carbon & nitrogen) and bone/cartilage development. Additionally, we performed lipidomics on these eggs to test whether their lipid profiles relate to and can be thereby used as a predictor for egg quality in fish.

Results and discussion

The potential egg quality descriptors we analysed all showed substantial variability between batches. The more conservative quality descriptors, such as larval length, egg diameter and egg weight, showed less variability than the more dynamic ones, such as the decrease in dry weight, or decrease in carbon content per egg between one- and 16-days post-fertilization. We quantified 245 different lipid species in the ballan wrasse eggs, of which 56 correlated to the 20 potential egg quality markers we used in this study (Figure 1). These markers can broadly be grouped into markers related to (a) size, (b) energy content, and (c) metabolic activity (Table 1). Those lipids which were related to the egg quality markers grouped into two main clusters: Cluster A (the lower cluster in the clustered image map Figure 1) showed a positive covariance with size related measures such as larval length at hatch, or yolk volume at 2 days post hatch, and negatively with measures related to metabolic activity, such as changes in respiration rate between fertilization and hatch or the decrease of nitrogen and carbon in the egg. This group consisted of phosphatidylcholines (PC), lysophosphatidylcholine (lysoPC), and triacylglycerides (TAG) and three different sphingomyelins (SM). Group B clustered related to the same measures, but in the opposite way with the exception that size related measures showed a weaker association. This group of lipid species contained several phosphatidylethanolamines (PE) and free fatty acids.

(Continued on next page)

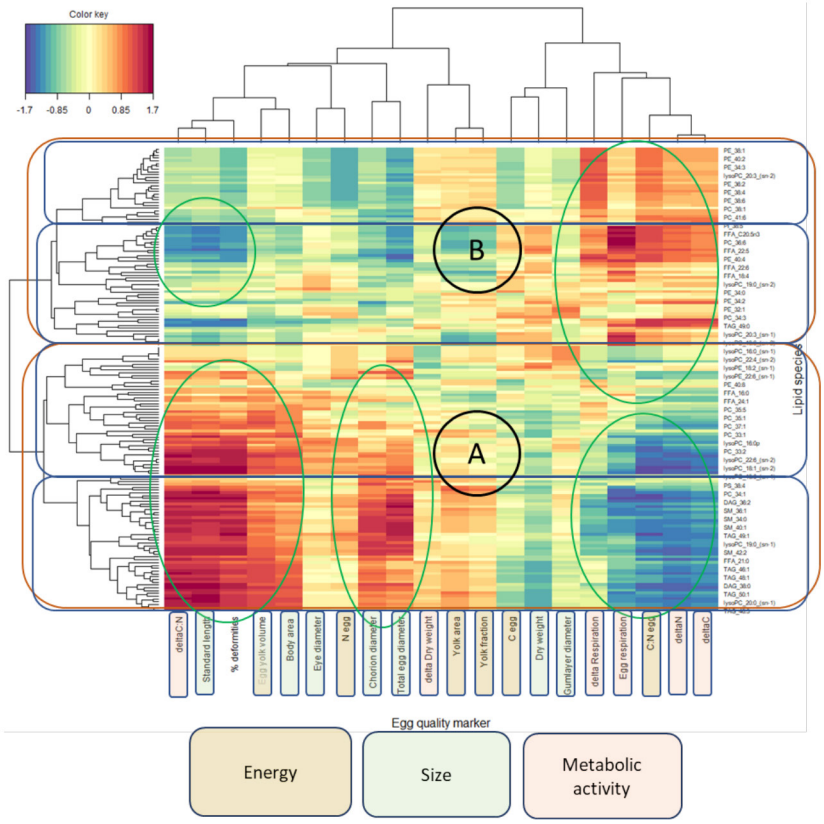


Figure 1: Clustered Image Map of the top lipid species that most contribute to the variance. (A) and (B) denotes the two main clusters.

Conclusions

The pattern of several lipid species being positively related to size and negatively to metabolic activity while another group is showing exactly the opposite deserves further investigation. To our knowledge this is the first study applying lipidomics approaches to assess its potential as an egg quality measure. This study only scratches the surface of this complex topic but can serve as a basis for further identifications of important groups of lipids. The future task would be to identify recurring patterns within species and to test to which extend these are species specific or general, and ultimately try to achieve a mechanistic understanding of the importance and the interplay of these groups

Acknowledgements

We thank FHF (Norwegian Seafood Research Fund) for funding of the work (#901561). The project war carried out in the framework of the national research infrastructure "Norwegian Center for Plankton Technology" (RCN #245937/F50).

References

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. 7, 387-416.

Srivastava, R., Brown, J., 2011. The biochemical characteristics and hatching performance of cultured and wild Atlantic salmon (Salmo salar) eggs. Can. J. Zool. 69, 2436-2441.

EPIGENETIC VARIATION IN GUT TISSUE CORRELATES WITH MICROBIOME COMPOSITION AND RESILIENCE DURING A TENACIBACULOSIS OUTBREAK IN ATLANTIC SALMON (*Salmo salar*)

Søren B. Hansen[1]*, D. Bozzi[2], J. A. Rasmussen[1], M. Kodama[1], M. T. Limborg[1]

[1]Center for Evolutionary Hologenomics, GLOBE Institute, University of Copenhagen, 1353 Copenhagen K., Denmark
[2]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan., Italy
E-mail: soren.blikdal.hansen@sund.ku.dk

Introduction

Infectious skin diseases, such as tenacibaculosis, occur frequently in salmon production and are a major challenge compromising fish welfare, sustainability and profit[1]. Tenacibaculosis are typically associated with the presence of an external pathogen which can be isolated from the ulcers. However, when an outbreak occurs, there are often large differences in disease susceptibility and response among fish within the same tank. Here, we investigate microbial and epigenomic variation in disease response in an acute outbreak of ulcer disease in Atlantic salmon (*Salmo salar*)[2].

Materials and methods

The fish investigated in this study were one year old Atlantic salmon from a commercial hatchery recently transferred to a land facillicy in northern Norway. In this standard aquacultural setting an acute outbreak of skin ulcer disease caused by *Tenacibaculum dicentrarchi,* occurred briefly after saltwater equilibration. The gut microbiome composition of 20 fish with and 20 fish without visual indications of tenacibaculosis were characterized with bacterial 16S rRNA gene metabarcoding (Fig. 1A). Genome wide DNA Methylation patterns were characterized in 10 sick and 10 healthy fish with different microbiome compositions using whole genome bisulfite sequencing (WGBS). Lastly, we resequenced key differentially methylated regions using nanopore cas-9 targeted sequencing (nCATS), to explore the potential of targeted DNA methylation analysis as a tool for monitoring fish health[3].

Results

A) Sampling of gut tissue and microbiota in healthy and sick fish. **B)** Relative abundance of intestinal bacteria. **C)** Principal component analysis of 16 million CpG sites (i.e. epigenetic methylation markers): PC1 values shown on the y-axis, random noise on x-axis.

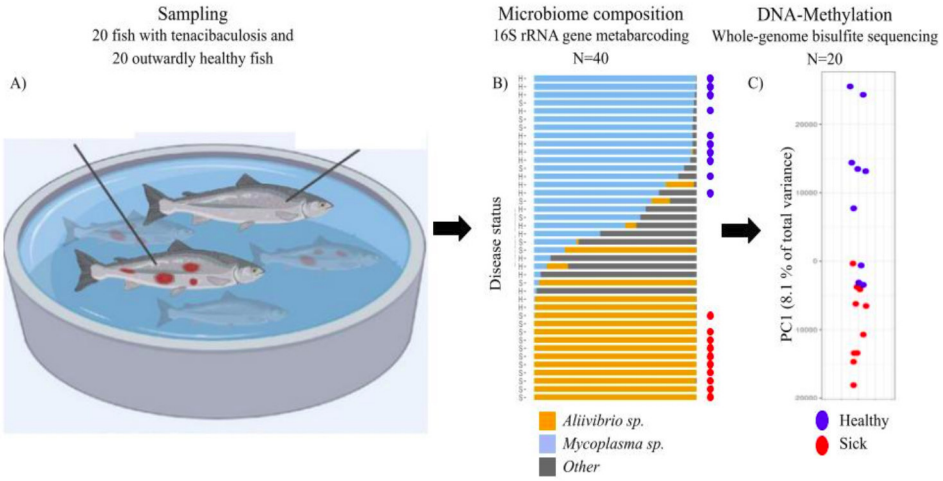


Figure 1. Summary of samples and preliminary results

The gut microbiomes were found to be dominated by two species of bacteria; *Mycoplasma sp*. and *Aliivibrio sp*. Interestingly, the sick fish were dominated by the *aliivibrio* species, while the healthy fish were dominated by the *Mycoplasma* species (Fig. 1B). Whole Genome Bisulfite Sequencing resulted in 16 million CpG sites with 5X coverage in all samples, meaning we could efficiently analyse more than a third of all the CpG sites in the salmon genome. Strikingly, the sick fish and the healthy fish showed global differences in DNA-methylation patterns (Fig. 1C), suggesting that epigenetic mechanisms were involved in the difference in tenacibaculosis susceptibility and/or response. The differences indicated large general methylation differences between the sick and healthy fish. Relative to the location of CpG sites in general, the differences between the sick and healthy fish were more often found in promoter regions and frequently multiple differences were found within the same promoter region. DNA-methylation level in promoters and other specific regions of genes is known to affect the transcription level of the gene. Therefore, investigating which genes were related to the epigenetic response of tenacibaculosis, could help to identify candidate genes involved in disease resilience. Our results suggest that especially genes involved in signaling and adhesion between cells are important for susceptibility and/or response to tenacibaculosis. In addition to presenting the analysis leading to our results, pilot results from DNA-methylation analysis of key differentially methylated nCATS regions will be presented and used to evaluate the method compared to today's golden standard method of WGBS.

Discussion

In Atlantic salmon with low genetic variation and raised in the same controlled environment, we found significant differences in microbiome composition and DNA-methylation between fish with tenacibaculosis and their more resilient healthy companions. There is increasing evidence of a symbiotic relationship between *Mycoplasma sp*. and its salmonid hosts, however the interactions between the two are still largely unexplored [4–6]. The results presented here suggest epigenomic regulation of host genes can be used to better understand interactions between the two and their influence on complex phenotypes. Exactly which mechanisms are causing the correlations and how the epigenome, microbiota, and phenotype are interconnected are still inconclusive. The results presented here, the manipulability and heritable nature of the epigenome highlights it as an interesting future target for monitoring complex phenotypes, including tenacibaculosis. On the methodological side, we hope the knowledge from our nCATS experiment can contribute to further development of strategies for analysing DNA-methylation profiles in a low cost and high throughput manner.

References

1. Takle, H. R. *et al*. *Sårproblematikk og hudhelse i laks- og regnbueørrettoppdrett*. *5* (2015).
2. Bozzi, D. *et al*. Salmon gut microbiota correlates with disease infection status: potential for monitoring health in farmed animals. *Anim. Microbiome* 3, 30 (2021).
3. Gilpatrick, T. *et al*. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat. Biotechnol*. 38, 433–438 (2020).
4. Holben, W. E., Williams, P., Saarinen, M., Särkilahti, L. K. & Apajalahti, J. H. A. Phylogenetic Analysis of Intestinal Microflora Indicates a Novel Mycoplasma Phylotype in Farmed and Wild Salmon. *Microb. Ecol*. 44, 175–185 (2002).
5. Cheaib, B. *et al*. Genome erosion and evidence for an intracellular niche – exploring the biology of mycoplasmas in Atlantic salmon. *Aquaculture* 541, 736772 (2021).
6. Rasmussen, J. A. *et al*. Genome-resolved metagenomics suggests a mutualistic relationship between Mycoplasma and salmonid hosts. *Commun. Biol*. 4, 1–10 (2021).

OPTIMIZING OFF-BOTTOM OYSTER CULTIVATION IN THE NETHERLANDS: IMPROVING YIELD WITH SITE-SPECIFIC OYSTER FARMING

Eva Hartog[1 *], Tony van der Hiele[1], Lotte Niemeijer[1], Wouter Suykerbuyk[2], Linda Tonk[2]

[1]HZ University of Applied Sciences, Edisonweg 4, 4382NW, Vlissingen, The Netherlands
[*]email: e.hartog@hz.nl
[2]WMR - Wageningen Marine Research, P.O. Box 77, 4400 AB Yerseke, The Netherlands

Off-bottom oyster cultivation in the Oosterschelde, the Netherlands, started in 2010. Off-bottom cultivation typically result in oysters (*Crassostrea gigas*) with faster growth and a higher meat quality in comparison with the conventional bottom oyster cultivation, due to a higher food quality and availability higher in the water column. Using triploid oysters, which invest more energy in growth, ensures a shorter cultivation process. In off-bottom cultivation oyster spat is placed at a high density (app. 5000 individual pieces) in fine-meshed bags or baskets and will be manually thinned in density during the growth process to consumption size. The starting material, oyster spat, can be of various origins and usually comes from hatcheries, as the Netherlands, Ireland and France.

Currently two different cultivation systems are being used by Dutch oyster farmers in the intertidal area in the Oosterschelde The first system consists of firm plastic bags (available in 4 different mesh sizes) which are placed on iron frames. The second system is the so-called BST system which consists of hanging baskets (available in 3 different mesh sizes) attached on longlines or on iron tables. Although these systems are widely and successfully used around the world, there seems to be quite some room to optimize this way of farming. Main issues are i) their labour intensiveness in combination with the relatively high wages in the Netherlands and ii) high losses caused by the variety wave- and flow dynamics in the Oosterschelde. It is questioned if other off-bottom cultivation systems that are also successfully deployed around the world would be a good addition and or alternative to improve efficiency and productivity of Dutch oyster cultivation.

In 2020 the European Marine and Fisheries funded project “Oyster yield improvement through knowledge transfer and monitoring” was initiated. The goal of the project is to optimize oyster farming and it should lead to efficiency improvements of sustainable oyster cultivation. In this project a comparative study between current off-bottom oyster cultivation and alternative off-bottom farming systems will be conducted. The effect of the farming systems on the performance of oyster growth, quality and shell shape will be examined for four different systems in three different off-bottom cultivation locations in the Oosterschelde. In a comparative study between the currently used systems and alternative off-bottom cultivation systems, additionally the following two methods are being tested:

1- SEAPA - hanging baskets
2- FlipFlop bags - horizontal hanging bags

For this study T15 triploid oysters, originating from the hatchery Roem van Yerseke, the Netherlands., were placed in four bags or baskets at 3 different locations in the Oosterschelde (Prinseplaat-C, Yerseke bank-74/75 and Hooge Kraaijer-46), see Table 1. The number of oysters per system is calculated according to the total volume of each individual system. All the systems and all the locations are sampled every 10 weeks for the period March 2021 till December 2021. During sampling, 1 bag or basket of all the systems (and of all three locations) will be collected. Per sample randomly 60 individual oysters are analyzed on - shell shape / growth: length, width, thickness, quality and wet fresh weight. The Condition Index (Rainer and Mann, 1992) is determined for 30 individual oysters per sample.

Monitoring and experiments are ongoing till December 2021. During the presentation the results of the study will be shown and discussed.

Table 1, Amount of oysters per system and number of treatments per location

		Location		
System	**Amount of triploid oysters**	**Prinseplaat-C**	**YB74/75**	**HK46**
Bags (current)	70 pc – T15 – 8-12 gram	* 4	* 4	* 4
BST – baskets (current)	120 pc – T15 – 8-12 gram	* 4	* 4	* 4
SEAPA - baskets	70 pc – T15 – 8-12 gram	* 4	* 4	* 4
Flip-Flop bags	120 pc – T15 – 8-12 gram	* 4	* 4	* 4

Europese Unie, Europees Fonds voor Maritieme Zaken en Visserij

COMPARISON OF MOLECULAR, IMMUNOCHROMATOGRAPHY AND IMMUNOHISTOCHEMISTRY METHODS FOR THE DETECTION OF BETANODAVIRUS

Fatemeh Hassantabar[1], Mohammad Jalil Zorriehzahra[2]*, Farid Firouzbakhash[1], Kim D. Thompson[3] and Mina Ziarati[4]

[1]Department of Fisheries Science, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, I.R. Iran
[2]Department of Information and Scientific Communication, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, I.R. Iran
[3]Aquaculture Research Group, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian, UK
[4] Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Jahrom, Iran

Introduction

Betanodavirus, the causative agent of the disease VNN (Viral Nervous Necrosis), has a substantial economic, social and environmental effect on aquaculture development in the world. Diagnostic tests, such as assays targeting the viral RNA based on reverse-transcription PCR (RT-PCR), Histopathology, diagnosis based on immunological assays, have been developed to detect the Betanodavirus in fish and for health certification purposes (Zorriehzahra et al., 2005), (Binesh et al., 2013).

Methods and Materials

In this study, a Real-time PCR assay, nested RT-PCR, immunohistochemistry (IHC) and immunochromatography (ICG) were compared in the diagnosis of Betanodavirus infection. The reverse transcription (cDNA synthesis) was carried out followed by the RNA extraction. The cDNA was screened with an SYBER Green Real-Time PCR using a pair of primers NF2/NR3and Nested RT-PCR using two pairs of primers F2/R3 and NF2/NR3 in two steps for detecting Betanodavirus infection. To visualise the location of the virus in the brain of infected fish, IHC test with rabbit polyclonal anti-Betanodavirus antibody and anti-rabbit IgG-HRP conjugate was performed. The ICG test strip was constructed using anti- Betanodavirus monoclonal antibody (mAb) labelled with colloidal gold (used as detection antibody) and the rabbit anti- Betanodavirus polyclonal antibody (PAb) (used as capture antibody), for rapid detection of Betanodavirus infection. Forty brain samples from symptomatic golden grey mullet fish and 20 brain samples from healthy golden grey mullet fish obtained from the coastal areas of the Caspian Sea. Real-time PCR was used to detect Betanodavirus in both groups. Symptomatic mullet with positive PCR were considered infected by Betanodavirus and the Real-time PCR technique was chosen as a gold standard test (An, et al.,2008), (Dai, et al., 2015)

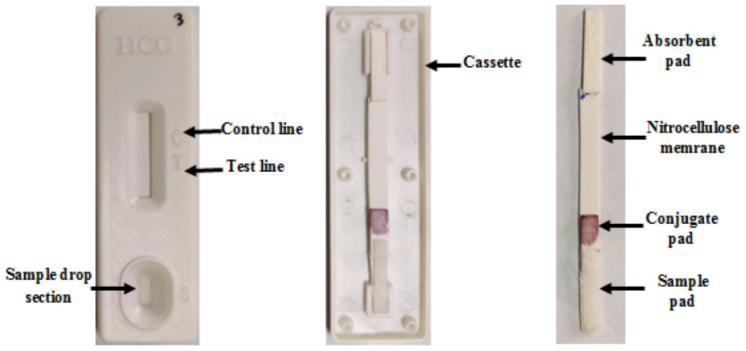


Fig.1. Most important Immunochromatography kit parts

(Continued on next page)

Result

IHC assay showed optimal diagnostic performance (100% sensitivity and specificity). ICG test identified 32 of 40 infected brain samples (80% sensitivity and 100% specificity) and 25 of 40 brain samples were positive only in nested RT-PCR (62.5% sensitivity and 95% specificity). Analysis of the results illustrate that ICG and IHC assays presented good sensitivity and specificity for diagnosing NNV; however, ICG may be more advantageous when a fast field test is required. The low overall sensitivity of nested RT-PCR may be due to high genetic variation within the T4 region occasionally leads to mismatches between primers and their targets and consequently the sensitivity (Fukuda et al.,,2009),(Dalla Valle et al.,2000)

References

An, D., Kim, T., Song, D., Kang, B., Park, B.)2008(An immunochromatography assay for rapid antemortem diagnosis of dogs suspected to have canine distemper. Journal of Virology. Methods 147, 244–249.

Binesh CP, Jithendran KP. (2013) Genetic characterization of betanodavirus isolates from Asian seabass Lates calcarifer (Bloch) in India. Archives of Virology 158:1543–7.

Chang S.F., Ngoh G.H. & Kueh S. (1997). Detection of viral nervous necrosis nodavirus by reverse transcription polymerase chain reaction in locally farmed marine food fish. Singapore Vet. J., 21: 39-44.

Dai, M., Gong, Y., Liu, A., Zhang, L., Lin, J., Zhang, M., Yu., X. (2015). Development of a colloidal gold-based lateral-flow immunoassay for the rapid detection of phenylethanolamine A in swine urine. Anal. Methods, 7: 4130–4137.

Dalla Valle L., Zanella L., Patarnello P., Paolucci L., Belvedere P. & Colombo L. (2000). Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. J. Fish Dis., 23, 321–327.

Fukuda, K., Kondo, M., Inagawa, H., Chongthaleong, A., Becerra, L., Nishi, K., Okuyama, A., Aoki, T.,Takahashi, Y. (2009). Development and Evaluation of an Immunochromatography Kit for Detection of White Spot Virus in Shrimp. Aquaculture Sci, 57)3), 437-447.

Zorriehzahra, M.E.J., Ghasemi, m., Ghiasi, M., Haghighi Karsidani , S., Nazari, A., Sharifpour, I., Rohani, M.S. (2005). Study of Viral Nervous Necrosis in Caspian Sea grey mullet *Liza auratus* and the evaluation of its infection and transition probability to other fish (sturgeon, *Rutilus frisi* and cultural fish) in Iran. Final report of national research proposal, Iranian Fisheries Research Organization (IFRO). P.186.

IMPROVED FILLET QUALITY IN HARVEST-SIZE ATLANTIC SALMON FED HIGH N-3 CANOLA OIL AS A DHA-SOURCE

Bjarne Hatlen[1]*, Thomas Larsson[1], Tone Kari Østbye[1], Odd Helge Romarheim[1], Laura Martinez Rubio[2], Bente Ruyter[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-9291 Tromsø, Norway
[2]Mowi Feed AS, Sandviksbodene 77AB, 5035 Bergen Norway

*bjarne.hatlen@nofima.no

Introduction

New sources of the very long-chain n-3 polyunsaturated fatty acids (LC-PUFA) are needed for salmon farming to grow, since the marine oils where these fatty acids are typically found, are of limited availability. High n-3 LC-PUFA canola oil has shown promise as a safe and effective source of DHA and other n-3 PUFA in small salmon (Ruyter et al., 2019). The present experiment was carried out to study the effects of long-term feeding of diets with graded levels of n-3 canola oil on performance and the fillet quality in large salmon in sea cages.

Materials and methods

A 12-months feeding experiment was carried out with Atlantic salmon growing from 0.7 to 4.7 kg in nine 5 x 5 m experimental cages. The fish were fed three diets containing graded inclusion levels of Aquaterra® omega-3 canola oil (Nuseed) resulting in levels of dietary DHA (22:5 n-3) of 4.0 % (Diet A), 6.6 % (Diet B) and 8.3 % of total fatty acids (Diet C) and total EPA + DHA of 6.4, 9.1 and 11.0 % of total fatty acids (weighted average of three pellet batches per diet). Feed intake and growth were measured, and at slaughter (4.7 kg) fish were analysed for tissue fatty acid composition and fillet quality. Fillet colour (CIE 1976 LAB color space) was measured instrumentally in 10 fish per cage using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc. Japan) and the same fish were pooled for astaxanthin analyses by HPLC. Thirty fish per cage were examined for black melanin spots in the fillets using a scoring scheme (Mørkøre, 2012).

Results

There were no significant differences between diets in overall fish growth and weight at harvest, although the fish fed Diet C had lower TGC in the last part of the study, when growing from 3 to 4.7 kg. No differences were seen in feed conversion ratio (FCR).

The FA-profiles in muscle mirrored those of the diets, giving increased DHA, EPA (20:5 n-3) and ALA (18:3 n-3) with increasing inclusion level of high n-3 LC-PUFA canola oil.

Fillet redness (a*), yellowness (b*) and chroma measured instrumentally (CIE 1976 LAB color space) were higher in the fish fed Diets B and C than Diet A (Fig. 1), suggesting a positive effect of DHA on muscle pigmentation. A similar trend, although not significant, was seen for astaxanthin measured in pooled muscle samples.

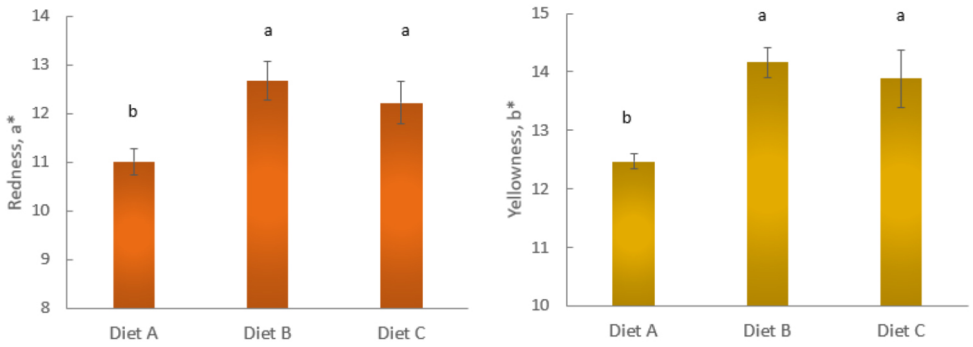


Figure 1 *Instrumentally measured redness (a*, left panel) and yellowness (b*, right panel) in fillets of salmon fed diets with graded level of DHA. Different superscripts[ab] indicate differences between diets.*

(Continued on next page)

The prevalence of dark melanin spots in the fillet was lower in fish fed Diet B than Diet A. The prevalence of more severe spots (score ≥ 2), average score and number of affected muscle segments were lower in fish fed both Diets B and C, compared to Diet A.

Conclusion

n-3 rich canola oil is an efficient source of DHA and other n-3 fatty acids for harvest-size Atlantic salmon. Increased dietary supply of DHA and other n-3 PUFA, above minimum requirement levels for DHA, improved fillet pigmentation and reduced fillet melanin spots, without compromising fish growth.

References

Ruyter B, Sissener NH, Østbye T-K, Simon CJ, Krasnov A, Bou M, Sanden M, Nichols PD, Lutfi E, Berge GM (2019) n-3 Canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon. British Journal of Nutrition 122, 1329-1345.

Mørkøre T (2012) Filet av oppdrettslaks: Kvalitetsavvik og årsakssammenhenger. FHF-prosjekt 900339. Report 17/2012, Nofima, Ås (in Norwegian).

FEED EFFICIENCY IN 35 FAMILIES OF ATLANTIC SALMON – THE RELATIVE IMPORTANCE OF BODY COMPOSITION AND ENERGY EFFICIENCY

Bjarne Hatlen[1]*, G.F. Difford[1], B. Gjerde[1], A. Norris[2] and A.K. Sonesson[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-9291 Tromsø, Norway
[2]Mowi Genetics AS, Sandviksboder 77A, NO-5035 Bergen, Norway
*Email: bjarne.hatlen@nofima.no

Introduction

Feed accounts for around half of the costs and 70-80% of the greenhouse gas emissions of Norwegian Atlantic salmon farming (Winther et al., 2019). Therefore, breeding more efficient animals may reduce the environmental footprint of the salmon industry and at the same time strongly increase its economic competitiveness.

Direct selection for feed efficiency (FE; gain/intake) requires measurements of feed intake in large numbers of animals. Measuring feed intake in individual fish with sufficient accuracy is difficult without interventions that may affect the measured variable. Exact feed intake measurements over time with minimal disturbance of the fish can be obtained in replicated family groups in tanks equipped for collection of the feed spill in the water outlet. However, the combination of high costs and loss of individual information prohibits the use of this method in breeding programmes.

Feed efficiency can be considered a product of i) energy efficiency and ii) weight gain per unit energy retained in the fish. Body fat and water content are inversely correlated in salmon and given similar energy efficiency lean fish (low energy density) can be predicted to have higher FE than fish with high body fat content (high energy density). Since fat content is relatively easy to measure, and may be estimated in live fish by non-destructive methods, this correlated response may be utilised for genetic selection for improved FE (e.g. Kause et al., 2016).

In order to unravel the relative importance of energy efficiency and body energy density for feed efficiency, an experiment was carried out to measure feed intake, growth, digestibility and body composition in duplicate groups of 35 Atlantic salmon families.

Materials and methods

Individually tagged and genotyped Atlantic salmon from 35 families were transported from Mowi Genetics AS (Øyerhamn, Norway) to the Nofima Research Station for Sustainable Aquaculture (Sunndalsøra, Norway) as 40 g parr. After one month of acclimation in small tanks, a random sample of 8 fish per family were taken for analyses and 25 individually weighed and length measured fish were distributed to each of two 150L experimental tanks per family. The fish were kept under continuous light and all tanks supplied with fresh water of stable temperature of 12.2 °C. The fish were then fed in excess for 7 weeks with water-stable extruded 3 mm feed pellets produced by extrusion at Nofimas Feed Technology Centre (Bergen, Norway). The feed was formulated according to commercial practices and added yttrium oxide (Y_2O_3) as an inert digestibility marker. All spill feed was collected from the water outlet and quantified daily after correction for recovery and dry matter content (Helland et al., 1996). At the end of the trial, the fish were individually weighed and length measured, and faeces collected by stripping (Austreng, 1978). The fish were then fasted for two days to empty the gut, euthanised and stored for analyses. Feces samples and initial and final samples of whole body were analysed for energy and crude protein, and their retention efficiency calculated.

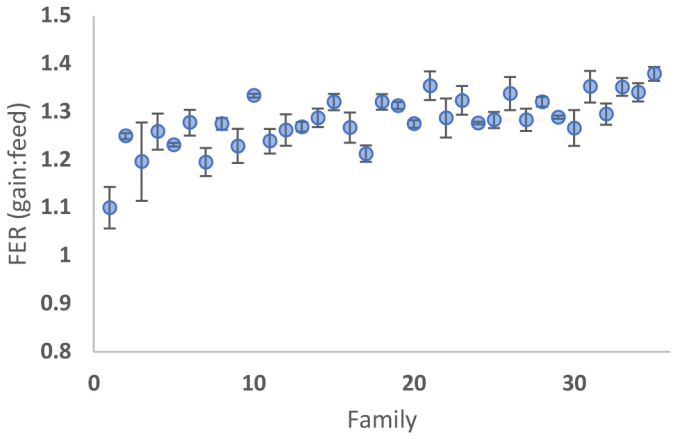
Results

To the best of our knowledge this is the first report of energy and protein efficiency in families from an elite breeding population of fish. The data showed variation in mean FE among the families from 1.10 to 1.38 (Fig. 1). Digestible energy efficiency varied from 55 to 66 % and digestible crude protein efficiency from 51 to 59 %. Although mean final body energy content among families varied within the narrow range of 8.32-9.24 kJ/g (corresponding to 10.5-12.8 % crude fat), there seemed to be a negative correlation between body energy and FE, as predicted. Statistical analyses to reveal the relative effects of energy utilisation and body composition on FE are underway and will be presented.

(Continued on next page)

Figure 1

Feed efficiency (FE) in 35 families of Atlantic salmon (mean ± s.e.m, N = 2 tanks/family)



Funding
This work was part of the EU project "NewTechAqua" (H2020 grant agreement No 862658).

References

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13, 265– 272.

Helland, S. J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture, 139, 157– 163.

Kause, A., Kiessling, A., Martin, S. A. M., Houlihan, D., and Ruohonen, K. (2016). Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (Oncorhynchus mykiss Walbaum). Br.J. Nutr. 116, 1656–1665. doi: 10.1017/S0007114516003603

Winther, U., Hognes, E.S., Jafarzadeh, S., Ziegler, F., 2020. Greenhouse gas emissions of Norwegian seafood products in 2017. Sintef report no. 2019:01505, 114 pp.

ARTIFICIAL INTELLIGENCE IN AQUACULTURE: APPLYING IMAGING ANALYSIS AND ACOUSTICS

S.S.W. Ende[1*], J.V. Apel[2], D. Ewald[2], J. Orellana[3], V. Edling[2], R. Thiele[1], M.S. Ur- Rehman[1], B. Wecker[4], K. Landsch[4], M.J. Slater[1] and J. Henjes[1]

[1]Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Sektion Marine Bioökonomie, Am Handelshafen 12, 27570 Bremerhaven
*Email: stephan.ende@awi.de
[2]MonitorFish GmbH, Hönower Str. 34, 10318 Berlin (Germany)
*Email: ewald@monitorfish.com
[3]Erwin Sander Elektroapparatebau GmbH, Am Osterberg 22, 31311 Uetze-Eltze (Germany)
[4]Förde Garnelen GmbH & Co. KG, Bülker Huk, 24229 Strande (Germany)

Introduction

In domestic shrimp farming, the monitoring of important production parameters (health status, biomass, feed rates and growth success of the animals) is still largely manual, which entails an enormous amount of time and high susceptibility to errors and also causes stress and, in some cases, physical damage to the animals.

The objective is to develop a digital monitoring technology (based on imaging and acoustics data) that enables a qualitative and quantitative assessment of the condition of the entire livestock population and provides the breeder with the data on a smartphone / tablet or computer via an interface. Real time recording and immediate automated decision making in land-based breeding systems for shrimp will make catch unnecessary, reduces hence stress and risk of injury to the animals and provides the farmer with information at all times about the actual biomass of its system as well as about the condition of the animals.

Material & methods

Shrimps are stocked in experimental units at various circumstances (single housed to commercial conditions in terms of density, size, light, etc…). Cameras (model) and hydrophones (icListen – 3500 m; 24-bit Smart Hydrophone – 200 kHz, Model Sb365-ETH, Software Program Marco, Ocean Sonics Ltd.) are installed above the experimental units. Imaging and acoustic recordings will be performed at various circumstances (from acoustically insulted settings to complex acoustic commercial environments, or, at various shrimp size and location, at feeding and absence of feeding). Further data recorded manually are number of shrimps, weight and biomass, mortality, Signs of illness / stress. In addition, the standard built-in sensors continuously record the water quality.

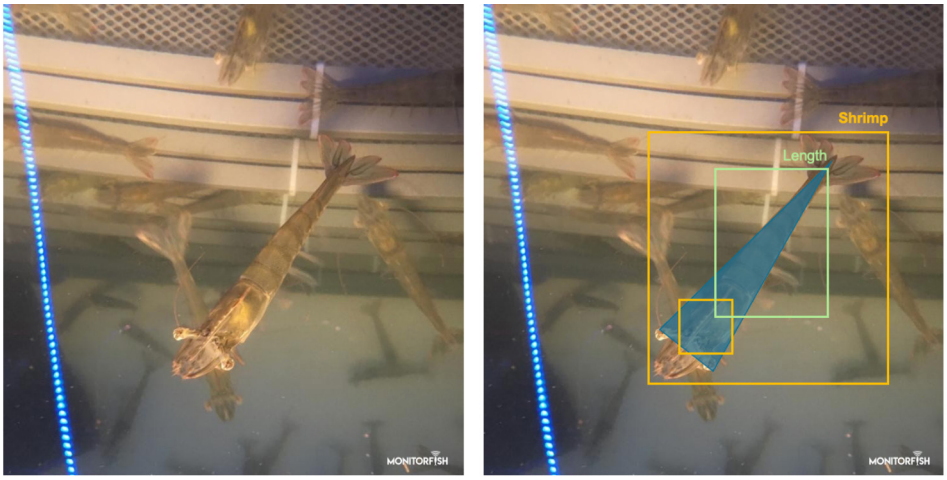
Results

At EAS 2021 preliminary results of imaging analysis and hydrophonic recordings will be presented. Experiments are currently conducted and first data will be available and analysed prior to this conference. We aim to present:

- Preliminary results of digital/automated counting the number of shrimp individual
- Preliminary results of intrinsic acoustic feeding signals from shrimp isolated in a disturbed acoustic environment

Discussion and conclusion

At EAS 2021 preliminary results on automated counting and acoustic feeding signals (considered key data for successful software development) will be presented. Reliability of first data (number of digital counts vs manual counts and acoustic feeding signal vs state of hunger) will be discussed and approaches for recognition algorithms that compares the metadata with a database on shrimp behavior will be presented.



IMPROVING PERFORMANCE OF SENEGALESE SOLE THROUGH DIET SUPPLEMENTATION WITH CURCUMIN EXTRACT

João Henriques[1]*, Maria Morais[1], Wilson Pinto[1], Maria J. Xavier[1,2], Sofia Engrola[2], Benjamin Costas[3], Jorge Dias[1] e Luís Conceição[1]

[1] SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
Email: joaohenriques@sparos.pt
[2] Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005-139 Faro, Portugal
[3] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal

Introduction

Senegalese sole (*Solea senegalensis*) is an emerging species for Southern European aquaculture. However, sole aquaculture faces bottlenecks regarding the development of strategies to control infectious diseases and the optimization of feeding and nutrition to improve growth rates. The growth performance of sole early life stages is highly dependent on nutritionally balanced diets. Diverse plant extracts containing bioactive compounds (e.g. polyphenols) are considered promising feed additives, with high potential to improve growth performance and also disease resistance [1]. This paper presents two trials that were conducted to evaluate the effect of a plant extract (curcumin) on the growth performance, immune status and oxidative parameters of Senegalese sole juveniles.

Material and methods

Both trials used two diets: an experimental microdiet, with inclusion of curcumin extract (CC) and a commercial microdiet (WINFlat, Sparos), as Control. Automatic feeders were used to continuously supply inert diets throughout both experiments. In the first trial, microdiets were introduced in the feeding regime of Senegalese sole postlarvae at 30 days after hatching (DAH) and kept during 4 weeks (64 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 30 DAH, 50 DAH and 64 DAH. In the second study, sole postlarvae were reared for 25 days, starting at 45 DAH. Sole postlarvae were kept in triplicate flat-bottom tanks (21 L) at a density of 3000 fish/m^2 and were sampled at 45 and 70 DAH.

Results

At the end of both trials, growth performance was improved in the groups fed CC diets. Senegalese sole fed on diets with curcumin extract presented higher (P<0.05) dry weight (Trial 1) and standard length (Trial 2) (P<0.05) when compared to sole fed the control diets (Fig. 1).

At the end of the second trial Senegalese sole fed on the diet with curcumin extract presented significantly (P<0.05) lower GST and HSP70 levels when compared to sole fed the control diet (Fig. 2).

Discussion and Conclusion

There is a growing interest in testing new feed ingredients and additives which can promote growth, and improve oxidative status and immune function in early stages of Senegalese sole and other fish species. Plant extracts such as curcumin contain phenolic compounds associated with high antioxidant capacity [2] hence posing high potential to be included as additives in fish feeds. The present results show that diets supplemented with curcumin extracts (CC) promote growth and improve oxidative status in sole postlarvae [4]. Previous studies showed that diet inclusion of curcumin improve sole growth performance through modulation in the expression of genes related to muscle growth [3]. Moreover, curcumin supplemented diets are also linked to improved growth performances and antioxidant activity in other fish species such as tilapia and trout juveniles [4, 5]. Overall results suggest that curcumin extracts at the tested levels are a viable growth promoter and can also contribute to improve oxidative status in early juveniles of Senegalese sole.

(Continued on next page)

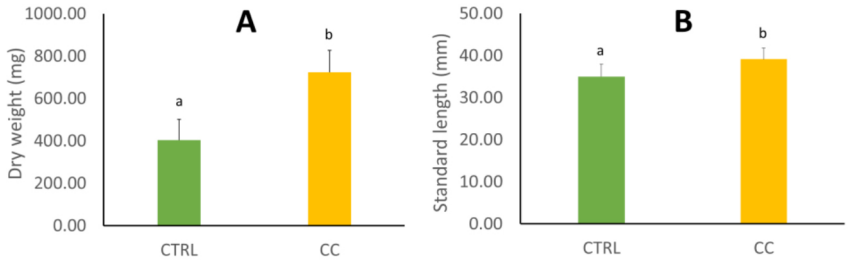


Figure 1. Dry weight of Senegalese sole juveniles fed a control (CTRL) or curcumin extract (CC) supplemented diet at: (A) 64 DAH, n=45 (Trial 1); and (B) 70 DAH, n=120 (Trial 2). Results are expressed as means ± standard deviation. Different superscript letters indicate significant differences between the dietary treatments (P<0.05).

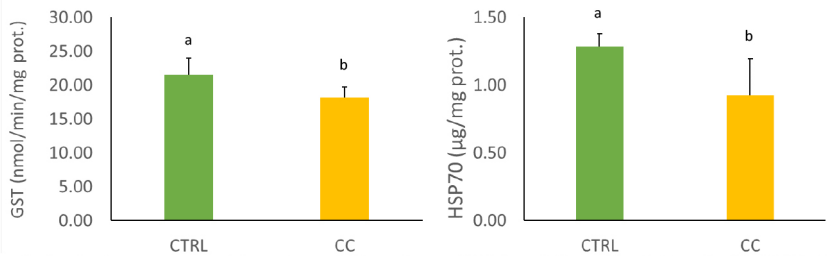


Figure 2. Antioxidant enzyme Glutathione-S-Transferase (GST) and Heat Shock Protein 70 (HSP70) levels in whole-body homogenates of Senegalese sole juveniles fed a control (CTRL) or curcumin extract (CC) supplemented diet, at the end of Trial 2. Results are expressed as means ± standard deviation. Different superscript letters indicate significant differences between the dietary treatments ($P<0.05$).

Acknowledgements

This work was funded by the Projects PATHAA, and UIDB/04326/2020 from the Foundation for Science and Technology of Portugal (FCT), and by project VALORMAR (ref. 024517) through Compete 2020, Lisboa 2020, CRESC Algarve 2020, Portugal 2020. Maria J. Xavier was supported by Grant PDE/0023/2013 (SANFEED Doctoral program, with support by FCT and SPAROS Lda., Portugal).

References

[1] P.M. Kidd, Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts, Altern Med Rev. 14(3) (2009) 226-46.

[2] I.O. Minatel, C.V. Borges, M.I. Ferreira, H.A.G. Gomez, C.-Y.O. Chen, G.P.P. Lima. Phenolic compounds: Functional properties, impact of processing and bioavailability. In: M. Soto-Hernández, M. Palma-Tenango, M.D.R Garcia-Mateos eds. Phenolic Compounds - Biological Activity, chapter 1. InTech (2017).

[3] M.J. Xavier, S. Engrola, L.E.C. Conceição, M. Manchado, C. Carballo, R. Gonçalves, R. Colen, V. Figueiredo, L.M.P. Valente, Dietary antioxidant supplementation promotes growth in Senegalese sole postlarvae, Front Physiol 11 (2020) 580600.

[4] Xavier, M.J., Conceição, L.E.C., Valente, L.M.P., Colen, R., Rodrigues, A.C.M., Rocha, R.J.M., Custódio, L., Carballo, C., Manchado, M., Engrola, S., 2021. Dietary Natural Plant Extracts Can Promote Growth and Modulate Oxidative Status of Senegalese Sole Postlarvae under Standard/Challenge Conditions. Animals. 11, 1398.

[4] H. Cui, B. Liu, X.-p. Ge, J. XiE, P. Xu, L.-h. Miao, S. Sun, Y. Liao, R. Chen, M. Ren, Effects of dietary curcumin on growth performance, biochemical parameters, HSP70 gene expression and resistance to Streptococcus iniae of juvenile Gift Tilapia, Oreochromis niloticus, Isr J Aquacult-Bamid 66 (2013) 986-996.

[5] M.E. Yonar, S. Mise Yonar, U. Ispir, M.S. Ural, Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (Oncorhynchus mykiss) against Aeromonas salmonicida subsp. achromogenes, Fish Shellfish Immun 89 (2019) 83-90.

IMPROVING SENEGALESE SOLE GROWTH PERFORMANCE THROUGH LOW LEACHING MICRODIETS

João Henriques[1]*, Afonso Valente[1,2], André Santos[1], Michael Viegas[1], Maria Morais[1], Wilson Pinto[1], Jorge Dias[1] e Luís Conceição[1]

[1] SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[2] Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005-139 Faro, Portugal
Email: joaohenriques@sparos.pt

Introduction

Production of high value flatfish species including Senegalese sole (*Solea senegalensis*) has recently increased in Southern Europe. Despite the research developments on sole weaning strategies, it is paramount to further adapt microdiet formulation and physical properties to the larvae feeding behaviour, while keeping good water quality [1]. Weaning microdiets with low particle size are prone to nutrient leaching, which can lead to significant nutrient losses thus impacting on water quality and larval performance. Several ingredients have binding properties, which have the potential to stabilize feed micro pellets, and thereby contribute to minimize leaching. The present study aimed at measuring leaching in microdiets with different binding ingredients and evaluating the effects on growth performance of Senegalese sole post-larvae.

Material and methods

Four diets were used in the trial: a Control microdiet (based on a commercial formula) and three experimental microdiets (Diets A, B and C) with different inclusions of binding ingredients. Leaching tests were performed to measure total protein loss. A sample of each feed was initially submerged in seawater for 2 minutes. After going through desalination and drying processes, the protein content of each sample was analysed and total protein leaching (%) was calculated. Microdiets were introduced in the feeding regime of Senegalese sole postlarvae at 31 Days After Hatching (DAH) and kept during 4 weeks (65 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 31 DAH, 52 DAH and 65 DAH.

Results

Leaching tests revealed that Diets A, B and C presented lower leaching level when compared to the control diet. Furthermore, diet C presented the lowest leaching level amongst the experimental diets (Fig. 1).

Discussion and Conclusion

There is scope to test alternative ingredients in microfeeds which can concomitantly contribute to minimize nutrient losses through leaching and allow keeping optimal biological performances of Senegalese sole and other fish species, reared in diverse production systems. Due to the non-proactive bottom-feeding behaviour of Senegalese sole postlarvae [2], the inclusion of ingredients with binding properties in weaning microfeeds is paramount to keep good pellet stability and minimize leaching, since lower sized feed particles are more susceptible to nutrient loss [1]. The present results indicate that diets containing different inclusions of alternative binding ingredients were linked to lower leaching levels and also promoted growth performance in sole postlarvae. Previous studies also showed that larval feeds with higher stability held more nutritional content [3]. Moreover, specific binders were also linked to higher pellet stability and improved performance of barramundi and tongue sole larvae [4, 5]. Overall, results suggest that alternative binding ingredients can reduce leaching in larval feeds which may contribute to promote growth performance in early juveniles of Senegalese sole.

(Continued on next page)

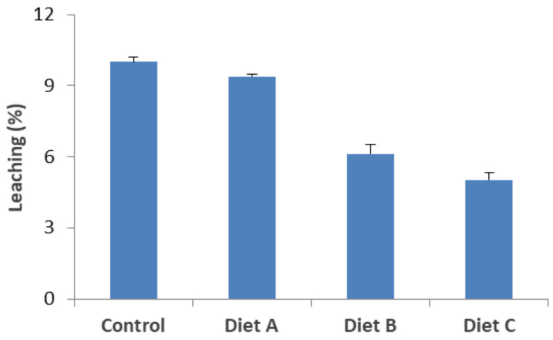


Figure 1. Leaching percentage of total protein in Control diet and experimental diets (Diets A, B and C, with different inclusion levels of binding ingredients). Results are expressed as means ± standard deviation (n=2).

At the end of the trial, growth performance in total length was improved in Senegalese sole groups fed diets B and C (P<0.05) when compared to sole fed the control diet (Fig. 2).

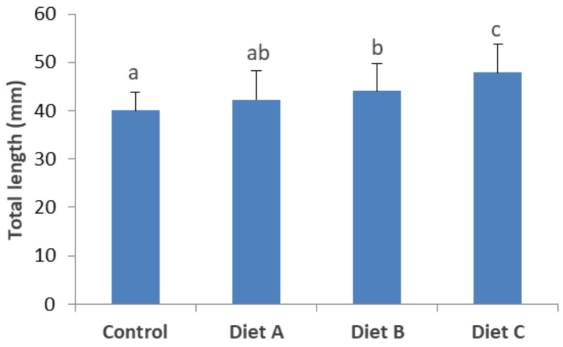


Figure 2. Total length of Senegalese sole post-larvae at the end of the trial (64 DAH, n=45) fed a control diet and experimental diets (Diets A, B and C, with different inclusion levels of binding ingredients). Results are expressed as means ± standard deviation. Different superscript letters indicate significant differences between the dietary treatments (P<0.05).

Acknowledgements

This work was funded by the Project SMART-HATCHERY, through the European Maritime and Fisheries Fund (EMFF), agreement EASME/EMFF/1018/BLUE-ECONOMY/863709.

References

[1] Pinto, W., Engrola, S., & Conceição, L. E. C. (2018). Towards an early weaning in Senegalese sole: A historical review. Aquaculture, 496, 1–9.

[2] Conceição, L. E. C., Ribeiro, L., Engrola, S., Aragão, C., Morais, S., Lacuisse, M., Dinis, M. T. (2007). Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). Aquaculture, 268(1-4), 64–81.

[3] Önal, U., & Langdon, C. (2004). Characterization of lipid spray beads for delivery of glycine and tyrosine to early marine fish larvae. Aquaculture, 233(1-4), 495–511.

[4] Partridge, G. J., & Southgate, P. C. (1999). The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. Aquaculture Research, 30 (11-12), 879–886.

[5] Liu, F., Ai, Q., Mai, K., Tan, B., Ma, H., Xu, W., LiuFu, Z. (2008). Effects of Dietary Binders on Survival and Growth Performance of Postlarval Tongue Sole, *Cynoglossus semilaevi*s (Günther). Journal of the World Aquaculture Society, 39(4), 500–509.

DIETARY FULL FAT AND DEFATTED *Zophobas morio* LARVAE MEAL MODULATES BOTH HAEMATOLOGICAL AND IMMUNOLOGICAL STATUS OF GILTHEAD SEABREAM *Sparus aurata*

M. Henry[1*], A. Asimaki [2], P. Psofakis [2], E. Golomazou[2], E. Fountoulaki[1], E. Mente[2], I.T. Karapanagiotidis[2]

[1]Institute of Molecular Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Anavyssos, Greece
morgane@hcmr.gr
[2]Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, University of Thessaly, Volos, Greece.

Introduction

Insects are suitable fishmeal replacers in animal feeds and seven species have recently been approved for use in European aquafeeds. Another interesting insect species, the giant mealworm, *Zophobas morio*, is a large tenebrionid beetle species with a high nutritive value (Rumbos & Athanassiou, 2021) and can successfully replace fishmeal in *Sparus aurata* diets (Asimaki et al., 2020 & 2021). In addition to a valuable protein source, insects are also being considered for their antimicrobial and immunomodulatory activities (Gasco et al., 2021). These recent insights called for the present study which examined the effects of dietary partial replacement of fishmeal with *Z. morio* on some haematological and immunological parameters of Gilthead seabream, *Sparus aurata*.

Materials and Methods

Six isonitrogenous (52%) and isoenergetic (21 Mj/Kg) diets were formulated where fishmeal protein of the control diet (FM) was replaced by full-fat *Z.morio* meal (39% crude lipid) at 5% (FF5) and 10% (FF10) or low-fat *Z. morio* meal (4% crude lipid) at 10% (LF10), 20% (LF20) or 30% (LF30). Diets were given for a period of 100 days to *S. aurata* juveniles kept in triplicate tanks in a RAS. At the end of the trial, 5 fish per tank, fasted and anaesthetized, were bled through the caudal vein. Heparinised blood samples were used to assess the blood profile of the fish (hematocrit, red and white blood cell counts, differential leucocytes counts). Serum samples were assessed for their antibacterial activity against the Gram-positive *Micrococcus luteus* (lysozyme, activity) and against a luminescent strain of the Gram-negative *E. coli* (complement activity) and for their anti-protease activity, nitric oxide concentration, myeloperoxidase and ceruloplasmin activities.

Results and Discussion

Dietary *Zophobas morio* meal, either full-fat or partially defatted, showed significant effect on both the haematological and immunological status of juvenile seabream (Table 1 & Figure 1). Fish fed LF10 did not show any difference in haematological or immunological parameters when compared with fish fed FM control or with the equivalent full-fat insect meal (FF10). When compared to control fish, fish fed LF20 showed increased neutrophil percentages, fish fed LF30 showed increased serum NO, and fish fed FF5 & FF10 showed increased complement antibacterial activity while fish fed FF5 and LF20 showed significantly decreased trypsin inhibition.

Although dietary *Z. morio* clearly modulates both haematological and immunological status of *Sparus aurata*, there was no clear indication of the optimal form and dietary dose of the insect meal to be used in seabream feed. Further study involving disease and/or stress challenge may clarify this aspect.

(Continued on next page)

Table 1. Differential leucocyte counts as the mean percentage of total leucocytes. Data are presented as mean ± S.E.M. Different letters, within the same raw, indicate a significant difference between diets (ANOVA; P<0.05). Data for red blood cells (RBC), white blood cells (WBC) and haematocrit are not presented because they were similar (P>0.05) among the dietary groups. (n=3).

	FM	FF5	FF10	LF10	LF20	LF30
Lymphocytes	69.9±2.9	62.5±2.9	61.9±5.5	61.1±5.5	56.7±4.9	58.9±2.3
Eosinophils	2.9±0.6	3.9±1.1	2.4±0.2	3.3±0.4	2.5±0.2	2.3±0.5
Neutrophils	26.4±2.5^{a}	33.3±2.1^{ab}	34.9±5.3^{ab}	35.0±5.7^{ab}	41.5±6.6^{b}	38.3±2.8^{ab}
Monocytes	0.7±0.2^{ab}	0.3±0.1^{ab}	1.0±0.1^{b}	0.6±0.2^{ab}	0.0±0.0^{a}	0.5±0.1^{ab}

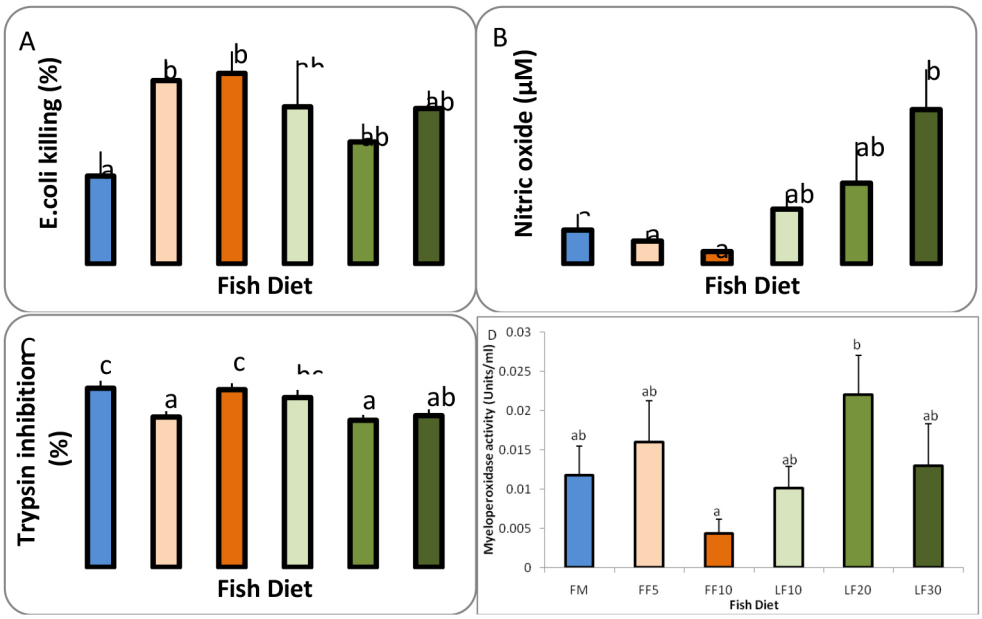


Figure 1: Complement associated bacterial killing (A), nitric oxide concentration (B), the anti-protease (C) and myeloperoxidase (D) activities in the serum of *S. aurata* fed the tested diets. Bars represent mean ± S.E.M. Different letters represent significant difference between dietary treatments. n=15.

Acknowledgements

This study is part of the project FInAl (MIS 5045804) co-financed by Greece and EU (EPAnEK - ESPA 2014-2020).

References

Asimaki, A., Psofakis, P., Neofytou, M.C., Gkalogianni, E.Z., Mente, E., Rumbos, C.I., Athanassiou, C.G., Fountoulaki, E., Henry, M., Karapanagiotidis, I.T. 2020. Effects of fishmeal replacement by defatted *Zophobas morio* larvae meal on growth and feed efficiency of gilthead seabream (*Sparus aurata*). Proc. EAS 2020: 38-39.

Asimaki A., et al., 2020. Evaluation of *Zophobas morio* larvae meal as fishmeal replacer for gilthead seabream diet. 3rd Intl Conf. Insects to Feed the World. Quebec. J. Insects as Food Feed., 6 (S1): S24.

Gasco, L., Josefiak, A., Henry, M, 2021. Beyond the protein concept: health aspects of using edible insects on animals. J. Insects as Food Feed. In press.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future.Anim. Feed Sci. Technol. 203: 1-22.

Rumbos, C.I. & Athanassiou, C.G. 2021. Insects as Food and Feed: If You Can't Beat Them, Eat Them! - To the Magnificent Seven and Beyond J. Insect Sci. 21(2):1-5.

TRANSVERSAL APPROACH IN MANAGING MULTISPECIES COMMERCIAL BREEDING PROGRAMS FOR COOKEAQUA INC

M. Herlin[1]*, J.A.K. Elliott[2], K. P. Ang[2], F. Powell[2], L. Gonzalez[1]

[1] Culmarex, C/ Don Carnal, 13, P.I. El Labradorcico 30889 Águilas, Murcia, Spain
2 Kelly Cove Salmon Ltd., Cooke Aquaculture Inc., Saint John E2L 3H3, Canada
Email: marine.herlin.absa@vculmarex.com

Cooke Aquaculture Inc. (CAI) is a vertically-integrated global aquaculture corporation based in Blacks Harbour, New Brunswick (NB), Canada. The company currently manages five breeding programs worldwide: one program on North American Atlantic salmon (*Salmo salar*) in East Canada, two programs on European seabass (*Dicenthrachus labrax*) and gilthead seabream (*Sparus aurata*) in Spain and two programs on Whiteleg shrimp (*Penaeus vannamei*) in Nicaragua. Over the past six years, the company has developed strategic synergies between its breeding programs while integrating critical technical knowledge on genomic selection. In the meantime, collaborations with both private companies and International Research Institutes prompted the rapid development of customized genomic toolboxes for North American Atlantic Salmon, European seabass and Gilthead seabream. These tools were first used, back in 2016, to select salmon broodstock candidates for both improved growth and disease resistance. Five years later, the company is seeing significant increases in the annual genetic gains obtained for salmon.

These positive results have encouraged CAI to now consider applying genomic selection in both its seabass and shrimp breeding programs. The transversal approach in managing multispecies breeding programs will enable the company to rapidly bring all its breeding programs to the same technical level while seeking economies of scale for subcontracted services such as genotyping.

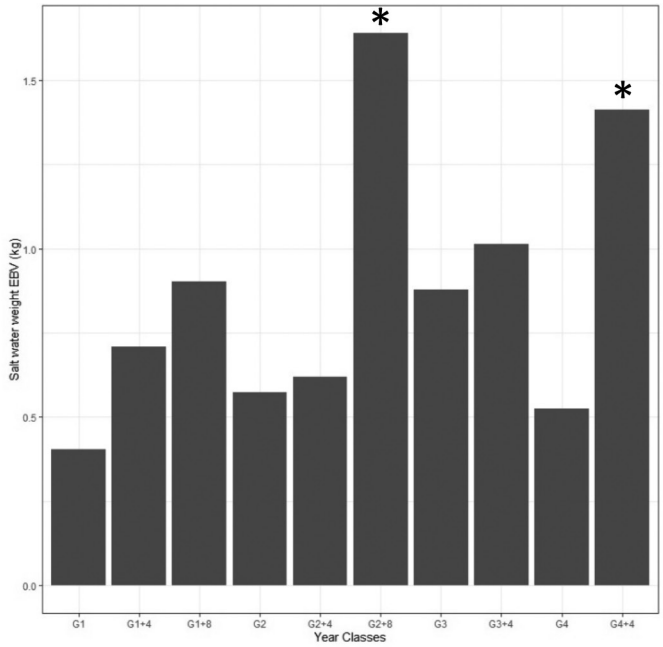


Figure 1. Evolution of historic salt water weight estimated breeding values (EBVs) in CookeAqua North American Atlantic salmon breeding program. (*) Application of genomic selection.

(Continued on next page)

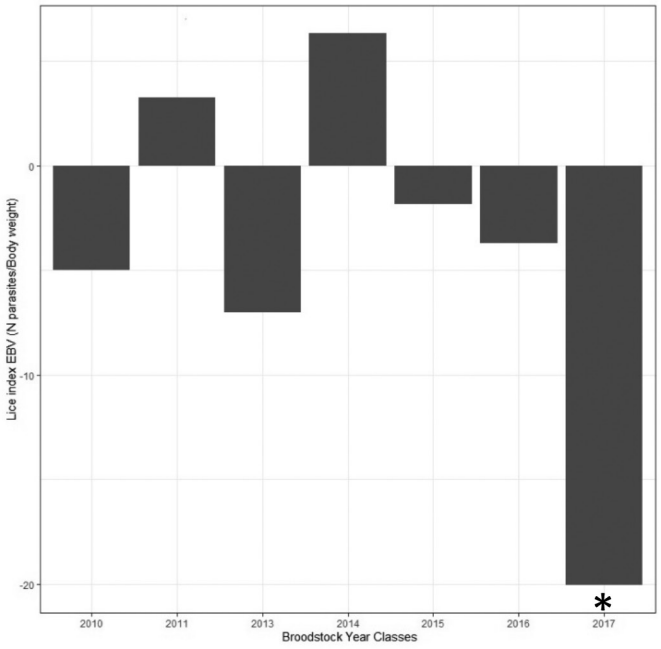


Figure 2. Evolution of sea lice index (N lice/body weight) EBVs in CookeAqua North American Atlantic salmon breeding program. (*) Application of genomic selection.

EFFECTS OF THE CONCENTRATION AND FEEDING PERIOD OF AMINO ACID SUPPLEMENTS ON FISH STRESS SYSTEM

Salamanca, N., Herves, M. A., Moreno, O., Herrera*, M.

IFAPA Centro Agua del Pino, El Rompido-Punta Umbria rd., 21459 Cartaya (Spain)
natalia.salamanca@juntadeandalucia.es.

It is known that some dietary amino acids can mitigate stress markers in fish. In this work, the effects of amino acid supplements on the stress response have been evaluated in order to determine amino acid concentration and feeding period for achieving the attenuation of stress response. Our findings indicate that the best feeding strategy (lesser stress indicator variations) was that containing 4% amino acid supplement and providing during 4 days.

Introduction

Stress is the most studied physiological process to assess the welfare state of farmed fish. For this reason, some experiments have aimed at reducing the stress response through the use of food additives, such as essential amino acids (Herrera et al., 2017). It seems that phenylalanine and tyrosine have mitigating effects on the stress system, probably due to their participation in the formation of catecholamine hormones (adrenaline and noradrenaline) (Herrera et al., 2017; Salamanca et al., 2020). Therefore, the objective of this work was to determine the optimal amount of phenylalanine (Phe) and tyrosine (Tyr) in the diet and the feeding time.

Material and methods

Seabreams with an average weight of 29.65±1.72g were stocked at 30 fish/tank in twelve 500 L flat-bottom circular tanks at 5 kg m^{-3}. The experimental treatments consisted of different types of feeding: control, Phe-enriched (5%, 7.5%, 10%), and Tyr-enriched (5%, 7.5%, 10%) food, for 2, 4 or 8 days each. Fish feed was provided through automatic feeders and the ration was adjusted to 3% tank biomass daily. At the end of the experiment, blood samples were taken from basal (non-stressed) fish, and 2 hours after stressing by air exposure (3 min). The samples were analyzed using commercial kits, measuring plasma cortisol, glucose, lactate, adrenaline and noradrenaline.

Results and discussion

Table I shows the p-values obtained after comparing the basal and the 2h post-stress states. The conditions whose stress indicators remained more stable (less significant differences) were the diet with a content of 5% and 10% of Phe for 4 days. In the case of the diets supplemented with Tyr, those containing 5% of the amino acid were for 2 days, for 4 days for all the amounts of Tyr in the diet and for 8 days those diets with the amount of 5% and 10% of Tyr.

Table II shows the values of the stress-related metabolites in those specimens fed the diet supplemented with Phe (5% and 10%). The adrenaline values presented significant differences between the specimens in basal state fed with 5% and 10% of the amino acid and between the specimens fed with both amounts of Phe but subjected to stress, noradrenaline only had differences in basal state.

Cortisol values in those specimens fed for 2 days with 5% Tyr were significantly lower than the rest, the minimum glucose was obtained after 4 days of feeding in the same way as in lactate and noradrenaline, adrenaline was obtained after 8 days of feeding (Table III).

In seabream, the values of plasma metabolites are modified with the addition of 5% of Phe and Tyr in the diet, in the same way it occurs in Salamanca et al., (2020) where 5% of amino acids in the diet modulate the metabolites plasmatic. The increase in epinephrine in basal state may be due to an accumulation of this hormone in plasma (Peter, 2011). Our results show that the best diet to mitigate stress is the one supplemented with 5% Phe or Tyr for 4 days of feeding.

(Continued on next page)

Table I. P-value obtained after performing two-way ANOVA (amino acid content – days). Significance level 0.05

			Cortisol	Glucose	Lactate	Adrenaline	Noradrealine
Phe	2 Days	5%	0,01	0,003	0	NS	0,02
		7,5%	NS	0,023	0	0	0,034
		10%	NS	0	0,002	0	0,001
	4 Days	5%	NS	NS	0	0	0
		7,5%	NS	0	0	0,04	0,021
		10%	0,029	0,027	0	NS	NS
	8 Days	5%	0,02	0	0	0	NS
		7,5%	0,014	0	0	0	0,005
		10%	0,005	0,001	0	0	0
Tyr	2 Days	5%	NS	0	0	0,008	NS
		7,5%	0,016	0	0	NS	0,009
		10%	NS	0	0	0,005	0,023
	4 Days	5%	NS	0	0	0,03	NS
		7,5%	0,016	NS	0,003	0,01	NS
		10%	0	NS	NS	0,04	NS
	8 Days	5%	NS	0,041	0	0	NS
		7,5%	0,02	0,004	0	NS	0,01
		10%	NS	0,009	0	NS	0

Table II. Cortisol, glucose, lactate, adrenaline and noradrenaline values of the specimens fed with the diet supplemented with Phe (mean ± SEM). The asterisks indicate significant differences between the different diets.

	Cortisol	Glucose	Lactate	Adrenaline	Noradrenaline
Basal 5%	0,42 ± 0,05	115,38 ± 15,65	33,86 ± 1,19	19,31 ± 16,70*	8,75 ± 6,06*
Basal 10%	0,76 ± 0,22	111,00 ± 20,71	2,77 ± 0,83	46,51 ± 27,59	19,34 ± 13,43
Stress 5%	0,84 ± 0,22	123,98 ± 20,13	20,13 ± 10,04	88,51 ± 12,66	62,69 ± 15,71
Stress 10%	3,67 ± 2,95	128,09 ± 8,80	28,67 ± 4,42	47,26 ± 5,92*	30,75 ± 2,82

Table III. Cortisol, glucose, lactate, adrenaline and noradrenaline values of the specimens fed with the diet supplemented with Tyr (mean ± SEM). Different letters indicate significant differences between the different days of feeding

	Cortisol	Glucose	Lactate	Adrenaline	Noradrenaline
2 Days	0,70 ± 0,35^{a}	120,94 ± 3,85^{a}	32,02 ± 24,78^{a}	66,25 ± 33,46^{a}	156,82 ± 69,18^{a}
4 Days	10,62 ± 2,03^{b}	90,63 ± 21,91^{b}	16,50 ± 11,25^{a}	30,97 ± 28,70^{b}	13,76 ± 8,05^{b}
8 Days	1,26 ± 0,60^{a}	121,40 ± 26,38^{a}	20,44 ± 15,08^{a}	17,41 ± 12,89^{b}	18,44 ± 8,94^{b}

Acknowledgments

The authors acknowledge to INTERREG V A Espanha Portugal (POCTEP) program for funding through project 0750_AQUA_AMBI_2_5_P and project 0240_AQUA_AMBI_5_P. N. Salamanca's pre-doc contract is cofinanced by the European Social Fund (FSE) through the call "Ayudas para contratos predoctorales para la formación de doctores 2017" from the AEI.

References

Herrera, M., Herves, M. A., Giráldez, I., Skar, K., Mogren, H., Mortensen, A., & Puvanendran, V. (2017). Effects of amino acid supplementations on metabolic and physiological parameters in Atlantic cod (*Gadus morhua*) under stress. *Fish Physiology and Biochemistry*, 43(2), 591–602.

Peter, M. S. (2011). The role of thyroid hormones in stress response of fish. *General and Comparative Endocrinology* 172(2), 198-210.

Salamanca, N., Morales, E., Ruiz-Azcona, P., & Herrera, M. (2020). Endocrine and metabolic effects of Trp-enriched diets for attenuation of chronic stress in the Senegal soles (*Solea senegalensis*). *Aquaculture*, 523 (73), 51-73.

EFFECTS OF THE CONCENTRATION AND FEEDING PERIOD OF AMINO ACID SUPPLEMENTS ON FISH STRESS SYSTEM

Salamanca, N., Herves, M. A., Moreno, O., Herrera*, M.

IFAPA Centro Agua del Pino, El Rompido-Punta Umbria rd., 21459 Cartaya (Spain)
natalia.salamanca@juntadeandalucia.es

It is known that some dietary amino acids can mitigate stress markers in fish. In this work, the effects of amino acid supplements on the stress response have been evaluated in order to determine amino acid concentration and feeding period for achieving the attenuation of stress response. Our findings indicate that the best feeding strategy (lesser stress indicator variations) was that containing 4% amino acid supplement and providing during 4 days.

Introduction

Stress is the most studied physiological process to assess the welfare state of farmed fish. For this reason, some experiments have aimed at reducing the stress response through the use of food additives, such as essential amino acids (Herrera et al., 2017). It seems that phenylalanine and tyrosine have mitigating effects on the stress system, probably due to their participation in the formation of catecholamine hormones (adrenaline and noradrenaline) (Herrera et al., 2017; Salamanca et al., 2020). Therefore, the objective of this work was to determine the optimal amount of phenylalanine (Phe) and tyrosine (Tyr) in the diet and the feeding time.

Material and methods

Seabreams with an average weight of 29.65±1.72g were stocked at 30 fish/tank in twelve 500 L flat-bottom circular tanks at 5 kg m^{-3}. The experimental treatments consisted of different types of feeding: control, Phe-enriched (5%, 7.5%, 10%), and Tyr-enriched (5%, 7.5%, 10%) food, for 2, 4 or 8 days each. Fish feed was provided through automatic feeders and the ration was adjusted to 3% tank biomass daily. At the end of the experiment, blood samples were taken from basal (non-stressed) fish, and 2 hours after stressing by air exposure (3 min). The samples were analyzed using commercial kits, measuring plasma cortisol, glucose, lactate, adrenaline and noradrenaline.

Results and discussion

Table I shows the p-values obtained after comparing the basal and the 2h post-stress states. The conditions whose stress indicators remained more stable (less significant differences) were the diet with a content of 5% and 10% of Phe for 4 days. In the case of the diets supplemented with Tyr, those containing 5% of the amino acid were for 2 days, for 4 days for all the amounts of Tyr in the diet and for 8 days those diets with the amount of 5% and 10% of Tyr.

Table II shows the values of the stress-related metabolites in those specimens fed the diet supplemented with Phe (5% and 10%). The adrenaline values presented significant differences between the specimens in basal state fed with 5% and 10% of the amino acid and between the specimens fed with both amounts of Phe but subjected to stress, noradrenaline only had differences in basal state.

Cortisol values in those specimens fed for 2 days with 5% Tyr were significantly lower than the rest, the minimum glucose was obtained after 4 days of feeding in the same way as in lactate and noradrenaline, adrenaline was obtained after 8 days of feeding (Table III).

In seabream, the values of plasma metabolites are modified with the addition of 5% of Phe and Tyr in the diet, in the same way it occurs in Salamanca et al., (2020) where 5% of amino acids in the diet modulate the metabolites plasmatic. The increase in epinephrine in basal state may be due to an accumulation of this hormone in plasma (Peter, 2011). Our results show that the best diet to mitigate stress is the one supplemented with 5% Phe or Tyr for 4 days of feeding.

(Continued on next page)

Table I. P-value obtained after performing two-way ANOVA (amino acid content – days). Significance level 0.05

			Cortisol	Glucose	Lactate	Adrenaline	Noradrealine
Phe	2 Days	5%	0,01	0,003	0	NS	0,02
		7,5%	NS	0,023	0	0	0,034
		10%	NS	0	0,002	0	0,001
	4 Days	5%	NS	NS	0	0	0
		7,5%	NS	0	0	0,04	0,021
		10%	0,029	0,027	0	NS	NS
	8 Days	5%	0,02	0	0	0	NS
		7,5%	0,014	0	0	0	0,005
		10%	0,005	0,001	0	0	0
Tyr	2 Days	5%	NS	0	0	0,008	NS
		7,5%	0,016	0	0	NS	0,009
		10%	NS	0	0	0,005	0,023
	4 Days	5%	NS	0	0	0,03	NS
		7,5%	0,016	NS	0,003	0,01	NS
		10%	0	NS	NS	0,04	NS
	8 Days	5%	NS	0,041	0	0	NS
		7,5%	0,02	0,004	0	NS	0,01
		10%	NS	0,009	0	NS	0

Table II. Cortisol, glucose, lactate, adrenaline and noradrenaline values of the specimens fed with the diet supplemented with Phe (mean ± SEM). The asterisks indicate significant differences between the different diets.

	Cortisol	Glucose	Lactate	Adrenaline	Noradrenaline
Basal 5%	0,42 ± 0,05	115,38 ± 15,65	33,86 ± 1,19	19,31 ± 16,70*	8,75 ± 6,06*
Basal 10%	0,76 ± 0,22	111,00 ± 20,71	2,77 ± 0,83	46,51 ± 27,59	19,34 ± 13,43
Stress 5%	0,84 ± 0,22	123,98 ± 20,13	20,13 ± 10,04	88,51 ± 12,66	62,69 ± 15,71
Stress 10%	3,67 ± 2,95	128,09 ± 8,80	28,67 ± 4,42	47,26 ± 5,92*	30,75 ± 2,82

Table III. Cortisol, glucose, lactate, adrenaline and noradrenaline values of the specimens fed with the diet supplemented with Tyr (mean ± SEM). Different letters indicate significant differences between the different days of feeding

	Cortisol	Glucose	Lactate	Adrenaline	Noradrenaline
2 Days	$0,70 \pm 0,35^{a}$	$120,94 \pm 3,85^{a}$	$32,02 \pm 24,78^{a}$	$66,25 \pm 33,46^{a}$	$156,82 \pm 69,18^{a}$
4 Days	$10,62 \pm 2,03^{b}$	$90,63 \pm 21,91^{b}$	$16,50 \pm 11,25^{a}$	$30,97 \pm 28,70^{b}$	$13,76 \pm 8,05^{b}$
8 Days	$1,26 \pm 0,60^{a}$	$121,40 \pm 26,38^{a}$	$20,44 \pm 15,08^{a}$	$17,41 \pm 12,89^{b}$	$18,44 \pm 8,94^{b}$

Acknowledgments

The authors acknowledge to INTERREG V A Espanha Portugal (POCTEP) program for funding through project 0750_AQUA_AMBI_2_5_P and project 0240_AQUA_AMBI_5_P. N. Salamanca's pre-doc contract is cofinanced by the European Social Fund (FSE) through the call "Ayudas para contratos predoctorales para la formación de doctores 2017" from the AEI.

References

Herrera, M., Herves, M. A., Giráldez, I., Skar, K., Mogren, H., Mortensen, A., & Puvanendran, V. (2017). Effects of amino acid supplementations on metabolic and physiological parameters in Atlantic cod (*Gadus morhua*) under stress. *Fish Physiology and Biochemistry*, 43(2), 591–602.

Peter, M. S. (2011). The role of thyroid hormones in stress response of fish. *General and Comparative Endocrinology* 172(2), 198-210.

Salamanca, N., Morales, E., Ruiz-Azcona, P., & Herrera, M. (2020). Endocrine and metabolic effects of Trp-enriched diets for attenuation of chronic stress in the Senegal soles (*Solea senegalensis*). *Aquaculture*, 523 (73), 51-73.

IMPROVING THE CONDITIONS OF LABOR AND HUMAN RIGHTS IN THE FEED INGREDIENTS FOR AQUACULTURE INDUSTRY

Hidalgo M[1], Short K[2]

1Onboard Social Accountability, Utrecht, The Netherlands
[2] Onboard Social Accountability, Wellington, New Zealand

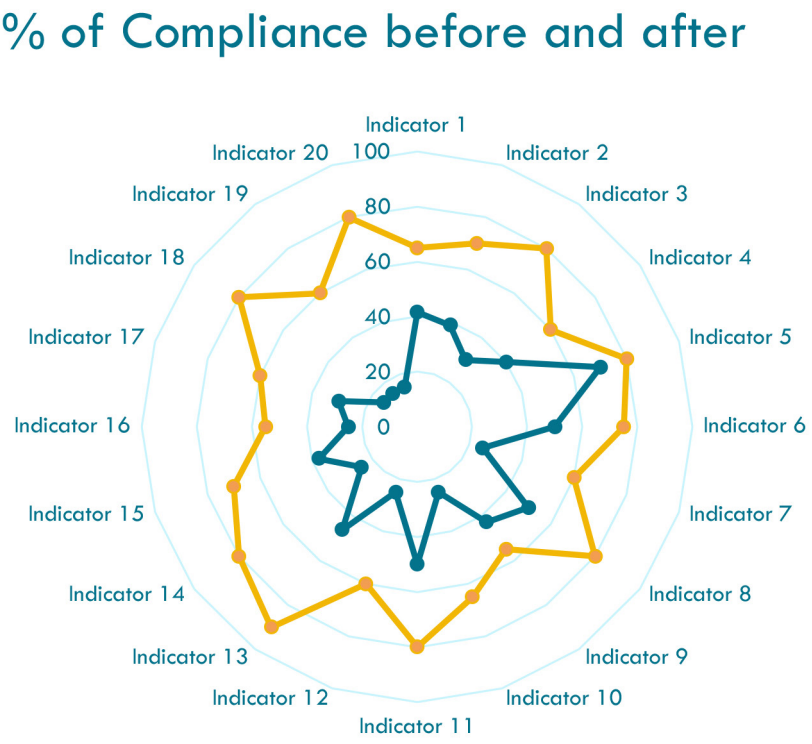
Onboard Social Accountability, Newtonlaan 115, ZEN Building, Utrecht, 3584 BH, Netherlands
Email: marcelo@osainternational.global

Introduction

Globally, awareness concerning poor working conditions and labour abuses on the Aquaculture Industry has significantly in the last decade. The various parts of the responsible global aquaculture system are responding with agreements, regulations, tools, frameworks, certifications and policies. The beginnings of standards to link labour on board fishing operations of marine ingredients for aquaculture are also emerging. However, a globally agreed Standard is not yet in place given the various jurisdictional, legal and operational technical challenges.

Through growing consumers and market expectations, the global communities' zero tolerance for onboard labour abuses poses significant brand and reputational risk. Key markets are demanding independent proof that seafood products are free from labour abuse and their traceability. For example, in August 2018 the Marine Stewardship Council introduced new requirements to provide transparency for labour practices at sea. All MSC certified fisheries now report publicly on the measures they are taking to address forced and child labour. In June 2021, the Aquaculture Stewardship Council launched the ASC feed standard that also include requirements for human rights and labour onboard fishing vessel

Crew members on board are also key actors to key the health of the Ocean, a tailored approach to assess the need of each fishing operation is needed to ensure that Marine Ingredients are coming from a fishery where there is not slavery, forced labour, child labour and human rights are respected.



(Continued on next page)

Methods and Materials

A unique social fingerprint tool for assessing the treatment and conditions of labor onboard fishing vessels has been developed that takes into account the major international frameworks (FFA FFC106, ILOC188, NGO criteria, Worker Voice Indicators, AENOR 195006, Responsible Fishing Scheme, the Geneva Declaration on Human Rights at Sea, SA8000, UNCLOS, UN FAO Code of Conduct for Responsible Fisheries, Aquaculture Stewardship Council, BAP, Global Gap, and BSCI).

Leading seafood sector companies have completed social labor assessments using this tool, and are developing management systems and policies and procedures to improve the conditions for labor on board.

This assessment and fingerprint tool has been trialed and developed for first time in a Tuna purse seiner fleet in Papua New Guinea in 2014 and the results were presented in 2015 at the Pacific Tuna Forum in Fiji; later in a Marine Stewardship Council certified Patagonian Toothfish fishing company (2016-2018) and further projects are underway with fishing companies operating in the Marine Stewardship Council certified Western Central Pacific tuna fishery, also the tool is able to assess fleet fishing pelagic fish for instance in Senegal and Mauritania. Building the capability within the seafood sector to manage this issue is critical.

A risk analysis, a desktop, audits on board at the port and interviews (30 crewmembers per vessel) to three toothfish multi-purpose fishing vessels and six tuna seiners fishing in the Indic, Arctic and the Pacific Ocean as well as carriers transporting frozen fish from the Western Central Pacific Ocean to Thailand, Vietnam and Philippines were assessed

Results and Conclusions

Several examples of the use of the tool, the results of the assessments in fleet fishing marine ingredients for Aquaculture, and of how capability is being built will be presented. This includes 20 criteria based on more than 150 indicators

FUNCTIONAL DIETS BASED ON ALGAE BIOMASS CAN IMPROVE GUT HEALTH IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

M. Hinzmann[1*], D. Peixoto[1,2], L. Ramos-Pinto[1], C. Marques[4], F. Soares[4], M. Barata[4], P. Pousão-Ferreira[4], J. Silva[6], H. Abreu[7], J. Dias[3], L. Conceição[3], A.T. Gonçalves[3,5], B. Costas[1,2]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal
[2] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[3] SPAROS Lda, Área Empresarial de Marim, Lote C, Olhão, Portugal
[4] IPMA - Instituto Português do Mar e da Atmosfera/EPPO – Estação Piloto de Piscicultura de Olhão; Av. Parque Natural da Ria Formosa, Olhão, Portugal
[5] GREENCOLAB- Associação Oceano Verde, Campus de Gambelas, Faro, Portugal
[6] ALLMICROALGAE - Natural Products, SA, Pataias, Portugal
[7] ALGA+ - AlgaPlus Lda, Ílhavo, Aveiro, Portugal
*E-mail: hinz@ciimar.up.pt

Introduction

Gilthead seabream (*Sparus aurata*) is a crucial species for Mediterranean countries' aquaculture. Seabream, like most farmed fish, faces stress and pathogens during its life, linked to the rearing conditions. One of the strategies to ensure animal welfare, is the diet, crucial to improve animal's performance and immune status. Special feeds in which selected ingredients are added, that trigger the immune system are important in certain periods of fish farming (e.g. high densities, grading, reproduction, transfer, transportation, vaccination), so their resilience against pathogens or other stressors is enhanced, diminishing the impact of disease outbreaks, mortality, and overuse of chemicals to restore the stock. Macroalgae and microalgae have been included in diets for farmed aquatic animals as sources of bioactive compounds, since they possess a high quantity of antioxidant molecules, and present high-quality protein content, promoting optimal animal growth, and overall health. In fish, like in most animals, the status of health of an animal is deeply connected with the health of its gastrointestinal tract, the gut can be used as a target tissue to evaluate the immune status of animals, as a mirror of overall health. In the present work, the synergistic effects of including a blend of *Gracillaria* sp. and *Nannochloropsis* sp. were tested on immune and oxidative stress status in the gut, and overall performance of gilthead seabream juveniles.

Materials and methods

Gilthead seabream juveniles with an initial body weight of around 1g, were randomly distributed over 12 fiberglass circular tanks (300L) in a flow-through seawater system with daily monitorization of abiotic parameters (salinity, temperature, and oxygen saturation) at IPMA/EPPO facilities (Olhão). Tanks were randomly attributed one of the four isonitrogenous and isolipidic diets to be tested in triplicate: CTRL1 (a control commercial-type formula), CTRL2 (an improved formula with no algae biomass), BLEND3 (a formula with 3% algae biomass inclusion: 1.5% *Nannochloropsis* sp.+ 1.5 % *Gracillaria* sp.) and BLEND6 (a formula with 6% algae biomass inclusion: 3% *Nannochloropsis* sp.+ 3 % *Gracillaria* sp.). Microalgae biomass was obtained from Allmicroalgae whereas macroalgae biomass was produced by Algaplus. All diets were formulated and manufactured by SPAROS. The experimental diets were supplied *ad libitum* to the fish through 4 daily meals, for 8 weeks. Sampling took place after 4 weeks (early sampling) and at the end of week 8 (final sampling). At each sampling time, fish were anesthetized with phenoxyethanol, growth was evaluated by weight, gut tissue was collected to evaluate immune and antioxidant response. Samples were stored at -80ºC for immune and biomarkers assays, and samples for gene expression were kept in RNAlater at -20ºC.

Homogenates of the gut were prepared to assay oxidative stress biomarkers (i.e. reduced glutathione: oxidized glutathione ratio, catalase, lipid peroxidation, glutathione S-transferase) and immune (immunoglobulins, and peroxidase) related parameters. A fragment of the anterior intestine was used for gene expression analysis (i.e. *il1β*, *il10*, *il34*, *hep*, *hspt70*, *igm*, *csfr1*, *tnfα*, *sod* (Mn), *gsp2*, *cd8α*, *muc*, *vim*, *occl*, and *tub*), *18s* worked as reference gene. Data analysis was performed using SPSS statistics 26 (IBM).

Results and discussion

Survival and relative growth rate were not altered by the dietary treatments, all fish developed well, presenting similar output to commercial and improved formula.

Oxidative stress biomarkers at early sampling showed no significant differences, only a slight increase, mainly in fish fed the BLEND6 diet. At final sampling, a significant reduction of total glutathione was detected, in all diets in comparison to CTRL1, more evident in BLEND6 treatment.

(Continued on next page)

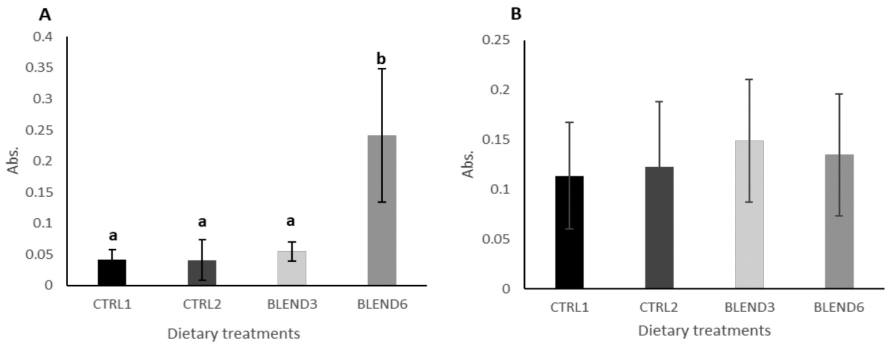


Fig.1 – Quantification of IgM levels on the gut of *Sparus aurata* juveniles fed dietary treatments: A – early sampling, B- final sampling, OD 450nm. Values are presented as means ± SD (n = 12). P-values from ANOVA one way, followed by Tukey's post-hoc test ($p \leq 0.05$). Different lowercase letters stand for significant differences among treatments.

The gut immune status recorded an increase in immunoglobulins (IgM) after 4 weeks in the diets with algae inclusion, mainly the BLEND6 (Figure 1). No significant changes were observed in total peroxidase levels. The mRNA expression levels of immune-related genes suggested that no inflammatory response was triggered. Most genes did not differ significantly between treatments; however, the BLEND3 diet promoted an increase of expression of intestinal mucins, linked to gut permeability.

Conclusion

Data from this study support the already consensus idea that algae have enormous potential as functional ingredients in fish diets. Small percentages of inclusion of microalgae and macroalgae can be crucial in terms of antioxidant capacity and growth promotion, which translates into good welfare of farmed fish. Overall, gilthead seabream juveniles fed diets supplemented with algae blends presented normal ranges for growth performance and survival. Gut oxidative stress biomarkers and immune parameters revealed that the blends improved the antioxidant system and have an immunomodulatory effect. *Nannochloropsis* sp. is known to have an immunostimulant effect, improving the innate defense mechanisms in the fish, while *Gracillaria* sp. may work as a prophylactic agent against chemotherapeutics compounds and also as an immune enhancer. According to these results, these algae biomasses combined seem to be promising candidates for inclusion in diets for gilthead seabream juveniles, mainly for stressful periods.

Acknowledgments

This work was supported by VALORMAR project (POCI-01-0247-FEDER-024517) funded by Compete 2020, Lisboa 2020, Algarve 2020, Portugal 2020, and the European Union through ERDF. BC was supported by FCT - Foundation for Science and Technology (IF/00197/2015).

POLYETHYLENE MICROPARTICLES MAY CAUSE THE DISRUPTION OF THE RAINBOW TROUT'S IMMUNE SYSTEM

Hodkovicova Nikola[1*], Hollerova Aneta[1,2], Faldyna Martin[1], Svobodova Zdenka[2]

[1]Veterinary Research Institute, Department of Infectious Diseases and Preventive Medicine, Brno, Czech Republic
* hodkovicova@vri.cz
[2]University of Veterinary Sciences Brno, Department of Animal Protection and Welfare & Veterinary Public Health, Brno, Czech Republic

Introduction

Despite efforts to reduce the need for plastics, the worldwide production and use of plastic products is enormous. In addition to their ability to mechanically damage the digestive system of aquatic invertebrates and vertebrates, another major risk of plastic particles is their capability to disintegrate into smaller particles, so-called microparticles, which are commonly defined as particles <5 mm in size. Some authors have already described the ability of these microparticles to further disintegrate in the digestive tract and penetrate the tissues of the various organisms. One of the main tasks of our study was whether plastic particles in microsizes can cause inflammatory changes in the body organs and possible disruption of the immune system.

Materials and Methods

We performed a test in accordance with OECD 215 methodology (Fish Juvenile Growth Test) in which polyethylene (PE) particles (size 40–48 μm) were incorporated into rainbow trout's commercial feed pellets. A total of 80 individuals were divided into control and three experimental groups fed by 0.5%, 2% and 5% PE concentration from their feed ratio / day. The experiment lasted six weeks and ended by taking the biometric data, blood and samples of tissues for subsequent laboratory analyses. The aim was to evaluate the changes in haematological and biochemical profile, changes in markers of oxidative stress and selected genes via their mRNA expression and to support obtained result with histological and electron microscopy images. All methodological and statistical procedures of samples and data processing are referred in detail by Hodkovicova et al. (2021).

Results and discussion

One of the most interesting findings of this experiment was the presence of numerous skin lesions on the body surface of exposed groups. Disruption of the skin barrier can have a serious impact on an individual's innate immunity. These findings were also confirmed by histological images in which an increased presence of mucinous cells was observed in response to innate-immunity stimulation. Oral intake of the PE particles also disrupted the intestinal mucosa, on which visible cracks and an increased amount of mucus were detected by electron microscopy in an attempt to prevent mechanical damage to the intestine integrity. However, the most affected organs by PE were gills, liver and kidneys, i.e. organs important for metabolic and detoxification processes.

Inflammatory lesions and extensive structure alterations were observed in gills of all PE exposed groups; this could negatively affect oxygen exchange with the external environment. In liver, wide hepatodystrophies were observed histologically for all PE groups and the pro-inflammatory cytokines, tumour necrosis factor alpha (*tnfa*) and interleukin 8 (*il8*), had increased mRNA amounts in 2% PE. Same cytokines were also increased in cranial kidneys (5% PE) together with the multiple pigment deposits and hyalinosis of tubules observed histologically in all PE exposed groups. Hyaline droplets are considered to be a marker of degeneration process, tubular necrosis, functional disorders or even neoplasia. Both *tnfa* and *il8* are related to a positive immune response that suggest that the diet supplementation with PE may cause tissue inflammation. Moreover, pathological processes found in gills, liver and kidney together resulted in reactive oxygen species release which was confirmed by the significantly increased antioxidant capacity of plasma in 2% PE indicated by the ferric reducing ability of plasma, i.e. FRAP marker.

A leukocyte amount decreased with the increasing PE concentration which could be a result of leukocyte migration to the site of the inflammatory process. This was supported also by increased mRNA amounts of *tnfa* and *il8* in organs which are responsible, among other, for regulation of leukocyte migration and phagocytosis. Given all the results obtained during experiment, authors can state that there was a significant disruption of the immune system caused by PE, especially in higher tested concentrations.

(Continued on next page)

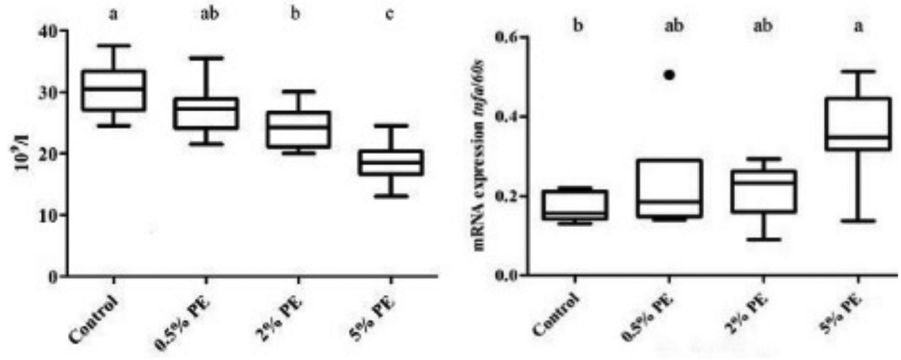


Figure 1. The decrease of total leukocyte count in plasma (left) and the increase of mRNA amount of *tnfa* in cranial kidneys (right) after oral exposure to polyethylene (PE) microparticles: 0.5%, 2% and 5%. Data are given as mean ± SD (n = 8); significance is stated as means with different letters of superscript (a, b).

Acknowledgments

Authors would like to thank the ERDF/ESF "Profish" [*no. CZ.02.1.01/0.0/0.0/16_019/0000869*] for supporting this research.

References

Hodkovicova, N., Hollerova, A., Caloudova, H., Blahova, J., Franc, A., Garajova, M., Lenz, J., Tichy, F., Faldyna, M., Kulich, P., Mares, J., Machat, R., Enevova, V., Svobodova, Z., 2021. Do foodborne polyethylene microparticles affect the health of rainbow trout (*Oncorhynchus mykiss*)? Science of the Total Environment 793:148490.

PACKAGING SOLUTIONS FROM LAND-BASED MACROALGAE AQUACULTURE

L.C. Hofmann[1]*, I. Cardoso[1], J. Henjes[1], I. Bartsch[1], M. Heins[2], R. Bosse[3], L. Klusmann[3], F. Reimold[3], I. Enders[4], W. D. Hoffmann[4], B. H. Buck[1]

[1]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[2] Roval GmbH Hauptstraße 26, 27404 Rockstedt, Germany
[3] University of Applied Sciences, An der Karlstadt 8, 27568 Bremerhaven, Germany
[4]NORDSEE GmbH, Herwigstraße 16, 27572 Bremerhaven, Germany
Email: laurie.c.hofmann@awi.de

Introduction

The use of single-use packaging materials has increased dramatically in recent decades in parallel with increasing trends in convenience and fast-food. Most of these packaging materials are made of non-biodegradable, petroleum-based polymers that have degradative impacts on the environment and contribute to the global plastic pollution crisis. Finding alternative packaging materials is an important step towards building a bio-based circular economy. Sustainable land-based macroalgae cultivation can provide a solution, as it eliminates land-use pressure on coastal areas, doesn't interfere with recreational activities or agriculture, reduces seasonal limitations, allows for complete control over product quality, and ensures consistent quality and traceability. Here, we present the success story of land-based macroalgae production for sustainable packaging solutions in the food industry via the Mak-Pak and Mak-Pak Scale-Up projects.

Materials and Methods

An initial screening of local macroalgae species was conducted based on detailed knowledge of growth rates, seasonality, geographic range, edibility, iodine content, biochemical properties, bioactivity, robustness and ease of cultivation. Different combinations of selected macroalgae were tested to develop a biodegradable, edible packaging prototype that was rated by consumer tests. In a follow-up project, we are focusing on eliminating the biggest bottleneck: scaling-up biomass production. We have partnered with a local, innovative farmer to sustainably scale-up and optimize biomass production for our sustainable, biodegradable macroalgae-based packaging material for the food industry.

Results

Several species of suitable macroalgae were selected based on the screening protocol and a method for using different combinations of selected species is described in a patent application for the packaging prototype. The packaging prototype was positively reviewed in consumer tests, where the consumers were pleasantly surprised by the neutral taste and smell. We could also show that certain components of the macroalgae that are important for packaging functionality (e.g. antioxidant activity) could be optimized during land-based production in artificial seawater. Currently we are in the early stages of scaling-up production and selecting strains to optimize growth rates and robustness, where we can complete the life cycle of one selected species from single cells to mature gametophytes within 6 weeks. With controlled induction of reproduction, we can continually provide material for transplantation to large-scale systems.

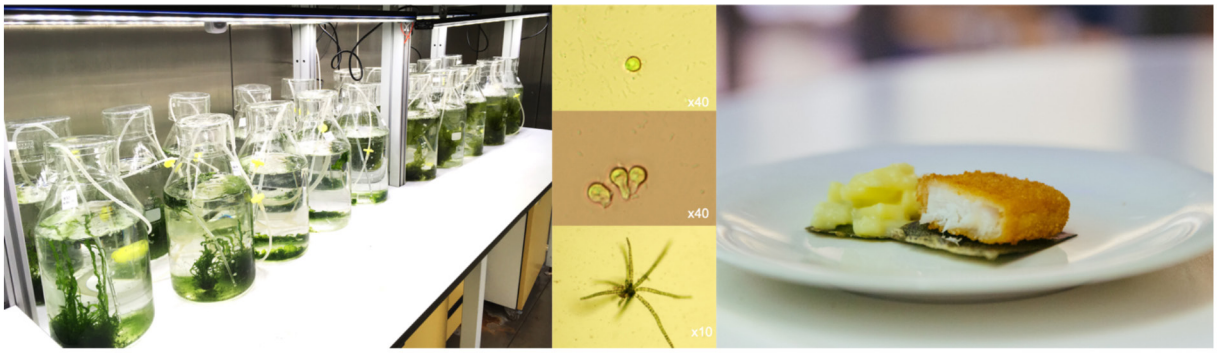


Figure 1 Macroalgal cultures in lab-scale (left), development of macroalgae from single cells to multicellular germlings (center), and the macroalgae-based packaging prototype (right). Photo credits from left to right: Laurie C. Hofmann/AWI, Isabel Cardoso/AWI, Ramona Bosse/Hochschule Bremerhaven.

(Continued on next page)

Discussion

The Mak-Pak and Mak-Pak Scale-Up projects have been featured in numerous news articles, exhibitions, and podcasts throughout Germany, Europe and even New Zealand. Our experience has shown that there is a lot of public interest in macroalgae-based packaging solutions. Consumers have become aware of the plastic pollution crisis and are open to alternatives to plastic packaging. Consequently, we have recently seen rapid changes in packaging trends in the cosmetic and food industries. Here we show that it is possible to produce a biodegradable, edible packaging from macroalgae biomass for the food-industry. Not only is this a success story for sustainable aquaculture, but also for macroalgae cultivation in general. This project has increased public awareness of macroalgae and contributed to a dialogue about the diversity of products and services that macroalgae can provide as we strive towards a sustainable, circular economy. However, optimization of the raw material production as well as the packaging itself is still underway. Furthermore, limitations in the food-industry require that our raw material meets high quality standards. In other industries where the quality of the raw material is not a limiting factor, there is enormous potential for macroalgae-based packaging solutions.

THE EFFECT OF ORAL APPLICATION OF POLYSTYRENE MICROPARTICLES ON RAINBOW TROUT'S (*Oncorhynchus mykiss*) HEALTH PARAMETERS

A. Hollerova[1,2]*, N. Hodkovicova[2], J. Blahova[1], M. Faldyna[2], T. Novotna[1] & Z. Svobodova[1]

[1]Department of Animal Protection and Welfare & Veterinary Public Health, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, 612 42 Brno, Czech Republic
[2]Department of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, 621 00 Brno, Czech Republic

Email: hollerova@vri.cz

Introduction

Plastic pollution is a global problem caused by an excessive use of this material, its high resistance in the environment and poor waste management. In the environment, larger-sized plastic particles may be transformed into smaller particles, so-called microplastics (< 5 mm), which have been discovered in the bodies of various organisms, across all trophic levels of the aquatic environment. The oral intake of plastic pollutants may cause mechanical damage of digestive tract; moreover, smaller particles are able to permeate the intestinal wall and transport to other tissues and organs (Provencher et al., 2017).

Materials and methods

The experiment was performed in accordance with Fish Juvenile Growth Test (OECD 215). The polystyrene microparticles (52.5 ± 11.5 μm) were incorporated into commercial feed pellets of experimental groups and their effects were compared to the control group (without microplastics). A total of 128 rainbow trout individuals were tested, divided into control and three experimental groups as follows – polystyrene microplastics in concentration of 0.5%, 2% and 5% of daily feed intake. The whole experiment lasted 8 weeks including 14-day long period of acclimatization and 6 weeks of experimental phase. At the end of the experiment, all fish were stunned with a blow to the head and killed by spinal transection. During the autopsy, samples of the liver tissue and gills (n = 8 per one concentration) were taken for the real-time quantitative polymerase chain reaction (qRT-PCR). The methodology of the qRT-PCR was adopted according to Hodkovicova et al. (2021) likewise as the primer sequences of genes of interest – interleukin 1β (*il1β*) and 8 (*il8*), thyroid hormone receptor α (*thrα*), tumour necrosis factor α (*tnfα*) and reference gene (*60S*). The difference was considered as statistically significant and highly significant if $p < 0.05$ and $p < 0.01$. Box plot graphs were constructed using median with whiskers of maximum 1.5 interquartile range and outliers denoted as points.

Results and discussion

During the experiment lasting, the expression of genes *il8* ($p < 0.01$), *thrα* and *tnfα* ($p < 0.05$) increased in the group exposed to the highest PS concentration (5%) in the liver tissue. In gills, the expression of *il1β* ($p < 0.05$) increased as well in 5% PS. The results are expressed in Figure 1.

The results of our experiment showed that PS microparticles may negatively affect the immune system of the rainbow trout. The increased expression of the pro-inflammatory cytokines, *il1β*, *il8* and *tnfα*, may cause some severe chronic diseases and could lead to changes in leukocyte migration ability, altered neutrophil activation and phagocytosis. Increase in *thrα* expression could negatively affect cell proliferation and differentiation. Altered function on thyroid hormones could result in angiogenesis and proliferation on cancer cells (Gionfra et al., 2019). The inflammation discovered in liver and gills by gene expression analysis will be further verified by histopathological examination. As the liver and gills are main detoxifying, metabolic and osmotic organs, their prolonged weakening can cause severe homeostatic and immunity changes with consequent susceptibility to infectious diseases and increased fish mortality (Hodkovicova et al., 2021).

(Continued on next page)

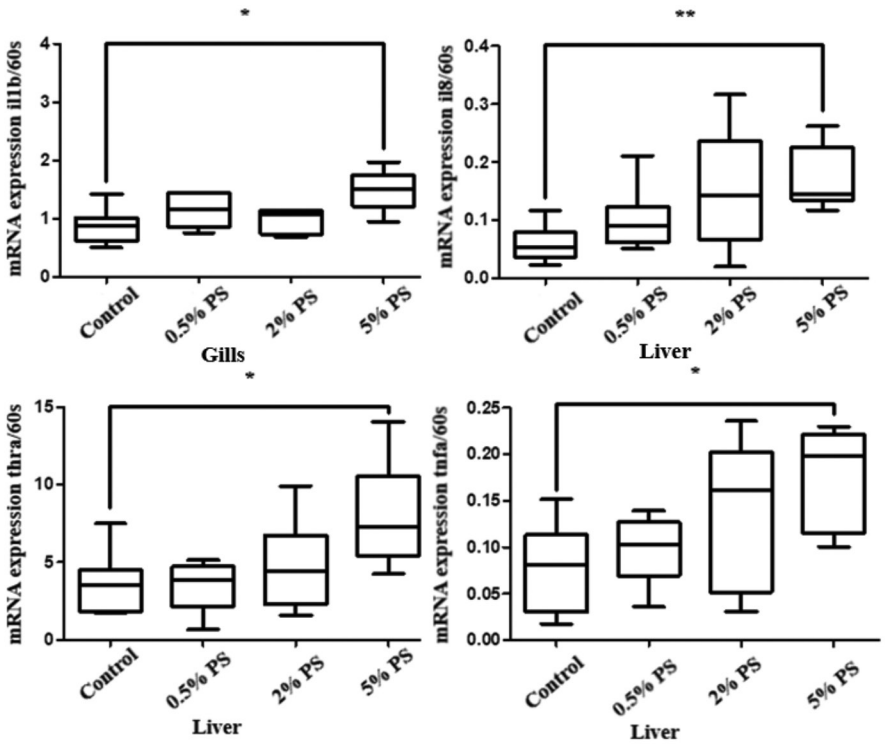


Figure 1. The mRNA expression of *il8*, *thrα*, *tnfα* in liver and *ilb* in gills of rainbow trout

Acknowledgments

This research was supported by the ERDF/ESF "Profish" [*no. CZ.02.1.01/0.0/0.0/16_019/0000869*].

References

Gionfra, F., De Vito, P., Pallottini, V., Lin, H.-Y., Davis, P.J., Pedersen, J.Z., Incerpi, S. (2019). The role of thyroid hormones in hepatocyte proliferation and liver cancer. Frontiers in Endocrinology 10, 532.

Hodkovicova, N., Hollerova, A., Caloudova, H., Blahova, J., Franc, A., Garajova, M., Lenz, J., Tichy, F., Faldyna, M., Kulich, P., Mares, J., Machat, R., Enevova, V., Svobodova, Z. (2021). Do foodborne polyethylene microparticles affect the health of rainbow trout (*Oncorhynchus mykiss*)? Science of the Total Environment 793, 148490.

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Rebolledo, E.L.B., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A. van Franeker, J.A. (2017). Quantifying ingested debris in marine megafauna: a review and reccomendations for standardization. Analytical Methods 9, 1454–1469.

DOES SPERM CONCENTRATION MATTER IN CARP SPERM CRYOPRESERVATION?

Á. Horváth*, B. Pataki, G. Mészáros, Z. Marinović, N. Kitanović, B. Urbányi

Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Hungary
E-mail: Horvath.Akos@uni-mate.hu

Introduction
Sperm cryopreservation has been recognized as an important tool in preservation of genetic resources in aquaculture as well as species conservation. Prior to freezing, sperm is typically diluted with an extender containing the cryoprotectants. In aquatic species dilution is generally done to a given ratio (e.g. 10×), while the importance of dilution to a given sperm concentration has been emphasized in several studies (Dong et al., 2007; Nynca et al., 2017; Judycka et al., 2018). Sperm concentration can be determined using several techniques: hemocytometers, absorbance measurement, computer-assisted sperm analysis (CASA) systems.

In this study we investigated whether absorbance or CASA systems give a better prediction of sperm concentration as opposed to cell counting in a hemocytometer and if standardization of sperm concentration has an effect on post-thaw motility and fertilizing capacity of common carp (*Cyprinus carpio*) sperm.

Materials and methods
Carp sperm was collected following hormonal stimulation of spermiation. Sperm concentration was determined at 1000× dilution using a Bürker-Türk type hemocytometer or using an AndroVision CASA system at 100× dilution. Absorbance of the same samples was determined either with a cuvette or a plate spectrophotometer at 100× dilution. Linear regression was used to determine the relationship of sperm concentration calculated following counting in a hemocytometer to that calculated by the CASA system as well as the absorbance measured by the two types of spectrophotometer.

Carp sperm was cryopreserved according to the protocol developed previously for that of the grayling (Horváth et al., 2012). Prior to freezing, sperm was diluted to the final concentration of 0.5; 1; 2; 4×10^9 spermatozoa per ml as well as in a 10× ratio. Following thawing, sperm motility parameters were determined using CASA. Fertilization tests were carried out using cryopreserved sperm and the fertilization percentage of eggs was determined.

Results
A significant negative linear relationship was detected between absorbance measured with cuvette spectrophotometer and sperm concentration (p=0.0002, r^2=0.7072, y=-3.416 $\times 10^{11} x - 6.658 \times 10^9$). A significant positive linear relationship was observed between absorbance measured in the plate spectrophotometer and sperm concentration (p=0.0022, r^2=0.7602, y=1.363×10^{11}x+1.576×10^9). The concentration values measured with CASA showed a significant linear relationship (p<0.0001, r^2=0.8559, y=0.7317x+8.555×10^8) with sperm concentration counted in a hemocytometer.

No significant main effect of sperm concentration was found on any of the parameters measured by CASA with the exception of LIN (p = 0.0112) where the post-hoc test found a significant difference (p = 0.0056) between linearity value for the sperm concentration of 0.5×10^9 spermatozoa mL^{-1} (0.86±0.03) and that for the 10× dilution ratio (0.74±0.08). A significant main effect (p = 0.0156) of cell concentration on the fertilizing capacity of cryopreserved common carp sperm was found with a significant difference (p = 0.0121) between the fertilization percentage of batches fertilized with sperm frozen at a cell concentration of 4×10^9 spermatozoa mL^{-1} (66 ± 6%) and the positive control (sperm diluted at a 10× ratio, 49 ± 5%).

Discussion
Results of this study demonstrated that in case of common carp sperm, CASA systems provide a reliable alternative for the determination of sperm concentration to the slow and cumbersome counting of sperm in a hemocytometer. Spectrophotometry was found also to be a good predictor of sperm concentration when CASA systems are not available. Sperm concentration, however, had little influence on the post-thaw motility an fertilizing capacity of carp sperm.

(Continued on next page)

Acknowledgements

This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12), the EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union and the European Social Fund as well as the NKFIH K129127 project.

References

Dong, Q., Huang, C., Tiersch, T.R., 2007. Control of sperm concentration is necessary for standardization of sperm cryopreservation in aquatic species: Evidence from sperm agglutination in oysters. Cryobiology 54, 87–98.

Horváth, Á., Jesenšek, D., Csorbai, B., Bokor, Z., Raboczki, É., Kaczkó, D., Bernáth, G., Hoitsy, G., Urbányi, B., Bajec, S.S., Snoj, A., 2012. Application of sperm cryopreservation to hatchery practice and species conservation: A case of the Adriatic grayling (*Thymallus thymallus*). Aquaculture 358–359, 213–215.

Judycka, S., Nynca, J., Liszewska, E., Dobosz, S., Grudniewska, J., Ciereszko, A., 2018. Optimal sperm concentration in straws and final glucose concentration in extender are crucial for improving the cryopreservation protocol of salmonid spermatozoa. Aquaculture 486, 90–97.

Nynca, J., Judycka, S., Liszewska, E., Dobosz, S., Ciereszko, A., 2017. Standardization of spermatozoa concentration for cryopreservation of rainbow trout semen using a glucose-methanol extender. Aquaculture 477, 23–27.

THE PHENOTYPIC AND IMMUNE RESPONSE IMPACT OF ACUTE THERMAL SHOCKS ON DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar*) EYED EMBRYOS

C. Howard*, J. Taylor, M. Bekaert, B. Craig, M. Mommens, M. Medina, H. Migaud

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
E-mail: callum.howard@stir.ac.uk

Introduction

The only currently accepted methods of producing sterile salmon for human consumption in Europe is triploidy (Benfey, 2016). Whilst research into the use of triploid salmon in aquaculture extends as far back as the mid-1980's (Benfey & Sutterlin, 1984), triploids are yet to be accepted as a viable alternative to diploids. Much of this stems from the fact that triploids are historically believed to be inferior to diploids (Leclercq et al., 2011; Benfey, 2016), however new knowledge on triploid biology has led to improved husbandry and nutrition and subsequently performance of triploid stocks (Fraser et al., 2013; Taylor et al., 2013). Triploids require lower egg incubation temperatures than diploids for optimal bone health (Fraser et al., 2015; Clarkson et al., 2020) and they are more sensitive to higher temperatures later in life (Atkins & Benfey, 2008; Hansen et al., 2015). This study aimed to study the impact of acute thermal shocks during egg incubation and explore how these impact egg dropout, growth and survival, immune response, and tolerance to a thermal challenge later in life (possible thermal programming and memory). Increasing the understanding of how early life history impacts phenotypes will support the industry in the development of ploidy specific husbandry for triploid Atlantic salmon.

Material and Methods

A total of 7,200 Atlantic salmon eggs were stripped from a single female (Aquagen) and split into 2 batches. After fertilisation by a single male, 1 batch was triploidised using hydrostatic pressure (9500 psi for 6.25 min at 8°C, 37.5 min post-fertilisation) both batches were incubated at 4 °C until eyeing (357 °/days) at which point they were transferred to the Institute of Aquaculture (IoA), University of Stirling, Scotland. Eggs were split into 24 tanks (300 eggs per tank) and after 24hr acclimation at 6 °C, three thermal shock treatments consisting in either 1 hr, 6 hr or 1 hr daily for 5 consecutive days at 10 °C were applied in triplicate with a control (egg handling but no thermal shock). During the thermal shock the eggs were subjected to an acute temperature shock from 6 to 10 °C and then back down to 6 °C. After the shocks, eggs were taken back to the tank, the temperature remained at 6 °C until just before first feeding at which point it was raised to 12 °C (1 °C/day). Fish were grown out for 7 more months. To test for the impact of the early temperature shock (thermal programming and memory), fish were challenged to an increase temperature of 16 °C (1°C/day) for three weeks. Feed intake was recorded daily in each tank throughout the experiment. Finally, to test for fish immune response, a PAMPS challenge (I.P. injection of 5 µg/g of Poly I:C, n=3, 6 fish per treatment with an additional 6 control fish injected with PBS) was performed.

Growth performances were analysed and X-rays were taken from 48 fish per tank for deformity analysis. Blood samples were taken before and after the thermal challenge for stress biomarker analysis. Head kidney samples were taken before and after (24 hrs) Poly I:C injection for qPCR analysis of immune related genes.

Results

The repeated shock treated diploids exhibited significantly more premature hatching (<430 **°/days)** than the control (*P*=<.001). Thermal treatment did not affect radiological vertebral deformities. In the triploid groups total feed intake and final weight were higher in the 6 hr and 1 hr treated fish but not significantly. Total specific growth rate was highest for the 6 hr treatments of both ploidies at both temperatures but not significantly. Biological FCR (bFCR) was lower in the 6 hr treatment than the control for diploids at both temperatures while in triploids, bFCR appeared to be lowest in the control at 12°C but lowest for the 6 hr treatment at 16°C.

Triploids suffered more pins and runts and weighed less at 1st feeding. Triploids also hatched earlier, with all tanks reaching 90% hatch 2 days earlier than any diploid tank. At both 12 °C and 16 °C triploids ate more than diploids overall, although this difference disappeared at 16 °C. At the end of the experiment triploids weighed significantly more than diploids. Radiological vertebral deformities were relatively low but were significantly higher in triploids than diploids. No cases of aplasia of the septum transversum, previously reported in the literature, were observed despite the thermal treatments. qPCR (PAMPS challenge) analyses are underway and results will be presented at the conference.

(Continued on next page)

Discussion

The thermal shocks had a limited negative impact on the performance of both ploidies. With the exception of higher premature hatch in the repeatedly shocked diploids and higher egg drop out in triploids, no differences between treatments in terms of deformity or later mortalities were observed. Given the known sensitivity of triploids to higher incubation temperatures (Clarkson et al., 2020), this result is promising and suggests that triploids are reasonably tolerant to short term acute changes from optimal incubation temperatures after eyeing. Whilst there was a significantly higher level of radiological vertebral deformities in triploids, this was considerably lower than seen in previous studies (Fraser et al., 2015), this is likely due to the fact that eggs were incubated at 4 °*C until eyeing.* Overall triploids growth and performance did not appear to be significantly impacted by the thermal shocks, and whilst there was a higher level of pin fry, survival between ploidies was comparable. Some evidence of thermal programming was apparent with the triploid 6 hr group final weight being on average 6.5 % higher than the control, the diploid 6 hr group having the lower average bFCR than the control at both temperatures and the triploid 6 hr group having a lower average bFCR than the control at the higher temperature. Ongoing qPCR analysis of the PAMPS challenge will shed more light onto the impact of the thermal shocks to immune response.

Bibliography

Atkins, M. E., & Benfey, T. J. (2008). Effect of acclimation temperature on routine metabolic rate in triploid salmonids. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 149(2), 157-161.

Benfey, T. J., & Sutterlin, A. M. (1984). Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (Salmo salar L.). Aquaculture, 36(4), 359-367.Clarkson, M., Taylor, J. F., McStay, E., Palmer, M. J., Clokie, B. G. J., & Migaud, H. (2021). A temperature shift during embryogenesis impacts prevalence of deformity in diploid and triploid Atlantic salmon (Salmo salar L.). Aquaculture Research, 52(3), 906-923.Fraser, T. W., Hansen, T., Skjæraasen, J. E., Mayer, I., Sambraus, F., & Fjelldal, P. G. (2013). The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. Aquaculture, 416, 255-264.

Fraser, T. W. K., Hansen, T., Fleming, M. S., & Fjelldal, P. G. (2015). The prevalence of vertebral deformities is increased with higher egg incubation temperatures and triploidy in Atlantic salmon Salmo salar L. Journal of fish diseases, 38(1), 75-89.

Hansen, T. J., Olsen, R. E., Stien, L., Oppedal, F., Torgersen, T., Breck, O. & Fjelldal, P. G. (2015). Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperature. Aquaculture, 435, 354-360.

Leclercq, E., Taylor, J. F., Fison, D., Fjelldal, P. G., Diez-Padrisa, M., Hansen, T., & Migaud, H. (2011). Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (Salmo salar) post-smolts. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 158(1), 116-125.

Taylor, J. F., Sambraus, F., Mota-Velasco, J., Guy, D. R., Hamilton, A., Hunter, D. & Migaud, H. (2013). Ploidy and family effects on Atlantic salmon (Salmo salar) growth, deformity and harvest quality during a full commercial production cycle. Aquaculture, 410, 41-50.

IMPACT OF THERMAL SHOCKS DURING INCUBATION ON EGG DEVELOPMENT AND JUVENILE PERFORMANCE IN DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar*) SIBLINGS

C. Howard*, J. Taylor, M. Bekaert, B. Craig, M. Mommens, M. Medina, H. Migaud

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
E-mail: callum.howard@stir.ac.uk

Introduction

Triploidy remains the only accepted method of producing sterile salmon for human consumption in Europe (Benfey, 2016). Triploids are historically believed to be inferior to diploids (Leclercq et al., 2011; Benfey, 2016), however new knowledge on triploid biology led to improved husbandry and nutrition and subsequently performance of triploid stocks (Fraser et al., 2013; Taylor et al., 2013). Triploids require lower egg incubation temperatures than diploids for optimal bone health (Fraser et al., 2015; Clarkson et al., 2020) and they are more sensitive to higher temperatures later in life (Atkins & Benfey, 2008; Hansen et al., 2015). This study aimed to study the impact of acute thermal shocks during egg incubation and explore how these impact the transcriptome, epigenome, and performance later in life. Such new fundamental knowledge will contribute to understanding how early life history may impact on long term phenotypes and support the development of ploidy specific husbandry in triploid salmon produced commercially.

Material and Methods

A total of 7,200 Atlantic salmon eggs were stripped from a single female (Aquagen) and split into 2 batches. After fertilisation by a single male, 1 batch was triploidised using hydrostatic pressure (9500 psi for 6.25 min at 8°C, 37.5 min post-fertilisation) and the other was handled similarly but not triploidised. Both batches were incubated at 4 °C until eyeing (357 °/days) at which point they were transferred to the Institute of Aquaculture (IoA), University of Stirling, Scotland. Eggs were split into 24 tanks (300 eggs per tank) and after 24hr acclimation at 6 °C, three thermal shock treatments consisting in either 1 hr, 6 hr or 1 hr daily for 5 consecutive days at 10 °C were applied in triplicate with a control (egg handling but no thermal shock). During the thermal shock the eggs were subjected to an acute temperature shock from 6 to 10 °C and then back down to 6 °C. After the shocks, eggs were taken back to the tank, the temperature remained at 6 °C until just before first feeding at which point it was raised to 12 °C (1 °C/day). Fish were grown out for 7 more months. To test for the impact of the early temperature shock (thermal programming and memory), fish were challenged to an increase temperature of 16 °C (1°C/day increase) for three weeks. Feed intake was recorded daily in each tank throughout the experiment. Finally, to test for fish immune response, a PAMPS challenge (I.P. injection of 5 µg/g of Poly I:C, n=3, 6 fish per treatment with an additional 6 control fish injected with PBS) was performed.

Samples (eggs) were taken before and after shocks (7 days) for Reduced representation bisulfite sequencing (RRBS) and RNA-seq analysis to analyse the effects of the thermal treatments on both genome-wide methylation and transcriptomic profiles. Growth performances were analysed and X-rays were taken from 48 fish per tank for deformity analysis. Blood samples were taken before and after the thermal challenge for stress biomarker analysis. Liver samples were taken before and after thermal challenge for RNA-seq. Head kidney samples were taken before and after (24 hrs) Poly I:C injection for qPCR analysis of immune related genes.

Results

Diploids exposed to the repeated thermal shock treatment exhibited significantly more premature hatching (<430 °/**days)** than the control (*P*=<.001). Thermal treatment did not affect radiological vertebral deformities. In the triploid groups, total feed intake and final weight were higher in the 6 hr and 1 hr treated fish but not significantly so. Total specific growth rate was highest for the 6 hr treatments of both ploidies at both temperatures but not significantly.

Triploids suffered more pins and runts and weighed less at 1st feeding. Triploids also hatched earlier, with all tanks reaching 90% hatch 2 days earlier than any diploid tank. At both 12 °C and 16 °C, triploids ate more than diploids overall, although this difference disappeared at 16 °C. At the end of the experiment triploids weighed significantly more than diploids. Radiological vertebral deformities were relatively low but were significantly higher in the triploid than the diploids. No cases of aplasia of the septum transversum were observed despite the thermal treatments.

qPCR (PAMPS challenge), RRBS (egg temperature treatments) and RNA-seq (high temperature challenge) analyses are underway and results will be presented at the conference.

(Continued on next page)

Discussion

The thermal shocks had a limited negative impact on the performance of both ploidies. With the exception of higher premature hatch in the repeatedly shocked diploids and higher egg drop out in triploids, no differences between treatments in terms of deformity or later mortalities were observed. Given the known sensitivity of triploids to higher incubation temperatures (Clarkson et al., 2020), this result is promising and suggests that triploids are reasonably tolerant to short term acute changes from optimal incubation temperatures after eyeing. Whilst there was a significantly higher level of radiological vertebral deformities in triploids, this number was considerably lower than seen in previous studies (Fraser et al., 2015), this is likely due to the fact that the eggs were incubated at 4 *°C until eyeing.* Whilst there was a higher level of pin fry in triploids, survival between ploidies was comparable. Some evidence of thermal programming was apparent with the triploid 6 hr group final weight being on average 6.5 % higher than the control. Ongoing qPCR, RNA-seq, and RRBS analyses will shed more light onto the epigenetic and transcriptomic impact of the thermal shocks.

Bibliography

Atkins, M. E., & Benfey, T. J. (2008). Effect of acclimation temperature on routine metabolic rate in triploid salmonids. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 149(2), 157-161.

Clarkson, M., Taylor, J. F., McStay, E., Palmer, M. J., Clokie, B. G. J., & Migaud, H. (2021). A temperature shift during embryogenesis impacts prevalence of deformity in diploid and triploid Atlantic salmon (Salmo salar L.). Aquaculture Research, 52(3), 906-923.Fraser, T. W., Hansen, T., Skjæraasen, J. E., Mayer, I., Sambraus, F., & Fjelldal, P. G. (2013). The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. Aquaculture, 416, 255-264.

Fraser, T. W. K., Hansen, T., Fleming, M. S., & Fjelldal, P. G. (2015). The prevalence of vertebral deformities is increased with higher egg incubation temperatures and triploidy in Atlantic salmon Salmo salar L. Journal of fish diseases, 38(1), 75-89.

Hansen, T. J., Olsen, R. E., Stien, L., Oppedal, F., Torgersen, T., Breck, O. & Fjelldal, P. G. (2015). Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperature. Aquaculture, 435, 354-360.

Leclercq, E., Taylor, J. F., Fison, D., Fjelldal, P. G., Diez-Padrisa, M., Hansen, T., & Migaud, H. (2011). Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (Salmo salar) post-smolts. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 158(1), 116-125.

Taylor, J. F., Sambraus, F., Mota-Velasco, J., Guy, D. R., Hamilton, A., Hunter, D. & Migaud, H. (2013). Ploidy and family effects on Atlantic salmon (Salmo salar) growth, deformity and harvest quality during a full commercial production cycle. Aquaculture, 410, 41-50.

THE IMPACTS OF DIETARY PHOSPHORUS ON WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS

Xiaoyu Huang*, Anne Johanne Dalsgaard, Sanni-Leea Aalto, Per Bovbjerg Pedersen

Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark.
E-mail: xiahu@aqua.dtu.dk

Introduction

The objective of this study was to investigate the impacts of dietary phosphorus on water quality in recirculating aquaculture systems (RAS).

Dietary phosphorus (P) requirement for rainbow trout (*Oncorhynchus mykiss*) is around 0.6–0.7% (Coloso et al., 2003; Dalsgaard et al., 2009). However, limitations in digestibility and body retention rate will drive up the P inclusion in feeds and consequently increase phosphate excretion (Coloso et al., 2003). High phosphate contents generally do not threat fish welfare, thus there is no internal treatment deployed in RAS to remove phosphate. Nevertheless, as a eutrophication contributor, phosphorus in aquaculture wastewater is highly regulated. On the other hand, as an essential element for cellular growth, high P concentrations may also contribute to internal eutrophication and promote microbial growth in RAS. Alteration in microbial water quality can reduce nitrification rates, introduce opportunistic pathogens, and increase fish stress (Rojas-Tirado et al., 2018). Some researchers have studied the mechanisms of phosphate excretion and its relation to dietary P in flow-through systems (Coloso et al., 2003; Sugiura et al., 2006). However, to the best of our knowledge, no study has investigated the performance of dietary P in RAS, especially with the focuses on micro particles and microbial water quality.

Materials and methods

Three type of feeds were formulated to provide 46% crude protein and 25% crude fat. High P group (HP) contained 55% fishmeal from trimmings, while medium P (MP) and low P (LP) groups replaced it with processed fish trimming meal (by further removing carcass remnants) at 10% and 20% of dietary protein, resulting in different dietary P contents (1.43%, 1.16%, and 0.90% respectively). The trial was conducted in triplicate using 9 pilot RAS situated at DTU Aqua, Hirtshals, Denmark. Each system consisted of a 500 L tank stocked with 7.84±0.02 kg of juvenile rainbow trout (*Oncorhynchus mykiss*), a moving bed biofilter (75 L), and a sump (140 L). Following 3 weeks of acclimation, experimental feeds were given to fish for 5 weeks at a fixed daily feeding rate of 100 g/tank, and daily water exchange was fixed at 60 L/system. Water samples were collected on a weekly basis from the sump for water quality analysis, including free-living bacteria abundance (represented by flow cytometry), microbial activity (represented by H_2O_2 degradation rate), and accumulated quantity and surface area (reflecting particle-associated bacteria abundance) of micro particles (1-100 μm). All statistical significance levels were set at 0.05.

Table 1. Summary of feed conversation rates (FCR) (n=3) and average water quality from the last three weeks (n=9) (mean ± standard deviation). Lowercase letters indicate statistical difference with other treatment groups.

Parameters	HP	MP	LP	Units
FCR	0.98±0.03	0.93±0.02	0.94±0.02	-
Total ammonia nitrogen	0.10±0.01^{a}	0.10±0.02^{ab}	0.12±0.02^{b}	mg N/L
Nitrite	0.09±0.02	0.09±0.01	0.09±0.02	mg N/L
Nitrate	57.29±1.77	56.12±1.59	58.31±1.69	mg N/L
Phosphate	4.32±0.17^{a}	2.65±0.07^{b}	1.39±0.30^{c}	mg P/L
UV transmittance	46.18±2.51^{a}	48.27±2.46^{a}	50.96±1.54^{b}	%
Free-living bacteria	43.29±32.35	33.51±22.84	21.12±6.08	million/mL
Microbial activity	0.70±0.44	0.65±0.31	0.46±0.10	1/h
Particle quantity	1.56±1.06	1.28±0.82	0.86±0.67	million/mL
Particle surface area	19.06±5.85	19.55±4.72	17.42±5.16	mm^2/mL

(Continued on next page)

Results and discussion

Dietary treatments had significant impacts on accumulated phosphate concentrations in RAS (Table 1), which were positively correlated with dietary P inclusions. The result was in line with the correlations between phosphate excretion and dietary P discovered by Coloso et al. (2003) and Sugiura et al. (2006) in flow-through systems. In the last three weeks, LP group had an approximately 78% reduction in phosphate content and 10% enhancement in UV transmittance when compared to HP group. Meanwhile, feed conversion rates (FCR) and nitrogenous water quality were little affected (Table 1). In general, all treatment groups experienced big variation in micro-particle quantity and microbial water quality. Even though no difference between treatment groups was detected by the end of the trial, there was a tendency for microbial activity and free-living bacteria abundance to decrease with the reduction in available phosphate (Table 1).

This study also found a strong linear correlation between microbial activity and free-living bacteria (R^2=0.769), but no correlation between microbial activity and micro particle surface area, indicating that free-living bacteria had higher microbial activity than particle-associated bacteria in intensive RAS. The findings also supported the presumption made by Pedersen et al. (2017) that in pilot RAS free-living bacteria might cause the cessation of linear correlation between microbial activity and micro particle surface area found in semi-intensive model trout farms.

Conclusion

This study showed that in rainbow trout RAS reducing dietary P from 1.43% to 0.90% could successfully minimize phosphate accumulation and cause a tendency for microbial activity and free-living bacteria abundance to decrease, with little affected FCRs and nitrogenous water quality. The result also suggested that free-living bacteria had higher microbial activity than particle-associated bacteria in intensive RAS. However, to further minimize the phosphate accumulation and confirm its impact on microbes in RAS, further reduction in dietary P should be studied.

Reference

Coloso, R. M., King, K., Fletcher, J. W., Hendrix, M. A., Subramanyam, M., Weis, P., & Ferraris, R. P. (2003). Phosphorus utilization in rainbow trout (Oncorhynchus mykiss) fed practical diets and its consequences on effluent phosphorus levels. *Aquaculture*, *220*(1–4), 801–820. https://doi.org/10.1016/S0044-8486(02)00403-9

Dalsgaard, J., Ekmann, K. S., Pedersen, P. B., & Verlhac, V. (2009). Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-depleted juvenile rainbow trout (Oncorhynchus mykiss), and on the magnitude and composition of phosphorus waste output. *Aquaculture*, *286*(1–2), 105–112. https://doi.org/10.1016/j.aquaculture.2008.09.007

Pedersen, P. B., von Ahnen, M., Fernandes, P., Naas, C., Pedersen, L. F., & Dalsgaard, J. (2017). Particle surface area and bacterial activity in recirculating aquaculture systems. *Aquacultural Engineering*, *78*(April), 18–23. https://doi.org/10.1016/j.aquaeng.2017.04.005

Rojas-Tirado, P., Pedersen, P. B., Vadstein, O., & Pedersen, L. F. (2018). Changes in microbial water quality in RAS following altered feed loading. *Aquacultural Engineering*, *81*(December 2017), 80–88. https://doi.org/10.1016/j.aquaeng.2018.03.002

Sugiura, S. H., Marchant, D. D., Kelsey, K., Wiggins, T., & Ferraris, R. P. (2006). Effluent profile of commercially used low-phosphorus fish feeds. *Environmental Pollution*, *140*(1), 95–101. https://doi.org/10.1016/j.envpol.2005.06.020

SIMPLE FIELD STORAGE OF FISH SAMPLES FOR MEASUREMENT OF DNA CONTENT BY FLOW CYTOMETRY

Martin Hubálek*, Martin Flajšhans

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters; South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25, Vodňany, Czech Republic
Email: mhubalek@frov.jcu.cz

Flow cytometry is an effective and widely used tool for determination of ploidy in fish, but it is not always possible to access the fresh samples for analysis. We investigated the potential for extended storage of fish tissue with sterlet and tench as representative species of Chondrostei and Teleostei, using blood and fin of subadult/adult specimens and tail of larvae. Thirteen procedures for extending storage, selected for rapidity and simplicity in both field and laboratory conditions, were tested for each tissue sample. Flow cytometry was applied to fresh tissue immediately after sampling and to tissue subjected to experimental protocols, always along with species-specific standard, after 1, 5, and 10 days storage at 0–4°C or freezing at -80°C. The fluorochrome 4′,6-diamidine-2′-phenylindole dihydrochloride was used with excitation/emission maximum 358/461 nm. Based on the measurability of stored samples, evaluation of directly measured coefficients of variation of their DNA peaks and the changes in fluorescence intensity compared to fresh tissue, optimal procedures for extended storage of the selected tissue types of the model species are suggested.

FOODLAND: AQUACULTURE RESEARCH TO IMPROVE SUSTAINABLE FISH PRODUCTION IN AFRICA

F. Robinson, S. Hunter*, K. Hoevenaars T. Bardócz and M. Setti

*AquaBioTech Ltd, Central complex, Naggar Street,Targa Gap, Mosta, Malta
Email: fmr@aquabt.com

FOODLAND aims at enhancing the diversity of food production and consumption in six African countries displaying different stages of the nutrition transition. To this end, FOODLAND will create a network of 14 local Food Hubs that will aggregate relevant actors and serve as injection points for the introduction of innovations. FOODLAND has identified specific objectives addressing the organizational, technological, and nutritional needs of the local African food systems: 1. To detect behaviour and preferences of consumers and producers, in order to customize innovations to local sensitivities; 2. To develop and implement organizational innovations, aimed at boosting coordination among food operators; 3. To develop, test, and validate (open) technological innovations in the laboratory and in the field; 4. To disseminate knowledge of solutions towards malnutrition reduction and innovations.

The aquaculture research and validation activities of the project will ensure a solid knowledge base for overcoming the main problems in the development of aquaculture in Sub-Saharan Africa and will provide new methods and technologies for other countries in Africa. By developing aquaculture technologies for urban and peri-urban areas, the production is brought closer to the markets resulting in a shorter distribution chain that can be more competitive with imported products. The aquaculture Food Hubs to be developed in the project will therefore be less dependent on cold chain product distribution. New fish species will be valorised, and new fish processing methods tested to increase shelf life and value of the products and ensure a competitive advantage for the aquaculture sector. The project will focus on the research and development of the most efficient aquaculture technologies by applying the most advanced production methods in various systems while observing the local environment and low investment models. The possibilities of exploiting the integration of different aquaculture and agriculture systems will enable the reduction of production costs and use of imported fish feed. The specific RAS developed in this project will provide a system with low operational costs to ensure the supply of high-quality fingerling with affordable prices to small-scale fish farmers.

FOODLAND will empower smallholder farmers and food operators, foster nutrition-responsive and sustainable agro-biodiversity, reinforce the productivity and resilience of food supply chains, and will create new market opportunities at both the local and global scales, thereby encouraging the flourishing of rural communities. These achievements will benefit both African and European consumers by providing them with traditional-based, healthy, nutritious foods, while encouraging the diffusion of African diets and aiding the fight against malnutrition, particularly in women and children.

Acknowledgement
This research has received funding from the European Union's Horizon 2020 research and innovation program for the project Foodland.

FISH WELFARE IMPLICATIONS OF PROLONGED FASTING PERIODS IN ATLANTIC SALMON

Malthe Hvas*, Ole Folkedal, Frode Oppedal, Lars Helge Stien

Animal Welfare Research Group, Institute of Marine Research, 5984 Matre, Norway
E-mail: malthe.hvas@imr.no

Feed withdrawal is a widespread practice in Atlantic salmon (*Salmo salar*) aquaculture to empty the gut prior to major farming operations. Moreover, emerging production practises such as RAS and offshore farm sites may also occasionally subject fish to prolonged fasting periods. However, such extended fasting periods may conflict with ethical and legal obligations to farm animals. Presently, science-based recommendations on responsible fasting times that consider fish welfare are lacking for Atlantic salmon as well as for other finfish aquaculture species.

We have investigated some of the physiological, behavioural, growth, and welfare related effects of fasting periods in a series of three studies on growing Atlantic salmon post-smolts in seawater. The purpose of this work was do define useable welfare guidelines for allowable fasting periods in Atlantic salmon aquaculture by identifying thresholds for significant impairments in performance traits.

In the first study, we measured metabolic rates in responses to increasing fasting periods of up to 4-weeks and after 1-week of subsequent refeeding in fish of ~575 g and ~38 cm at 12°C. The standard metabolic rate decreased stepwise after 1 and 3 weeks, showing that Atlantic salmon gradually adapt a mode of energy saving when resources are limited. The increase in metabolic rates following acute stress was slightly reduced after 4-weeks, indicating that stress responses first become impaired at this point. Following refeeding metabolic rate traits reverted to control levels, showing that metabolic adjustments to prolonged fasting were rapidly reversed when regaining access to feed.

In the second study, we measured the critical swimming speed in fish of ~250 g and ~29 cm at 12°C that had been fasting for up to 4-weeks. In addition, we also measured blood parameters before, at fatigue, and after 3 and 24 hours of recovery from the swim test. The 4-week fasting period reduced condition factors from 1.03 to 0.89. However, the critical swimming speed remained statistically unaffected at ~3.5 body lengths s^{-1}. Exhaustive exercise caused large osmotic and ionic disturbances, and large increases in plasma lactate and cortisol. During subsequent recovery, the changes in osmolality and plasma ions took the longest to correct, suggesting that these parameters may be considered the most challenging stressors during strenuous exercise in seawater. However, only minor effects of fasting period on blood parameters in relation to the swim challenge were detected which included a repressed response in red blood cell recruitment and reduced cortisol response at fatigue. Nevertheless, Atlantic salmon maintained their full swimming capacity and their ability to respond and recover adequately to acute challenges following extended periods of food deprivation.

In the third study, Atlantic salmon of ~1200 g and ~46 cm were fasted for 8 weeks at 12°C and subsequently refed for 5 weeks, whereafter they were transferred to triplicate sea cages in a common garden setup with a control group until harvest size of ~6100 g and ~73 cm. At the end of the fasting period fish had lost 7.3% mass and the condition factor had decreased from 1.2 to 1.0. Furthermore, fasted fish were 544 g lighter and 3.8 cm shorter than fed controls, corresponding to a size difference of 50%. Following periods of refeeding, fasted fish eventually showed compensatory growth and at harvest weight and length were statistically like controls. At harvest, males were larger than females, and immature fish were larger than maturing fish. The proportion of maturing fish was 25% higher in the continuously fed control treatments. After the 8-week fasting period, fish welfare was scored based on the salmon welfare index model. Only minor deviations were found and at similar regularities between fasted and control fish, showing that prolonged fasting did not cause detrimental welfare conditions. To assess potential long-term impacts on welfare status, vertebral deformities in the spinal column were quantified with radiology after harvest. Frequency of skeletal deformities were low and similar between treatments. Hence, Atlantic salmon are highly flexible with regards to growth patterns in response to food availability, and a prolonged fasting period neither caused reduced welfare in the short or in the long term.

Based on these three studies we conclude that farmed Atlantic salmon are well adapted to cope with prolonged fasting periods without suffering poor welfare or other detrimental and persisting consequences to physiological and growth performances. When poor welfare is observed in fish that have been fasting for long periods, the underlying cause will most likely instead by suboptimal environmental conditions or infectious diseases, not fasting on its own. As such, formulating welfare guidelines for allowable fasting periods for farmed Atlantic salmon may therefore ultimately be redundant since the required time to initiate severe starvation takes much longer than any realistically encountered fasting period in modern aquaculture practices.

WHY TO BET ON DIETARY LYSO-PHOSPHOLIPIDS: DEEP ANALYSIS OF WAYS-OF-ACTION IN SALMON VIA INTESTINE AND LIVER INTERACTOMES

A. Ibarz*[1], W. Nuez-Ortín[2], I. Sanahuja[1], L. Fernández-Alacid[1]

1- Department of Cellular Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona (Spain)
2- Adisseo (Spain)
E-mail: tibarz@ub.edu

Introduction

Lipid supplies energy and acts as a structural component of cell membranes, and may spare protein in diets for aquatic animals. The inclusion of intact phospholipids in the diet could improve culture performance of various freshwater and marine fish species (Tocher et al., 2008). The primary beneficial effect was improved growth in both larvae and early juveniles, but also increased survival rates and decreased incidence of malformation in larvae, and perhaps increased stress resistance. Lyso-phospholipids or lyso-lecithin are produced from phospholipids by enzymatic hydrolysis. Lyso-phospholipids contain only one fatty acid tail. This structure makes them more hydrophilic than phospholipids, which translates into better emulsifying capacity. While lyso-phospholipid supplementation in animal feed mainly aims at improving fat emulsification, additional metabolic effects in fish have not been explored. The present study aimed to gain understanding of the mechanisms underlying the growth promoting effect of lyso-phospholipid supplementation (AQUALYSO®, Adisseo). Atlantic salmon was used as model species with intestine and liver as targets tissues.

Material and Methods

Middle intestine and liver (n =15) were dissected from adult Atlantic salmon fed a control and lyso-phospholipid (0.1% AQUALYSO®, Adisseo) supplemented feed for 12 weeks. Tissue protein purification was performed as described in Ibarz et al. (2010) with slightly modifications for the salmon tissue samples. Protein identification and quantitation were performed, via "shotgun analyses" from by nanoLC-MS/MS and the raw data were processed using the Proteome Discoverer 2.5.0.400 software (Thermo Scientific, Bremen, Germany). The complete map of interactions (interactome) was obtained from the differentially expressed proteins: DEPs (proteinteractome). For this purpose, the Search Tool for the Retrieval of Interacting Genes (via STRING program) public repository version 10.0 (https://string-db.org) was used. Protein-protein interaction (PPI) network for the differentially expressed proteins (DEPs) was conducted with a high-confidence interaction score. Gene ontology (GO) pathway enrichment analysis (Biological Processes; Reactome Pathways and Post-Translational Modifications) was also performed for the DEPs by STRING program.

Results and Discussion

Liver and mid intestine proteome were deep analysed to determine the main "ways-of-action" of the dietary lyso-phospholipid supplementation. The numbers of DEPs detected in each tissue were similar but differed in terms of the proportion of up-regulation and down-regulation (Fig. 1A). The most relevant interactomes were the up-regulated DEPs in liver and the down-regulated DEPs in intestine (Fig. 1B). They resulted from the STRING analyses and they were analysed by their significant clusters on Biological Processes and Protein Reactomes.

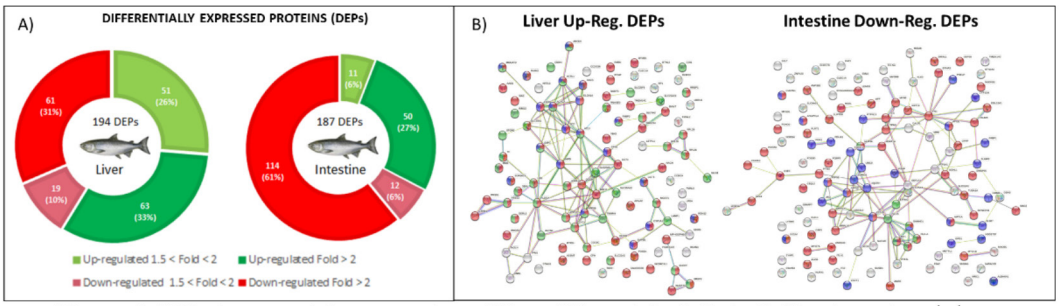


Figure 1. Effects on protein expression of Lyso-PL-enriched diet. A) Up- (green) and down- (red) regulated expressed protein distribution. B) Proteinteractome maps of liver up-regulated DEPs and intestine down-regulated DEPs.

(Continued on next page)

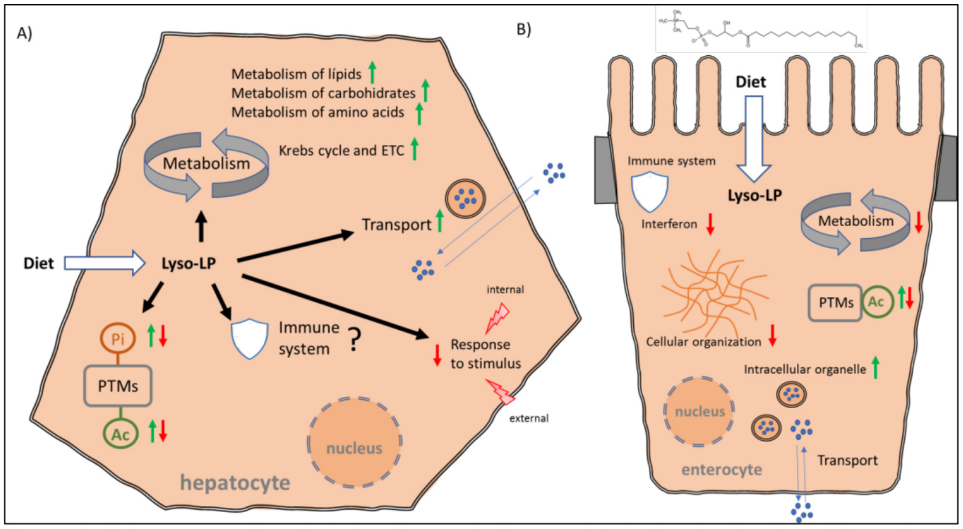


Figure 2. Scheme of main affected pathways in liver and intestine by the Lyso-LP-enriched diet. Green arrows indicated upregulation and red arrows indicated downregulation. PTMs (Post-Translational Modifications: Pi-phosphorylated, Ac-acetylated).

In liver (Fig. 2A), the Biological Processes enhanced by dietary lyso-phospholipids were related to the "metabolism" and specifically lipid, carbohydrates and amino acids metabolism together with mitochondrial activity. Moreover, intracellular "transport" was stimulated and the "response to stimulus" was downregulated. In intestine (Fig. 2B), the main effects were associated to an improvement of vesicle trafficking, whereas the "stress response" and the "interferon signalling" were downregulated. Interestingly, a vast number of DEPs in both tissues were significantly grouped in a PPI-enrichment of Post-Translational Modifications, mainly as acetylated or phosphorylated proteins. This could indicate a specific way-of-action of lyso-phospholipids also on important protein modification pathways following its biosynthesis.

In conclusion, shotgun proteomics points towards enhanced functionality of intestine and liver in fish following lyso-phospolipid supplementation.

References

Ibarz A, Martín-Pérez M, Blasco J, Bellido D, Oliveira E, Fernández-Borrás J, 2010. Gilthead sea bream liver proteome altered at low temperatures by oxidative stress. Proteomics 2010, 10, 963–975

Tocher DR, Bendiksen EA, Campbell PJ, Bell JG, 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280 (2008) 21–34

EVALUATION OF TWO ANAESTHETICS IMPACT ON SKIN MUCUS BIOMARKERS TO MONITOR RAINBOW TROUT CULTURE

M. Tejero[1], A. Ibarz*[1], I. Sanahuja[1], C. Madrid[2], C. Balsalobre[2], L. Fernández-Alacid[1].

1- Department of Cellular Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona (Spain)
2- Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona (Spain)
E-mail: tibarz@ub.edu

Introduction

Anaesthesia plays a necessary role in fish manipulation in both aquaculture and research conditions. In order to maintain fish welfare during several common procedures, a wide range of anaesthetic agents has been described and used. Since its introduction, Tricaine methane-sulfonate (MS-222) has become the most used anaesthetic and its properties and physiological effects have been exhaustively reviewed (Topic Popovic et al., 2012). Being very hazardous in case of inhalation and skin contact, personnel using MS-222 must follow some safety practices, less guaranteed and controlled in large-scale production. Also, due to its toxicity and potential contamination of near ecosystems, in some European countries, such as Spain, France and Greece, MS-222 use is not licensed for food fish production while others apply very specific limitations ang lengthy withdrawal periods. Therefore, experimental studies carried in European fish farms should take regional legislation into account before administrating MS-222, widely established in laboratories. In this regard, the aim of the present study is to assess the use of clove oil, less persistent and authorized in most European countries, as a suitable alternative to MS-222 for research studies in farmed rainbow trout (*Oncorhynchus mykiss*). For this purpose, potential differences in plasma and skin mucus biomarkers and antibacterial activity were evaluated for individuals anaesthetized with MS-222 or with clove oil.

Materials and methods

Forty-eight rainbow trout individuals (343,55 ± 13,46 g) were randomly sampled from three different cages of a local fish farm (Viveros del Segre, Peramola, Spain) and anaesthetized with MS-222 (100 mg/L) (Sigma-Aldrich, Madrid, Spain) or clove oil (10 mg/L) (Sigma-Aldrich, Madrid, Spain) in two supplementary tanks of 40L in consecutives caught of 4 fish each time. Once fish were sedated, skin mucus was collected from all fishes by gently sliding sterile glass slides along both sides of the fish in a front to caudal direction and stored in sterile tubes (2mL). Blood samples were obtained from 12 fishes per condition from the caudal vein using syringes impregnated with EDTA-Li as the anticoagulant. The other 12 fishes per condition were directly recovered in two 40L additional tanks with oxygenated water, in order to evaluate the recovery capacity from both anaesthetics. The main stress and physiological biomarkers analysed in mucus and plasma were soluble protein, glucose, lactate and cortisol levels and were measured according to Fernández-Alacid et al. (2018, 2019). Additionally, antibacterial activity of plasma and mucus were measured against *E. coli*, *A. hydrophila*, and *A. salmonicida* adapting the protocol described in Sanahuja et al (2019). Statistical analysis was performed using Student's *t*-test of SPSS software Version 22.0 (IBM Corp, Armonk, NY, USA).

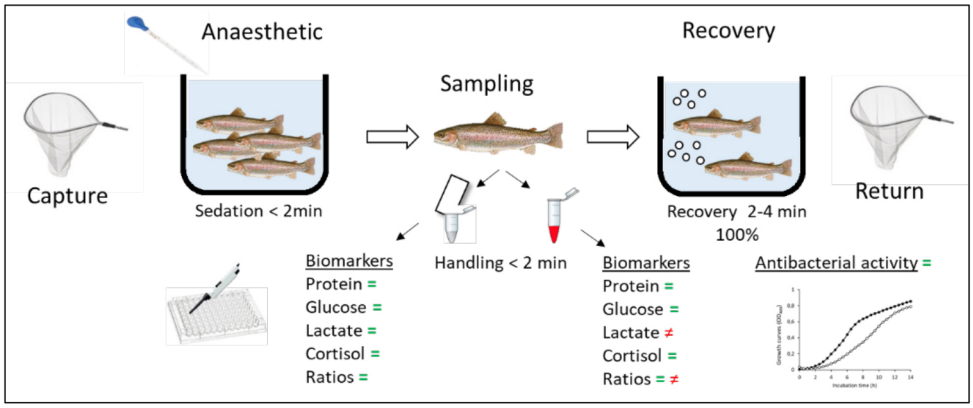


Figure 1. Schemetic representation of the whole process of anaesthesia, sampling and recovery of farmed rainbow trout. No significant differences (t-test) were observed in main biomarkers analysed (= symbol) except for plasma lactate (≠ symbol)

(Continued on next page)

Results and Discussion

In recent years, growing interest has been shown in the use of minimally-invasive methods to assess fish physiological status and welfare, such as fish skin mucus analysis. The use of MS-222 in fish research studies are not applicable to monitor farmed fish due to its hazardous. Clove oil, as an organic substance, does not require any withdrawal period in contrast to MS-222 (Topic Popovic et al., 2012). In this preliminary study we compared the main biomarkers of mucus and plasma when fish were anaesthetised with MS-222 or with clove oil (Figure 1).

Using the reported anaesthetic concentration, no differences were observed among the time for fish sedation (< 2 min) to be adequately handling and recovery (2-4 min for the 100% of returned fish). The stress-related biomarkers analysed, cortisol, glucose and lactate did not modify among clove or MS-222 for both mucus and plasma samples. Only plasma lactate was significantly higher (20%) in fish anesthetized with clove oil, which allow us to improve the time used to sampling the animals and avoid anaerobic metabolism. No significant differences of plasma antibacterial activity between anaesthetics were found for any of the bacterial strains tested (E. coli, A. hydrophila and A. salmonicida). Several previous studies suggested that the physiology of fish can be affected by changes in the haematology and biochemistry of exposed fish to clove oil. Current results have shown great promise for the use of epidermal mucus as a study and monitoring tool for trout culture.

In conclusion clove oil appears to be a strong alternative for farm aquaculture studies, specially using the mucus monitorization where sampled fish could be returned to farm in a remarkable short time (less than 10m).

References

Fernández-Alacid, L.; Sanahuja, I.; Grande, B.O.; Sánchez, S.; Viscor, G.; Gisbert, E.; Herrera, M.; Ibarz, A. Skin mucus metabolites in response to physiological challenges: A valuable non-invasive method to study teleost marine species. Sci. Total Environ. 2018, 644, 1323–1335

Fernández-Alacid, L.; Sanahuja, I.; Grande, B.O.; Sánchez-Nuño, S.; Herrera, M.; Ibarz, A. Skin mucus metabolites and cortisol in meagre fed acute stress-attenuating diets: Correlations between plasma and mucus. Aquaculture 2019, 499, 185–194

Sanahuja, I.; Fernández-Alacid, L.; Grande, B.O.; Sánchez, S.; Ramos, A.; Araujo, R.M.; Ibarz, A. Comparison of several nonspecific skin mucus immune defences in three piscine species of aquaculture interest. Fish Shellfish Immunol. 2019, 89, 428–436

Topic Popovic, N.; Strunjak-Perovic, I.; Coz-Rakovac, R.; Barisic, J.; Jadan, M.; Persin Berakovic, A.; Sauerborn Klobucar, R. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. Journal of applied ichthyology. 2012, 28, 553–564

NUTRITIONAL MITIGATION OF EXTREME HEATWAVE EXERTED STRESS IN FISH. THE CASE OF EUROPEAN SEABASS, *Dicentrarchus labrax*

M. Jakiul Islam[1,2*], A. Kunzmann[2], Matthew J. Slater[1]

[1]Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, 27570, Germany
[2] Leibniz Center for Tropical Marine Research, Bremen, 28359, Germany
Email: jakiul.islam@awi.de

Introduction

In the current scenario, global climate change is a reality that impacts all living species, including fish. The aquatic ecosystem, since it is the largest sink for global warming and high temperatures, is the most affected system and affects all types of aquatic life.

Materials and methods

Fish were fed on diets supplemented with vitamin C (0.40%) and E (0.35%), propolis (0.45%), phycocyanin (0.30%), and β-glucan (0.30%) along with a control diet for 56 days, followed by 18 days of extreme warm exposure. Growth performance, individual fitness, metabolic activities, nutrient assimilation capacity, and molecular stress responses were evaluated to understand dietary manipulation impacts.

Results

Final weight gain, weight gain, specific growth, and protein efficiency were higher in fish fed with propolis followed by phycocyanin, vitamin C & E, and β-glucan compared to the control diet. Besides, viscera somatic index, intestine somatic index, and hepatosomatic index values were higher in the control diet compared to tested diets. Significantly higher Na+ and Cl- levels were measured ($p<0.05$) in fish fed to control diet followed by vitamin C & E, propolis, phycocyanin, and β-glucan. While K+ ion level was found significantly lower ($p<0.05$) in fish fed with control and β-glucan supplemented diets during heatwave stress. Cholesterol and triglycerides levels were found significantly lower ($p<0.05$) in fish fed on vitamin C & E, propolis, and β-glucan supplemented diets compared to fish fed on the control diet. Blood urea nitrogen, creatine, and cortisol content were significantly lower ($p<0.05$) in fish fed with vitamin C & E, propolis, and phycocyanin supplemented diets compared to fish fed on the control diet. AST, ALT, and LDH contents were found higher in fish fed on Vitamin C & E supplementation followed by propolis, control, phycocyanin, and β-glucan supplemented diets. Transcriptomics result also supports the better health and physiological conditions in fish fed on vitamin C & E, propolis, and phycocyanin supplemented diets.

Discussion and conclusion

Diets with the increased dosage of Vitamins C and E, propolis extract, and phycocyanin were found to be beneficial for European seabass, *Dicentrarchus labrax* during extreme warm exposure. Diets containing high levels of either vitamin E (4.0 g kg-1) together with high levels of vitamin C (3.5 g kg-1), or propolis (4.5 g kg-1), or phycocyanin (0.30 g kg-1) were able to counteract the adverse effects of stress responses during extreme warm exposure. To some extent, these ingredients were found to improve fish growth, antioxidant capacity, and immune functions. In contrast, the inclusion of dietary β-glucan was found to be less functional in supporting fish growth, physiology, and metabolism.

SEASONAL SUCCESSION OF PLANKTON IN HYPERTROPHIC PONDS IS CONSTRAINED BY FISH DENSITY AND AVAILABLE PHOSPHORUS

A. Ivanova,[a*] J. Vrba,[b] M. Svitok,[bc] J. Regenda,[a] and O. Strunecký[a]

[a] University of South Bohemia in *České* Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Na Sádkách 1780, 370 05 *České* Budějovice, Czech Republic

[b] University of South Bohemia in *České* Budějovice, Faculty of Science, *České* Budějovice, Czech Republic

[c] Department of Biology and General Ecology, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, Slovakia
Email: aivanova@frov.jcu.cz

Human-induced input of nutrients to waters further increases eutrophication, resulting in hypertrophy. To understand the processes of plankton succession in hypertrophic water bodies, we analysed data from six hypertrophic fishponds in Czech Republic, 2008 – 2016. The significant source of total phosphorus was supplemental fish feed giving an overall mean of 0.15 mg L^{-1} in fishpond water. Carbon in zooplankton biomass ranged from 0.004 to 5.9 mg L^{-1} reaching maximum in late spring. Fish biomass of 300–900 kg ha^{-1} disrupted the plankton food web by top-down elimination of zooplankton. Carbon in phytoplankton biomass ranged from 0.1 to 12.3 mg L^{-1} and peaked in late summer. The phytoplankton was dominated by cyanobacteria, closely reflecting the total phosphorus concentration in water. Algae grew until they had consumed available nitrogen, whereas cyanobacteria continued to grow due to the fixation of dissolved nitrogen. The top-down forcing plays a more significant role than was previously expected. Fish biomass lower than 300 kg ha^{-1} increase phosphorus transfer from phytoplankton to fish via zooplankton, and potentially improve water quality. Such seasonal plankton succession caused both by high phosphorus concentration, and high fish density has not been described by previous ecological studies.

DIFFERENTIATION AND INNOVATION IN NORWEGIAN SALMON AQUACULTURE: BARRIERS AND OPPORTUNITIES

A. Cojocaru, A. Iversen*, O. Bergesen and R. Tveterås

Nofima, Postboks, 6122, Langnes 9291, Tromsø, Norway.

E-mail: Audun.iversen@nofima.no

Introduction

The level of control over the farming process in salmon aquaculture makes it possible, in principle, for farmed Atlantic salmon to be tailored on a number of dimensions to meet requirements from different customer groups at different stages of the value chain. However, production, harvesting and primary processing of salmon have resulted in largely homogeneous products being exported from Norway. Around 85% of Norwegian salmon is exported as whole, gutted salmon (Norwegian Seafood Council, 2019). Primary processing in Norway focuses on fresh whole salmon with few of the farming companies also selling processed salmon, mostly as fresh or frozen fillets, and a few making portions and "ready-to-eat" products. As so much of the salmon is sold whole, and with fillets being of very similar quality, products can mainly be considered commodities, with standardized specifications and sold in markets where the price is driven by supply and demand (Asche, Bremnes, and Wessells, 1999; Asche and Bjørndal, 2011). Innovation tends to be focused on efficiency, and less on product development. Differentiated products are thus relatively few and associated volumes are small (Asche, Cojocaru, and Roth, 2018). Cojocaru, Iversen, & Tveterås (2020) discuss to what extent salmon is differentiated, how the differentiation strategies answer customers' quality demands, and the degree to which various attributes offer competitive benefits, leading to advantages or price premiums. However, while having identified general barriers to differentiation, we also found that differentiation is understood and approached differently across industry players depending on where in the value chain they operate or what customers they are serving.

This paper identifies five major routes of differentiation for salmon, including the strategies for differentiation as understood from the points in the supply chain where they are executed today. The paper then discusses specific barriers for each route of differentiation, including opportunities for achieving differentiation in each of them. To obtain information with respect to differentiation strategies for salmon, we conducted in-person interviews with a range of Norwegian producers, the majority of them also operating internationally.

Results

"It´s not the Norwegians who have done a good job, it´s all the customers." This quote from one of our informants illustrates the fact that differentiation of salmon products has taken place at different stages in the value chain, and not with the producers themselves. It implies different relations between sellers and buyers, with varying power balance and opportunities for differentiation. The value chain for Atlantic salmon, sketched in Figure 1, resembles that of other (intensive) food production systems such as pork and poultry (Asche, 2008; Asche, Cojocaru, and Roth, 2018), There is also an increasing tendency towards vertical integration (Kvaløy and Tveterås, 2008; Asche et al., 2013), with producers responsible for all stages from smolt production to export, but with little to no activities past the stage of primary processing.

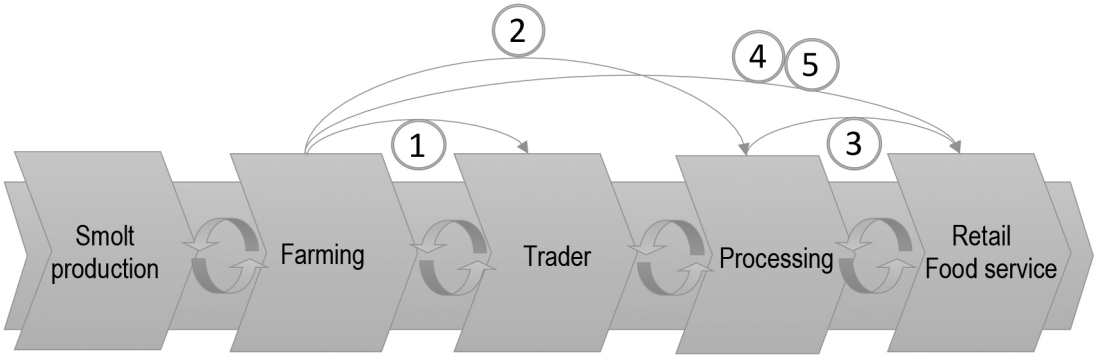


Figure 1. Simple representation of the Atlantic salmon value chain: with intermediate markets (or feedback loops) indicated between each stage. Numbered above: **Five different routes of differentiation** identified within the salmon value chain.

(Continued on next page)

The identification of differentiation routes throughout the value chain allowed for a classification of the differentiation strategies used. Figure 1 shows the five main routes of differentiation identified based on the interviews we conducted.

The first we termed *1) The commodity producer*, as most Norwegian salmon producers are large-scale producers of highly perishable products, implying a focus on low production costs and efficient production and logistics, with little differentiation. Slightly more differentiated products are sold by the *2) The industrial supplier*, supplying processors closer to the destination market, either in low-cost countries like Poland, or in the destination country. Processors, that either have their own brand or cater to private labels for retail chains, rarely ask for differentiated products, as they do their own differentiation, but choose suppliers based on industrial relations, where quality as supplier is as prominent as quality of products. *3) The marketing company*. Some traders make an effort to move away from trading the salmon as a commodity, towards marketing the salmon as something more distinctive. They move from sales to marketing. *4) The long-range producer* typically works with retail chains, contributing to a greater range and variety of products, which might be sold both under the producer´s own label or the chain´s label. *5) The branded producer*. A few of the largest salmon producers have launched their own brands, but only one has set off to create a global one. Brands are most prominent in the home market, or markets close to Norway, but are also slowly spreading to some of the major markets.

The routes identified above place an array of requirements on the firms, require different resources, or the firm's position in the value chain may lead to different probabilities of success. In practice, these differentiation route archetypes can vary from firm to firm. Some companies may also choose to pursue a number of routes in parallel, depending on the customer segments they serve. While (Cojocaru et al., 2020) identified general barriers for differentiation, this paper points to barriers for each of the identified routes of differentiation, and identifies responses to overcome these barriers.

The paper also discusses to what degree this categorization will be stable over time. Recent developments in new production techniques, with widespread innovation in new production processes (land-based farming, closed or semi-closed containment at sea, off-shore aquaculture, etc), might increase the focus on differentiation, and the share of production being marketed as differentiated products, as marketing of benefits with new production technologies, aiming to obtain premium prices, is meant to offset cost disadvantages and high investment cost in new concepts.

Table 1: Barriers and suggested responses for the defined routes of differentiation

Route of differentiation		Barrier(s)	Opportunities
1	**The Commodity Producer**	- Difficult and expensive to produce many varieties as production focuses on scale, efficiency and costs	- Origin and storytelling - Ecolabels - Shelf-life and value-added products
2	**The Industrial Supplier**	- Lacking demand for differentiated products (processing firms want to differentiate for themselves/their own products)	- Intrinsic qualities achieved through feed mix varieties - Service-oriented differentiation
3	**The Marketing Company**	- Little to no control over intrinsic properties	- Service- and concept-oriented differentiation
4	**The Long-Range Producer**	- Label-owners want to control differentiation - Supermarket chains interested in growing own private labels	- Growing category, room for more varieties, labels and certification - Intrinsic qualities achieved through feed mix varieties
5	**The Branded Producer**	- Costly, time-consuming, knowledge-intensive strategy	- Growing category, room for more varieties, value-added products, high-end products, brands, feed mixes

DESCRIPTION OF FIRST ESCAPE OF GREATER AMBERJACK (*Seriola Dumerili*, Risso 1810) IN THE MEDITERRANEAN SEA BY MEANS OF GENETICS, GEOMETRIC MORPHOMETRICS AND SOCIAL MEDIA

D. Izquierdo-Gomez[1]*, I. Talijančić[2], I. Žužul[2], T. Segvić-Bubić[2] Tanja decides the order

[1] Université de Pau et des Pays de l'Adour (UPPA), Collège STEE, Pau (France).
[2] Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21000 Split (Croatia)

* E-mail: david.izquierdo-gomez@univ-pau.fr

Introduction

The farming of the greater amberjack (*Seriola dumerili*, Risso 1810) in the Mediterranean Sea started in the 1980s in a capture-based fashion (FAO 2016-2021). Nowadays, as a result of the industrial diversification of marine fish in Mediterranean aquaculture, the rearing cycle of *S. dumerili* is fully carried out in captivity. However, it was not until 2017, when the industrial production and commercialization started, with Spain and Greece as the main producers (FishSTAT), and more recently Croatia in 2019 (No data available in FishSTAT; pers.obs Segvic-Bubic). Reared fish escaping from farms have a number of potential socio-economic and ecological implications, and some of them have been explored for *Sparus aurata*, *Dicentrarchus labrax* and *Argyrosomus regius* (Arechavala-Lopez et al., 2018).

This research describes the first escape of *S.dumerili* in the Mediterranean and aims i) to develop the identification techniques of the escaped individuals of *S. dumerili,* ii) to help understanding the potential influences of the escape events at a genetic and fisheries levels and, iii) to improve the aquaculture genetic management of the species.

Material and Methods

In the current research, captures of unusual individuals of *S.dumerili* were detected via pictures posted in Facebook groups with fisheries interest. Data on commercial fisheries landings of the artisanal fleet based on the marina of Santa Pola (38º11'12''N; 0º33'33''W, Alicante, Spain), was explored to identify abnormal captures of the *S. dumerili* at a temporal level. Local Ecological Knowledge of fishermen was obtained via personal interviews on Facebook messenger, to better understand the behavior and dispersion of the escaped fish.

Following a simplified mammalian DNA isolation procedure, a total of 492 individuals were successfully genotyped with 15 microsatellite markers developed for *S. dumerili* (Renshaw et al. 2006, 2007) from 10 populations sampled in both, the Adriatic and Balearic Seas, covering different fish origins (wild, farmed and escaped). Genetic diversity, differentiation and structure for each population were calculated and assessed by using FSTAT 2.9.3, Arlequin v.3., FreeNA, Structure 2.3 and Adegenet package in R.

A photo of the left side of each fish was taken at 1m (orthogonal distance), with a photo camera set at 50mm focal length. A 50 cm ruler was included in each picture for body length measurement in ImageJ software. Quantification and visualization of fish shapes were conducted by means of geometric morphometrics, using a total of 21 landmarks (TpsDig 2 and MorphoJ software and geomorph R package). Eventually, differences between fish groups of different origins were explored with canonical analysis.

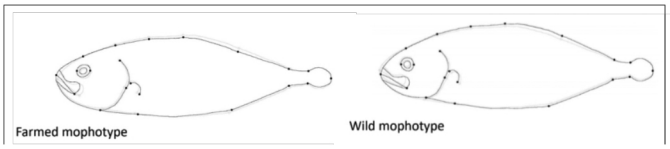


Figure 1. Farmed and wild *Seriola dumerili* morphotypes

(Continued on next page)

Results and conclusion

Arising from social media pictures and conversations/commentaries of fishermen, the escaped fish was darker than wild ones and some fish presented deformities and/or wounds. Approximately two months after the escape event, the presence of escaped *S. dumerili* still could be observed in the escape area. The maximum dispersion attained up to 100 km to the south and 90 km to the north from the potential escape locations, namely San Pedro del Pinatar (Murcia) and Calpe (Alicante), both in the SE of Spain. The captures of commercial and recreational fisheries were influenced by escaped fish. Significant genetic differentiation between farmed and wild fish groups were observed where farmed fish were characterised with reduced number of alleles, allelic richness and expected heterozygosity. Minimum estimates of effective population size, which may serve as a conservative estimate for wildlife management, were for two order of magnitude smaller in farmed (~ 3) than in wild (~ 300) populations, implicating urgent need for genetic improvement of broodstock menagment

Morphological differences between wild and reared fish existed, with a striking difference in the mouth position, which showed upwards-oriented in reared fish, compared to a more horizontal position of wild fish.

In first term, fish escapes should be managed from a prevention perspective followed by mitigation and monitoring programs. The development of a tool-kit to identify escaped individuals is suggested. The behaviour of escaped *S. dumerili* should be studied from a spatio-temporal, ecological, fisheries and management perspectives. In terms of aquaculture management, to increase the broodstock size it is suggested, in order to minimize inbreeding risks and potential genetic influences for the populations of wild counterparts.

References

Arechavala-Lopez, P., Toledo-Guedes, K., Izquierdo-Gomez, D., Šegvić-Bubić, T. and Sanchez-Jerez, P., 2018. Implications of sea bream and sea bass escapes for sustainable aquaculture management. Reviews in Fisheries Science & Aquaculture, 26, pp.214-234.

Cultured Aquatic Species Information Programme. Seriola dumerili. Cultured Aquatic Species Information Programme. Text by Jerez Herrera, S. and Vassallo Agius, R. In: FAO Fisheries Division [online]. Rome.

Renshaw, M.A., Patton, J.C., Rexroad 3rd., C.E., Gold, J.R., 2006. PCR primers fortrinucleotide and tetranucleotide microsatellites in greater amberjack, Serioladumerili. Mol. Ecol. Notes 6, 1162–1164.

Renshaw, M.A., Patton, J.C., Rexroad 3rd., C.E., Gold, J.R., 2007. Isolation andcharacterization of dinucleotide microsatellites in greater amberjack, Serioladumerili. Conserv. Genet. 8, 1009–1011.

Acknowledgements

This research has been funded by the projects AquaPop IP-2014-09-9050 (Croatian Science Fundation) and GLORIA (GLObal change Resilience In Aquaculture: FBIOPLEAMAR20-05). Eventually the authors would like to thank the personnel of the Santa Pola Fish market and the rearing facilities of CROMARIS (Croatia) and the Spanish division of the ANDROMEDA group.

FIRST EXPERIENCE OF NEWLY ISOLATED FRESHWATER ALGAE STRAINS AS IMMUNSTIMULANTS FOR STERLET (*Acipenser ruthenus*) JUVENILES

Zsuzsanna J. Sándor[1], Janka Biró[1], Ottilia Kóbori[2], Ágnes Dergez[2], Sai Divya Kanna[3], Ildikó Domonkos[3], Bettina Ughy[3], László Ardó[1]

[1] Research Centre for Aquaculture and Fisheries (HAKI), Hungarian University of Agriculture and Life Sciences – H-5540 Szarvas Anna-liget u. 35, Hungary
[2] Institute of Plant Biology, Biological Research Centre (BRC), Eötvös Loránd Research Network, H-6726 Szeged Temesvári krt.62, Hungary
[3] Bay Zoltán Nonprofit Ltd. for Applied Research, H-6726 Szeged Derkovits fasor 2, Hungary
* E-mail: jakabne.sandor.zsuzsanna@uni-mate.hu

Introduction

High density of fish in intensive aquaculture systems (recirculation system or cages) is permissive to growth of bacterial and non-bacterial pathogens. Bacterial infections are usually prevented and treated by utilization of chemical disinfectants and antibiotics, respectively, which pose a huge threat to environment and human health. Therefore, alternative strategies must be developed to improve fish health and to prevent fish diseases. For this purpose, the use of feed additives such as immunostimulant is considered to be a promising area in aquaculture. There is a great potential for freshwater algae to become feed components in intensive fish farming. In order to be used in aquaculture, a microalgae strain has to meet various criteria. It has to be easily cultured and non-toxic. It also needs to have high nutritional quality and digestible cell wall to make nutrients available. This study aimed to evaluate the suitability of new microalgae strains isolated from Hungarian water bodies in diets for sterlet (*Acipenser ruthenus*) juveniles.

Materials and Methods

The strains were selected based on their growth capacity and content of potential immunostimulant agents (like PUFA, carotenoids, beta glucan, etc.) against diseases specific to early-stage fish cultures. The immunostimulant potential of the algae strains were tested *in vivo* in juveniles of sterlet (initial weight 56.1g). The feeding experiment with different inclusion levels of algae biomass (1, 2 and 4% dry weight basis) were performed in intensive recirculation fish rearing system. The enrichment of the basal diet with algae suspension was done using simple kitchenware technique (Table 1.). The fish growth was recorded at the end of trial, on the 28th day. Blood samples were taken weekly from 2 individuals/tank in order to determine the non-specific immunological parameters as lysozyme, total protein and immunoglobulin levels. Beside these parameters the production parameters and nutrient utilization of the feeds were evaluated. At the end of the trial ten fish from each tank were transported to the infection recirculation system of the institute where bacterial infection with *Aeromonas hydrophila* was implemented and the mortalities were recorded during five consecutive days.

Results and Discussion

The determined growth parameters after feeding trial such as final weight, weight gain and specific growth rate ranged between 82-89g, 50-57% and 1.36-1.68 %/day, respectively, without significant differences between groups. However, the highest values were recorded in the group of fish with 4% of algae inclusion in all investigated parameters. Similar tendency was observed in the nutrient and protein utilization parameters (FCR and PER). Based on this we could conclude that the new algae isolate has favorable impact to the growth and nutrient utilization of juvenile sterlet.

The investigated non-specific immune parameters (Table 2.) revealed a slightly positive effect to the immune status of the fish fed with algae enriched feed compared to the control. Meantime significant increase was found in the parameters during the feeding period compared to the values measured before feeding. After infection the recorded mortalities were not significantly differing between groups but higher mortality rate was observed in the fish fed without algae inclusion.

Acknowledgement

The financial support of GINOP-2.3.2-15-2016-00058 project of Hungarian National Research, Development and Innovation Office is gratefully acknowledged.

APPARENT DIGESTIBILITY COEFFICIENTS (ADC) OF DIFFERENT INSECTS FOR JUVENILE AFRICAN CATFISH (*Clarias gariepinus*)

Zsuzsanna J. Sándor[1], Jenő Káldy[1], Vojislav Banjac[2], Rita T. Farkas [3], Nóra A. Kisbocskói[3], Janka Biró[1]

[1] Research Centre for Aquaculture and Fisheries (HAKI), Hungarian University of Agriculture and Life Sciences, Anna-liget u. 35, Szarvas, Hungary
[2] Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara br.1, Novi Sad, Serbia
[3] Institute for Food Science, Hungarian University of Agriculture and Life Sciences, Herman Ottó u. 15, Budapest, Hungary
* E-mail: jakabne.sandor.zsuzsanna@uni-mate.hu

Introduction

Several feeding experiments with insects were carried out on many aquaculture species in the last ten years, and the results so far are encouraging for their industrial production (Gasco et al, 2020). However the available nutritional characteristics of the insects are well investigated, knowledge on their digestibility in different fish species is limited. Together with chemical analysis, digestibility of the nutrients and energy may allow a more thorough estimation of the nutritive value of a particular protein source in a complete feed for fish. Scarce information exists on its utilization as feed ingredient in nutrition for intensively reared African catfish, an important and dominant aquaculture species in Hungary. Meals from insects such as the shea caterpillar (*Cirina butyrospermi*), housefly (*Musca domestica*) variegated grasshopper (*Zonocerus variegatus* L.) and black solider fly (*Helmetia illucens*) have been included in the diets of African catfish as alternate protein sources. The aim of our study was to study the digestibility of black soldier fly (BSL), yellow mealworm (*Tenebrio molitor*) (MW) and blue bottle fly (*Calliphora vicina*) (BBF) meals for African catfish juveniles.

Materials and Methods

The digestibility trial was conducted with African catfish hybrid (*Clarias gariepinus x Heterobranchus longifilis*) juveniles when we aimed to determine the apparent digestibility coefficients (ADC) for dry matter, protein, lipid, phosphorus, fatty acids and amino acids, chitin and gross energy. The feeds were formulated by using a control feed in 70% mixed with tested ingredients in 30% (as is). The control feed was prepared to be a high fish meal diet in order to be easyily digestible for fish. Nine hundred African catfish juveniles (average weight, 217.4 ± 9.5 g) were distributed in recirculation water system equipped with twelve 1m^3 fiberglass tanks. During feeding period, the fish were hand-fed to apparent satiation. At the end of feeding period 15 pc from the fish stock per tank were sacrificed in order to collect faeces from intestine. From each treatment the faecal samples were pooled, freeze dried and stored in exicator until analysis. The chemical compositions of test ingredients, feeds and faeces were analysed using standard analytical methods. The apparent digestibility coefficients for the test ingredients and diets were calculated as follows:

$$ADC_{diet} = [1 - (\{Y_{diet} / Y_{faeces}\} \times \{D_{faeces} / D_{diet}\})] \times 100$$

Where, Y_{diet} is the dietary yttrium level, Y_{faeces} is the faeces yttrium level, D_{diet} is the dietary nutrient level and D_{faeces} is the faeces nutrient level.
The apparent digestibility coefficients of the test ingredient (BSL, MW, BBF) were calculated as follows:

$$ADC_{ingredient} = ADC_{test\ diet} + [(ADC_{test\ diet} - ADC_{reference\ diet}) \times (0.7 \times D_{ref} / 0.3\ D_{ingredient}]$$

Where, D_{ref} is the % nutrient (or kJ g^{-1}) of reference diet (dry matter basis) and D_{ingr} is the % nutrient (or kJ g^{-1}) of insect ingredient (dry matter basis).

(Continued on next page)

Results and Discussion

The ADC of the test ingredients are presented in Figure 1. The protein digestibility of the BSL is in line with data presented for hybrid grouper (*Epinephelus fuscoguttatus* ♀ *x Epinephelus lanceolatus* ♂) (81-88%) (Mohamed-Zulkifli et al, 2019), higher than for turbot *Psetta maximus* (63.1%) (Kroeckel et al, 2012) and less than for Atlantic salmon (*Salmo salar*) (89%), but regarding MW our data are much lower compared to tilapia (Fontes, 2019) (85.4%). The chitin digestibility is relatively high for BSL (96%), and significantly differing from others. The ADC data on chitin in our study reflect that African catfish is able to digest the chitin from the investigated insects in different ratio, moreover these data are comparable with ADC_{chitin} data obtained in tilapia (Fontes et al, 2019). It was assumed that $ADC_{Protein}$ negatively correlate with chitin and acid detergent fibre (ADF) content of the feed (Marono et al, 2015). Chitin content of MW diet in our study is 2.64 % (as fed) and ADF level is high (10.1% as fed) compared to the control feed (2.13% as fed). The ADF content of TM used in this trial was much higher (27.7 % dry matter) than data reported in other studies (7-11% d.m Marono et al, 2015; 7.2% Piccolo et al, 2017). The chitinase activity was not measured in our case and there are no available data for African catfish as well, therefore explanation of the reduced digestibility could not be proven either with chitinase activity of the fish or with chitin content of the diet.

Comparing these ADCProtein data with other feed ingredients it surely could be stated that insects' meals are better digested than the plant feedstuffs.

Acknowledgement

This study has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2020-4.1.1-TKP2020 funding scheme.

References

Fontes, T.V., de Oliveira, K.R.B., Gomes Almeida, I.L., Maria Orlando, T., Rodrigues, P.B., Costa, D.V., Rosa, P. V. (2019). Digestibility of insect meals for Nile Tilapia fingerlings. *Animals*, *9*, 181.

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P. P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., & Roncarati, A. (2020). Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. *Italian Journal of Animal Science*, *19*(1), 360–372.

Kroeckel, S., Harjes, A. G. E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C. (2012). When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute - Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*, 345–352.

Marono, S., Piccolo, G., Loponte, R., Meo, C. Di, Attia, Y.A., Nizza, A., Bovera, F. (2015). In vitro crude protein digestibility of tenebrio molitor and hermetia illucens insect meals and its correlation with chemical composition traits. *Ital. J. Anim. Sci.* 14, 338–343.

Mohamad-Zulkifli, N. F. N., Yong, A. S. K., Kawamura, G., Lim, L. S., Senoo, S., Devic, E., Mustafa, S., Shapawi, R. (2019). Apparent digestibility coefficient of black soldier fly *(Hermetia illucens*) larvae in formulated diets for hybrid grouper (*Epinephelus fuscoguttatus* ♀ *x epinephelus lanceolatus* ♂). *AACL Bioflux*, *12*(2), 513–522.

Piccolo, G., Iaconisi, V., Marono, S., Gasco, L., Loponte, R., Nizza, S., Parisi, G. (2017). Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). *Animal Feed Science and Technology*, *226*, 12–20.

ASSOCIATING SEA BREAM, OYSTER, CLAM AND SHRIMP IN AN EARTHEN POND LOOP: TOWARD AN ENVIRONMENTALY FRIENDLY SYSTEM

Christophe Jaeger[1]*, Vincent Gayet[2], Joël Aubin[1]

[1] UMR SAS, INRAE, Institut Agro, 35000 Rennes, France
Christophe.Jaeger@inrae.fr
[2] Lycée de la mer et du littoral, rue William Bertrand, 17 560 Bourcefranc-le-Chapus, France

Introduction

In aquaculture, loss of nutrients in the surrounding environment is an important concern. The benefits of associating aquatic organisms to use nutrients released from fish farming is well reported in the literature[1], but in general the documented applications at a commercial scale remain scarse. In the same time, Fish oil and fishmeal included in feed are also pointed out to contribute to the depletion of natural resources and to be in competition with direct human consumption[2]. Hence, to maximize the use efficiency of nutrients, the system presented was designed to associate three different organisms of commercial interest and differing from seabream (*Sparus aurata*) for their trophic level, while using only local vegetal raw materials in the formulated fish feed. In addition, fresh discarded mussels, were directly supplied to the fish to balance their micronutrient and fatty acids needs. Thus, the aim of this study was to assess the performances of this system, by monitoring the water quality and the growth performances of the different organisms reared.

Materials and methods

The system was tested from 9th June to 7th October 2020. It was composed of 4 ponds, connected in cascade, to circulate the water by gravity, according to the following order (Figure 1): (i) in pond 7, seabreams were stocked at a mean weight of 210 g; (ii) in ponds 6 and 5, oysters (*Crassostrea gigas*) and shrimps (*Penaeus japonicus*) were stocked at a mean weight of 47.5 g and 0.52 g, respectively; (iii) in pond 4, clams (*Ruditapes decussatus*) and shrimps were stocked at a mean weight of 3.4 g and 0.52 g, respectively. From the pond 4, water was pumped back to the pond 7. Since salinity remained at an appropriate level in the system during the experiment, water was added from the inlet conduct (connected to the open sea) only to compensate evapotranspiration, avoiding discharge water toward the surrounding environment. During the experiment, the fish, shrimp and bivalves were weighed to estimate the growth. The water quality was weekly recorded to control temperature, oxygen, pH, turbidity and salinity. Once a month, water was sampled in each pond for concentrations in phytoplankton, nitrogen compounds and phosphorus compounds.

Results

The mean concentration in oxygen for the entire period of experiment was significantly ($p<0.05$) lower in the pond 7 (6.5 ± 1.8 mg/l, n=13) than in the ponds 5 (9.5 ± 2.6 mg/l, n=13) and 4 (9.8 ± 2.8 mg/l, n=13), and in the inlet water conduct (8.7 ± 1.4 mg/l, n=9). An increase of the concentration in total chlorophyll was observed in the system during the cycle of production (from 2.5 µg/l in June to 60.6 µg/l in October) contrary to that in the inlet water conduct, in which the concentration remained quite constant (between 5.1 µg/l in June and 11.0 µg/l in October). No significant differences were observed ($p<0.05$) between compartments, in the mean concentrations in N (N total, NH4, NO2 and NO3) and P (P total and PO4). Nevertheless, the level of the N total in the ponds of the system was higher compared to the inlet water channel, in which the N total remained almost stable along the production cycle time. The mean values of P-PO4, were significantly lower ($p<0.05$) in all the ponds of the system than that in the inlet water conduct. Despite a period of adaptation due to changes in feed and environmental conditions, seabreams grew well according to the expected performances. Thus, 1 169 seabream were harvested at a mean weight of 352.4 g, with a survival rate of 86.6%, and a corrected FCR of 1.9. At the harvest, the mean weight of the shrimps from the pond 4 was significantly ($p<0.01$) higher (35.2 ± 10.0 g, n=120) than that from the pond 5. Unfortunately, the week before the harvest, a storm event depleted the oxygen in the water and all shrimps in the pond 6 died. The rearing performances of oyster were higher than expected. Despite a summer mortality syndrome observed in the area, the survival rate of oysters observed was 91% for the pond 4 and 87% for the pond 5. At the harvest, the mean weight of the oysters was significantly ($p<0.01$) better in the pond 5 (88.4 ± 20.6 g, n=89) than in the pond 6 (71.2 ± 10.4 g, n=89), as well as the mean filling ratio (21.5 ± 3.3 %, n= 50 and 18.8 ± 2.5 %, n=50, respectively). The number of clams harvested was estimated to 8 102, representing a survival rate of 69%, for a mean weight of 11.4 ± 3.3 g (n=100). Harvested clams had a filling ration of 20.0 ± 3.6% (n=50) which was significantly ($p<0.01$) higher than that measured in June (16.1 ± 3.0%, n=20).

(Continued on next page)

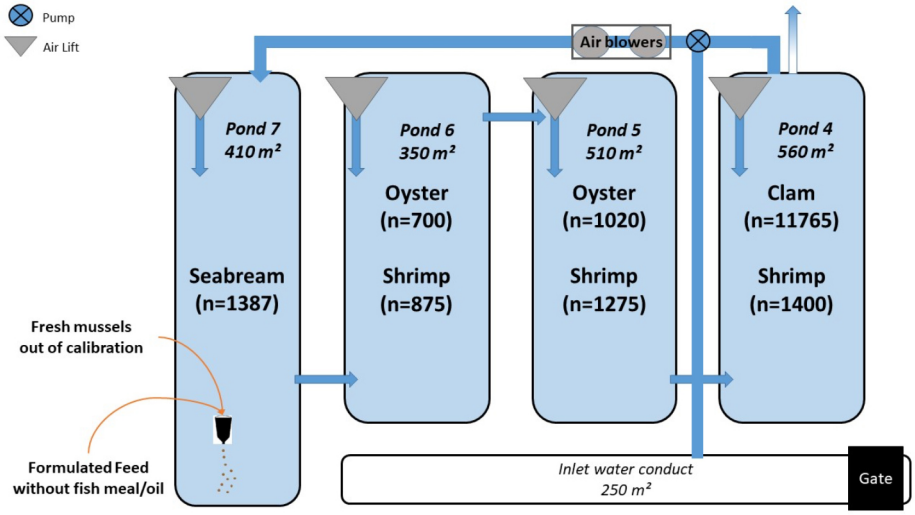


Figure 1: representation of the experimental design with the number (n) of individuals at the beginning

Discussion-Conclusion

The water concentration in oxygen was a key parameter of the system. In the fishpond, oxygen was at low levels in the morning despite the air adduction, so a special attention was paid to that in postponing the feeding time to the afternoon. The different concentrations in N and P, and physical indicators in water, like oxygen concentration, observed in the pond 4, reflected the ability of the system to improve the quality of the water released from the fishpond. Moreover, no water was discharged from the system along the rearing period, resulting in the save of water and in the preservation of the surrounding environment. In the system tested, good results were obtained for fish growth and feed conversion ratio, despite the lack of fishmeal and fish oil in the feed. Without additional specific source of nutrients in the system, except than the feed supplied to the sea bream, the body growth of the others organisms reared were similar and even higher (filling ratio and survival rate of the molluscs) compared to their usual monoculture in ponds (based on expert experience). Variations in the performances of the different species among the ponds give interesting perspectives in the improvement of this IMTA system, especially on optimization of animal densities and in adding a compartment dedicated to seaweed cultivation.

Gunning, D., et al., 2016. The Development of Sustainable Saltwater-Based Food Production Systems: A Review of Established and Novel Concepts. Water 8(12).

Tacon, A.G.J., Metian, M., 2009. Fishing for Feed or Fishing for Food: Increasing Global Competition for Small Pelagic Forage Fish. Ambio. 38, 294-302.

IMPAQT UNDERWATER ACOUSTIC TELEMETRY PLATFORM: RECEIVER DESIGN

Hamed Jafarzadeh, Marco Belcastro, Brendan O'Flynn

Tyndall National Institute, University College Cork, Cork, Ireland.
E-mail: hamed.jafarzadeh@tyndall.ie

Introduction

By 2050 world population will reach approximately 10 billion people, and it will requires more food production [1]. Seafood is one of the main sources of nutrition and consequently, its production should be increased to support the demand. Integrated multi-trophic aquaculture (IMTA) refers to the co-culture of the aquatic species including the extractive species that use the waste or feed leftovers of other species [2]. IMTA is gaining popularity as a sustainable aquaculture method that also minimizes environmental impacts and provides economical benefits. To maximize IMTA benefits, farmers need to gain a good insight on water quality parameters and chemical substances which can be done using manual sampling of the water or using off-the-shelf sensors.

As part of the European IMPAQT project, we are developing a universal telemetry platform that can be integrated with external sensors to collect the sensory information and transmit them wirelessly underwater to the receiver gateway, which will save the collected information for manual downloading or can transmit to an inland station using Long Range (LoRa) radios. The proposed telemetry platform enables the farmers to have relatively better and almost instant insight over the IMTA sites.

Platform

Our proposed platform, shown in Figure 1, consists of several miniaturized transmitter nodes and a receiver node that collects the sensory information of attached sensors. IMPAQT ultrasonic transmitters are designed in a miniaturized form factor that will run on a battery and they can be connected to external sensors using Serial Peripheral Interface (SPI) protocol or Universal asynchronous receiver-transmitter (UART) protocol to collect their information and transmit it in real-time to the receiver gateway. Transmitter design has been discussed in [3].

The receiver gateway is running on a battery, and it can be attached to a buoy with a connected hydrophone immersed into the water, and it receives information from the transmitter nodes. The received information can be collected on a SD Card and it also can be transferred to an inland receiver station for monitoring or further processing.

The receiver board has 3 main stages which is shown in Figure 2.

The signal reception stage receives the hydrophone signal, pre-amplifies the signal, and then drives the hydrophone signal for the filter stage. There is also a variable gain amplifier that is controlled via Inter-Integrated Circuit (I2C) protocol [4] by STM32 microcontroller to amplify the signal to the required level before feeding into the digitalization stage. We chose the On-Off keying (OOK) modulation technique as it is the most favourable to the battery life of the miniaturized transmitters and there is a relevant OOK demodulator on the receiver side to demodulate the received signal.

Results

The transmitter and receiver boards are manufactured, and tests were successful. We managed to connect external sensors to the platform and transmit the sensor's data underwater and receive it using the receiver board. We initially managed to achieve 40 bit per second bitrate at 42KHz carrier frequency. The battery life, bitrate, and deployment results will be presented in the oral presentation.

Future Work

Future work involves increasing the carrier frequency to shorten the active transmission period to increase the overall battery life and also increase the bitrate. It would be also interesting to evaluate binary-phase-shift keying modulation and compare the results with OOK demodulation regarding battery life and signal noise ratio between two. Also, there is an opportunity to perform edge-processing on the receiver board, to inform emergency events, for instance, early detection of excessive number of toxic substances in the sites.

(Continued on next page)

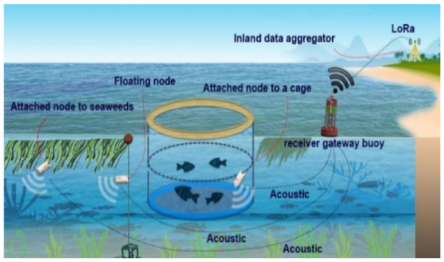
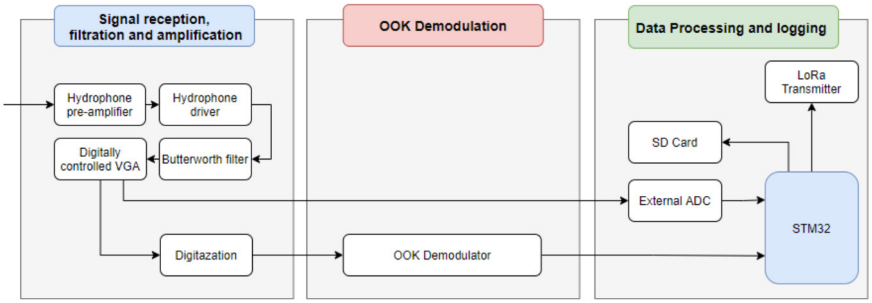


Figure 1. IMPAQT telemetry platform

Figure 2 IMPAQT Receiver system design



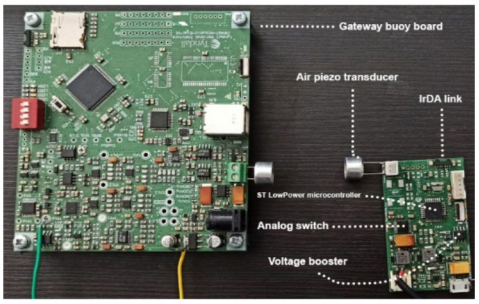


Figure 3 IMPAQT boards

Acknowledgments: This work is part of IMPAQT project (https://impaqtproject.eu/) – and it has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 774109. Aspects of this publication have been funded in part by a research grant from Science Foundation Ireland (SFI) - co-funded under the European Regional Development Fund under Grant Number 16/RC/3835 – VISTAMILK and 13/RC/2077 - CONNECT.

[1] "World Population Prospects - Population Division - United Nations." https://population.un.org/wpp/Graphs/DemographicProfiles/Line/900 (accessed Sep. 16, 2020).

[2] J. Rosa *et al.*, "Integrated multitrophic aquaculture systems – Potential risks for food safety," *Trends Food Sci. Technol.*, vol. 96, pp. 79–90, Feb. 2020, doi: 10.1016/j.tifs.2019.12.008.

[3] H. Jafarzadeh, M. Belcastro, and B. O'Flynn, "IMPAQT Miniaturized Underwater Acoustic Telemetry Platform: Transmitter Node System Design," Nov. 2020, pp. 34–37. Accessed: May 14, 2021. [Online]. Available: http://www.thinkmind.org/index.php?view=article&articleid=sensorcomm_2020_1_60_10031

[4] B.-C. Renner, J. Heitmann, and F. Steinmetz, "ahoi: Inexpensive, Low-power Communication and Localization for Underwater Sensor Networks and μAUVs," *ACM Trans. Sens. Netw.*, vol. 16, no. 2, p. 18:1-18:46, Jan. 2020, doi: 10.1145/3376921.

IMPACT OF TRANSPORT ON SUBSEQUENT ROE ENHANCEMENT OF THE SEA URCHINS *Stronylocentrotus droebachiensis* AND *Paracentrotus lividus*

P. James*, T. Evensen, C. Hannon, L. Gutiérrez

*Nofima, 9-13 Muninbakken, Tromsø, Norway
Email: philip.james@nofima.no

Introduction

There is growing interest in Europe and worldwide in the capture and roe enhancement of various sea urchin species. There has been a significant amount of research focused on feed development and protocols for sea urchin roe enhancement. Less focus has been directed on the protocols for, and the impact of, transport on subsequent survival, and roe enhancement of sea urchins. This is a crucial step in the value chain as sea urchins need to be transported from the point of harvest to enhancement facilities as well as onwards to markets (sometimes via live holding hubs). This study describes some of the research being undertaken to understand what the stressors are for sea urchins during transport, particularly when this is followed by roe enhancement.

Materials and methods

A series of experiments were run to determine the optimal transport methods for *Strongylocentrotus droebachiensis,* in terms of both 'in water' (road and sea) and 'out of water' (road and air) transportation. This presentation will focus on a simulated transport trial followed by a roe enhancement trial to measure the effect of transport stress on subsequent roe enhancement. Sea urchins were held in simulated transport (static seawater tanks with aeration) for 0 days, 7 days and 14 days at 2 different densities. They were then transferred to a roe enhancement system and enhanced for 8 weeks. At the conclusion of that period the increase in sea urchin gonad index (GI) and the biochemical quality of the roe was measured. Additional trials have been conducted on *Paracentrotus lividus* to see how they compare in terms of sensitivity to transport stress. This presentation will focus on the former species but will discuss possible implications for the latter.

Results and Discussion

Overall, sea urchins held in simulated transport (static seawater tanks with aeration) for 0 days, 7 days and 14 days showed no significant differences in GI after the enhancement period (Figure 1). There was also no significant difference in the final GI of sea urchins held at low (4kg) or high (8kg) densities during the transport or between sea urchins transported for 7 days or for 14 days prior to enhancement (Figure 1).

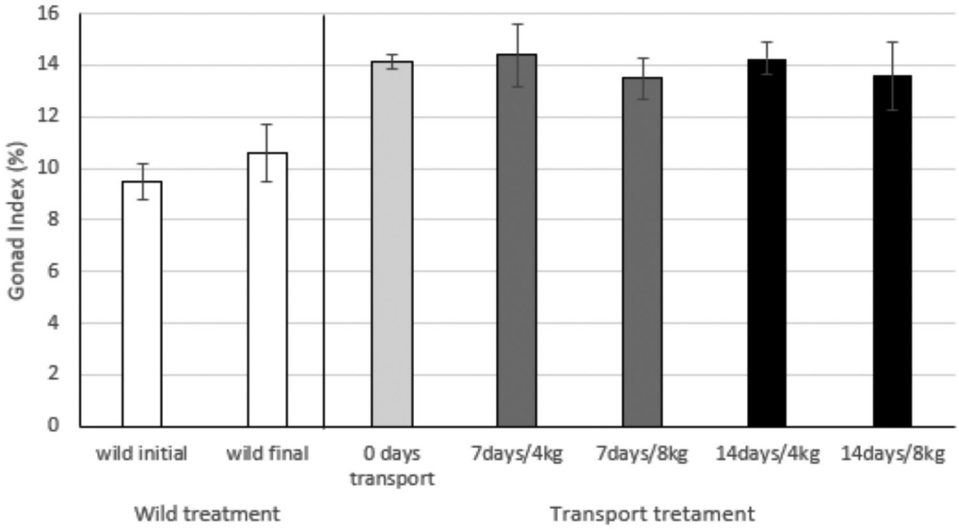


Figure1: The final GI of sea urchins collected from the wild compared to those held for 0, 7 and 10 days simulated transport followed by an 8 week enhancement period.

(Continued on next page)

Table 1: The % mortality of sea urchins held for 0, 7 and 10 days simulated transport followed by an 8 week enhancement period.

Treatment	Mortality (%)
0 days transport	2.0
7 days / 4kg	10.4
7 days / 8kg	8.3
14 days / 4kg	14.5
14 days / 8kg	10.41

However, there were differences in sea urchin mortality between sea urchins transported 0 days vs all simulated transport treatments. There was also higher mortality in sea urchins transported for 14 days compared to 7 days (Table 1). The biochemical results will also be presented (not yet available).

Preliminary testing on *P. lividus* suggest this species is much more sensitive to transport stress than *S. droebachiensis*. All the results from this and a series of studies will be discussed in terms of the future development of a sea urchin roe enhancement industry and what role transport plays in this.

EFFECTS OF BLUE MUSSEL MEALS WITH VARYING ASH LEVELS ON TROUT PERFORMANCE

J. Jaxion-Harm*

TripleNine Group, Trafikhavnskaj 9, 6700 Esbjerg, Denmark
E-mail: jjh@999.dk

Introduction

Mussels are an important fishery industry that has primarily focused on harvesting for human consumption. Undersized mussels are generally discarded or restocked for further growth. A special case of undersized mussel production has been used to reduce the effects of eutrophication, and this "mitigation mussel" culture is expanding. Nutrients leading to eutrophication is recaptured in the mussel tissue and can be used as a valuable source of protein, lipids, and other nutrients. There are limited studies testing the effects of blue mussel on aquaculture fish, and most replace fishmeal in high fishmeal diets (17%-50% of diet, e.g. Berg and Austreng 1989; Larsen et al. 2015). The present study aimed to uncover the effects that replacing fishmeal with blue mussel meal would have on commercially relevant (low fishmeal) trout diets. Furthermore, the study investigates two types of mussel meal- one with shell completely removed and one with shell only reduced.

Materials and Methods

Rainbow trout (starting weight 50g +-) were fed low fishmeal (10%) diets with 5% protein and 25% fat. Fishmeal was replaced with either 50% or 75% mussel meal. Two types of mussel meals were tested- mussel meal with shell removed before cooking (MM, ash =) and a whole mussel meal with sieving to reduce shell content after drying (SMM). The last diet was supplemented with fish-sourced phospholipids to the control diet level.

Rainbow trout (9kg/tank) were distributed in three 1.17x1.17x 0.9 m tanks per diet, and mean water temperature was 16ºC during the experiment. Diets were randomly distributed amongst tanks. Fish were sampled after 30 days and 60 days via individual weighing, while number of dead fish was recorded daily. At the start and the termination of the trial, HSI was also measured from 7 fish per tank.

Results and Discussion

Overall survival of rainbow trout in the present study was high (more than 98%) and was not affected by diet. However, growth was affected by diet: SGR of fish fed MM5% tended to be higher than the fishmeal control and was significantly higher than for fish fed MM7.5% and SMM7.5%. FCR of rainbow trout was similar with fish fed MM5% having the lowest FCR. Because of differences in ash content amongst diets, there was a slight variation of digestible energy. When FCRe was calculated (i.e. based on digestible feed intake, no significant differences were observed.

Previous research shows that effects of mussel meal replacement of fishmeal are dependent ash content of mussel meal. Replacement of fishmeal in high fishmeal diets of rainbow trout with whole mussel meal (with shell) resulted in decreased growth due to low digestibile energy of the feed from high ash content (Berg and Austreng 1989). However, full replacement of fishmeal with de-shelled mussel meal resulted in no differences in growth of rainbow trout fed ad-libitum (Larsen et al. 2015). Poor performance of trout fed SMM corroborates the need to reduce the ash content of mussel meal by removing shells as part of production is essential to improving the energy content of the ingredient.

Berg and Austreng (1989) found that fish fed whole mussel meal (high ash) resulted in increased liver size which was hypothesized to be due to physiological stress. However, the present study did not find any differences in hepatosomatic index (HSI) amongst diets.

The results verify that Blue Mussel meal can be used to replace fishmeal up to 50%, even in low fishmeal diets. With a growing aquaculture market needed to sustain the nutritional security of an increasing population, the need for sustainable marine ingredients is strong.

(Continued on next page)

References

Berge, G. M., & Austreng, E. (1989). Blue mussel in feed for rainbow trout. Aquaculture, 81(1), 79-90. doi:https://doi.org/10.1016/0044-8486(89)90232-9

Larsen, B. K., Tang Dalsgaard, A. J., Pedersen, P. B., Larsen, O. M., Poulsen, E., Jensen, R. F., . . . Jokumsen, A. (2015). *Substitution of fishmeal with mussel meal in rainbow trout diets*. Retrieved from

Weis and Buck 2017- no shell, turbot 50% fishmeal, could repalce up to 25% of FM

Lagel et al – Turbot. BMPC (blue mussel with extracted fat removed). 34% positive control, 17% fishmeal neg control. Replace 10, 20, 50% of neg contr fishmeal with BMPC. No changes in performance, only tendency for liver enlargement

FIRST EUROPEAN EEL LARVAE PRODUCED USING RECOMBINANT FSH AND LH - *IN VIVO* AND *IN VITRO* EFFECTS ON OOCYTE MATURATION AND REPRODUCTIVE SUCCESS

Pauline Jéhannet [a]*, Arjan P. Palstra [a], Ignacio Giménez Nebot [b], William Swinkels [c], Leon T.N. Heinsbroek [a,d], Hans Komen [a]

[a] Animal Breeding and Genomics, Wageningen University & Research, PO Box 338, 6700 AH Wageningen, The Netherlands
Email: pauline.jehannet@wur.nl.
[b] Rara Avis Biotec S.L., Calle Moratín, 17 - 4º, 46002 Valencia, Spain
[c] Palingkwekerij Koolen BV, Hongarijesedijk 12, 5571 XC Bergeijk, The Netherlands
[d] Wageningen Eel Reproduction Experts B.V., Mennonietenweg 13, 6702 AB Wageningen, The Netherlands

Introduction

The current protocol for the induced maturation of female European eel consists of long-term weekly injections with carp or salmon pituitary extract (C/S PE) to induce oocyte growth, a single booster PE injection and a single 17α,20β-dihydroxy-4-pregnen-3-one (DHP) injection to induce oocyte maturation and ovulation. However, egg quality is often poor and when larvae are produced they die before exogenous feeding. This study describes the third *in vivo* experiment in a series of ongoing experiments in our lab aiming to replace PE treatment by highly stable eel-specific recombinant gonadotropins (Peñaranda et al, 2018) in order to improve gamete quality and reproductive success. In an earlier study, we have been able to fully mature European eel and strip good quality eggs by treatment with recombinant gonadotropins but no larvae hatched (Palstra et al., 2019). In this third *in vivo* experiment, we have stimulated the sexual maturation by treatment with (i) CPE, (ii) recombinant FSH (recFSH) followed by CPE, and (iii) recFSH followed by recombinant LH (recLH), and compared the effects on reproductive success. Additionally, to focus the study on dose and effects of recombinant LH in boosting oocyte maturation, we have set-up an *in vitro* system and compared recLH with CPE as control.

Materials and methods

For the *in vivo* trial, experimental eels were feminised, subjected to simulated migration (Mes et al., 2016) and treated with a steroid implant (Thomson-Laing et al., 2019) containing 17-methyltestosterone and E2. Thirty eels were then divided over three groups and treated as described in Table 1. Oocyte maturation was boostered by injecting CPE or by injecting recLH (Table 1). Ovulation for eels in all groups was induced by injecting DHP (2 mg kg^{-1}). Eels were stripped for eggs which were fertilised and reared.

For the *in vitro* trial, experimental eels were feminised, subjected to simulated migration and treated with a steroid implant. Ten females were weekly injected with CPE to induce oocyte growth. Just before the CPE booster, ovarian tissue (~5g) was retrieved and kept on ice-cold culture medium. After the oocytes had been dispersed by pipetting the ovarian pieces, approximately 60 oocytes were placed per well in triplicate per treatment in a 24-well culture containing 1mL of hormone-free media and 1mL medium supplemented with the treatment (CPE: 1.25, 12.5 and 125 ug/mL; RecLh: 10, 100, 1000 ng/mL). Just before incubating, oocytes were sampled for microscopy, histology, RTPCR and medium analysis for DHP measurements. Culture plates were then incubated at 16°C for 12 and 18h. After incubation, oocytes were sampled for the same purposes.

Table 1. The three groups and the weekly injections they received. CPE= carp pituitary treatment. recFSH= recombinant follicle stimulating hormone. recLH= recombinant luteinising hormone.

Group	weeks	recFSH (µg)	recLH (µg)	CPE (mg kg^{-1})
1	1-12			20
	13-15			20
	16-18			20
	>18			20
	Booster			20
2	1-12	12		
	13-15	12		10
	16-18	6		20
	>18			20
	Booster			20
3	1-12	12	10	
	13-15	12	20	
	16-18	6	20	
	>18		20	
	Booster		20	

(Continued on next page)

Table 2. Reproductive success of eels from the three treatment groups (CPE, recFSH-CPE and recFSH-recLH) with the number of eels that started the treatment, died during the treatment, matured, died after DHP, gave eggs, embryos and larvae. Larval longevity (i.e. the number of days post hatch that larvae survived - dph) was daily recorded.

Treatment	Start	Died	Mature	Died DHP	Eggs	Embryos	Larvae	Longevity (dph)
CPE	10	1	9	5	4	2	2	6, 18
recFSH-CPE	10	4	6	4	2	1	1	2
recFSH-recLH	10	3	7	4	3	1	1	2

From the *in vitro* trial, analyses are currently being executed but results will be presented.

Results and discussion

Of the ten eels that were treated with CPE *in vivo*, one eel died during treatment, seven matured after 7-9 weeks, two matured after 12-13 weeks. Four of these eels could be stripped and two eels gave larvae that survived up to 6 and 18 days post hatch (Table 2). Of the ten eels that were treated with recFSH and CPE, four eels died during treatment, others matured much later after 15-22 weeks. Two of these eels could be stripped and one eel gave larvae (Table 2). Of the ten eels that were treated with recFSH and recLH, three eels died during maturation, others also matured after 15-22 weeks. Three eels could be stripped and one eel gave larvae (Table 2). Three eels that had been treated with recFSH died after DHP injection with GSI values of 75, 77 and 80 which are enormous in comparison with the regular average values of 40-50 after CPE treatment.

From the *in vitro* trial, analyses are currently being executed but results will be presented.

Acknowledgements: The authors thank the DUPAN foundation; The Dutch Ministry of Economic Affairs and the European Union, European Maritime and Fisheries Fund, and partners of the international EELRIC consortium (www.eelric.eu).

References

Peñaranda, D.S., Gallego, V., Rozenfeld, C., Herranz-Jusdado, J.G., Pérez, L., Gómez, A., Giménez, I., Asturiano, J.F., 2018. Using specific recombinant gonadotropins to induce spermatogenesis and spermiation in the European eel (Anguilla anguilla). Theriogenology 107, 6–20.

Palstra, A.P., Giménez Nebot, I., Jéhannet, P., Lokman, P.M., Swinkels, W., Heinsbroek, L.T.N., 2019. Testing recombinant gonadotropins for the propagation of European eel (Anguilla anguilla), pretreated by feminization, simulated migration and steroid implants. Aquaculture Europe, October 7-10, Berlin (Germany).

Mes, D., Dirks, R.P., Palstra, A.P., 2016. Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*). Aquaculture 452, 367–372.

Thomson-Laing, G., Damsteegt, E.L., Nagata, J., Ijiri, S., Adachi, S., Todo, T., Hiramatsu, N., Lokman, P.M., 2019. Synergistic effects od estradiol and 11-ketotestosterone on vitellogenin physiology in the shortfinned eel (*Anguilla australis*). Biol. Reprod. 100, 1319–1332.

Salicornia ramosissima BIOMASS INCORPORATION IN DIETS FOR JUVENILE SEA BASS (*Dicentrarchus labrax*): EFFECTS ON GROWTH, SURVIVAL, WHOLE-BODY COMPOSITION AND NUTRIENT DIGESTIBILITY

D. Jerónimo[*1]; B. Silva[1]; F. Cruz[2], A. Couto[2]; J. Dias[3]; B. Costas[2]; R.J.M. Rocha[1,4]

[1]RIASEARCH Unipessoal Lda, Murtosa (Portugal)
[2]CIIMAR, Matosinhos (Portugal)
[3]SPAROS Lda., Olhão (Portugal)
[4]CESAM & DeBio, Universidade de Aveiro (Portugal)
Email: danieljeronimo@riasearch.pt

Introduction

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 cultivation areas. Their richness in bioactive secondary metabolites, which can have health promoting effects for the consumer, is a distinctive quality of these plants. The tenderest stems of Salicornia can be directed for human nutrition and the remaining parts of the plant, which can be considered as a residual co-product, have great potential for animal nutrition. Valorization of such co-products is paramount to develop a sustainable aquaculture feeds following principles of circular economy. Although fairly low in protein (9-10%), this Salicornia co-product may potentially serve as a source of carbohydrates in fish feeds, allowing for a reduction on the use of cereal eatable crops like wheat. European sea bass (*Dicentrarchus labrax*) is one of the most representative species from the aquaculture industry and therefore, the present study aimed to evaluate the potential of aerial *S. ramosissima* biomass to integrate aquafeed formulations for juveniles of this species. The sea bass growth performance, survival, body composition and nutrient digestibility were evaluated during this work.

Methods

Four experimental diets were tested in triplicate: a commercial like diet (CTRL) and three experimental diets containing *S. ramosissima* biomass at 2.5%, 5% and 10% inclusion levels (SL2.5, SL5 and SL10, respectively), at the expenses of wheat. European sea bass juveniles (initial mean wet weight 7.3 g) were kept at around 22 ºC and fed *ad libitum* for 62 days. At the end of the trial, fish were weighed and counted for growth performance and survival determination. Additionally, a digestibility trial was conducted and feces collected daily according to the Guelph system (each tank provided with a feces settling column). Fish were fed *ad libitum* 7 days a week, and temperature maintained at 22ºC.

Results and discussion

No significant differences in growth performances, feeding and survival were observed among treatments (Table 1), suggesting that the inclusion of *S. ramosissima* biomasses in the levels tested did not compromise the nutritional adequacy of the diets. The SL2.5 and SL5 diets revealed only some significant differences to control diet regarding some indexes of whole-body composition and digestibility coefficients (see Table 2 and 3, respectively). The SL10 did not show any significant differences for control diet regarding whole body composition and digestibility coefficients. The above-mentioned results suggest that it is viable to incorporate values up to 10% of Salicornia biomass in European sea bass juvenile diets, without any jeopardy for the species and producer.

Conclusion

Data from this study indicates that *S. ramosissima* biomass can be included in diets for juvenile European sea bass up to 10% of their composition, with no detrimental effects to growth performances, survival, whole body composition and digestibility coefficients. The valorization of Salicornia as biomass to incorporate aquafeed diets is of utmost importance, especially if it is produced under principles of sustainability and circular economy.

(Continued on next page)

Table 1. Initial and final weight, relative growth rate (RGR), feed conversation ratio (FCR), feed intake and survival of sea bass juveniles fed the experimental diets for 62 days.

	CTRL	SL2.5	SL5	SL10
Final weight (g)	43.70 ± 0.32	43.30 ± 1.28	43.60 ± 0.98	43.50 ± 0.95
RGR (%day^{-1})	2.93 ± 0.01	2.93 ± 0.05	2.93 ± 0.03	2.93 ± 0.03
FCR	1.00 ± 0.03	0.99 ± 0.01	0.99 ± 0.02	1.01 ± 0.00
Feed intake (%ABW day^{-1})	2.32 ± 0.04	2.30 ± 0.02	2.30 ± 0.03	2.36 ± 0.02
Survival (%)	94.60 ± 4.10	97.10 ± 1.60	97.90 ± 1.20	95.80 ± 1.60

Results expressed as mean ± standard deviation (n = 3 experimental units). No significant differences ($P < 0.05$) between treatments in a One-way ANOVA.

Table 2. Whole-body composition (% fresh weight) of sea bass fed the experimental diets.

Diets	Initial	CTRL	SL2.5	SL5	SL10	*P* value
Dry matter (%)	33.48	29.54 ±2.54	32.37 ± 1.05	32.36 ± 0.92	30.87 ± 0.66	0.125
Protein (%)	20.18	14.46 ± 1.58	15.98 ± 1.31	16.88 ± 1.43	15.65 ± 1.20	0.549
Lipids (%)	7.64	12.69 ± 1.16	12.91 ± 0.13	11.78 ± 0.63	12.40 ± 0.65	0.111
Ash (%)	8.21	4.47 ± 0.74	5.74 ± 0.65	5.61 ± 0.59	5.37 ± 1.11	0.652
Energy (kJ g^{-1})	7.88	7.77 ± 0.83^{a}	8.90 ± 0.31^{b}	8.96 ± 0.03^{b}	8.46 ± 0.22^{ab}	0.018

Results expressed as mean ± standard deviation (n = 3 experimental units); Different letters in the same row indicate statistical differences between diets; $P < 0.05$.

Table 3. Apparent digestibility coefficients (ADC) of the experimental diets.

Diets	Control	SL2.5	SL 5	SL10	*P* value
Dry matter[1]	81.37 ± 0.49^{a}	84.16 ± 0.61^{b}	83.19 ± 0.62^{b}	80.17 ± 0.83^{a}	<0.001
Protein[2]	96.41 ± 0.45^{ab}	97.05 ± 0.04^{b}	96.89 ± 0.25^{ab}	96.25 ± 0.32 ^{a}	0.034
Lipids[2]	98.89 ± 0.47	98.62 ±0.51	98.62 ± 0.38	98.37 ± 0.62	0.664

Results expressed as mean (n = 3 experimental units). Different superscript letters in the same row indicate statistical differences ($P<0.05$) between treatments in a One-way ANOVA followed by Tukey's multiple range test. *Statistical artifact without biochemical meaning; [1]ADC = 100 − 100 × (% yttrium in feed / % yttrium in feces). [2]ADC = 100 − 100 × (% yttrium in feed / % yttrium in feces) × (% nutrient in feces / % nutrient in feed).

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No. 86283 (project AQUACOMBINE). This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein. BC was supported by FCT - Foundation for Science and Technology (IF/00197/2015).

DEVELOPING AN IMTA VALUE CHAIN: SEA-BASED FARMING OF MACROALGAE AND MUSSEL IN SALDANHA BAY, SOUTH AFRICA

C.L.W. Jones*[1], A. Wu[1] and D. Weich[2]

[1]Department of Ichthyology and Fisheries Science, Rhodes University, South Africa; [2]Marifeed (Pty) Ltd, South Africa
Email: c.jones@ru.ac.za

Introduction

Commercial-scale mussel farming is well established in Saldanha Bay, South Africa. The strong winds along the Atlantic coastline of the country, coupled with the upwelling of cold, nutrient-rich water provides the ideal condition for mussel production, within the confines of a sheltered bay. Numerous farms produce mussel in the bay, where the bivalves are seeded onto vertical twisted ropes, attached to rafts and left to filter-feed on the plankton in the bay for approximately eight months. There is already a well-established and complete value-chain for this monoculture production system.

The environmental conditions that are conducive to the filter-feeding production of mussel are also conducive to the production of various species of macroalgae; yet they are not being farmed in Saldanha Bay. The potential of culturing macroalgae, using a sea-based integrated multitrophic aquaculture (IMTA), on the existing mussel rafts had not been investigated prior to this study.

The macroalgae take up dissolved nutrients from the water column, so they do not compete for food with the mussels, but sequester the dissolved metabolic waste of the mussel. Furthermore, the production of macroalgae on the mussel rafts would result in the more efficient utilization of the existing aquaculture facilities, making this a first attempt at sea-based IMTA-produced macroalgae and mussel in South Africa.

Materials and methods

Three rafts in Saldanha Bay, that were already fully stocked with mussel, were loaded with additional twisted ropes that had been seeded with *Gracilaria gracilis* at 15 g/site along a 3.0 m rope at 40 cm intervals. The twisted ropes were placed below the rafts, either horizontally or vertically in the water column, and were positioned in the current on either the south (i.e., facing the oncoming current) or north side of the raft (i.e., with its back to the current). After three months (September 2020) and after six months (November 2020), the ropes were lifted out of the water and approximately 80 % of the tufts of Gracilaria were removed and weighed. The remaining tufts of algae were left in their original position and the ropes were returned to the sea.

Results

The position of the twisted ropes on the raft, in relation to the oncoming current had a significant effect on the mean weight of the seaweed, and this was the same after three and six months of growth (Figure 1). In both instances, algae on the south-side of the raft (i.e., algae that was placed in the oncoming current) was significantly larger than that on the leeward side of the raft.

There was also a significant interaction between the depth that the *Gracilaria* were seeded and the vertical/horizontal position of the ropes in the water column (Figure 2). *Gracilaria* that was seeded just below the surface of the water grew similarly on ropes that were placed vertically and horizontally at both sampling times (Figure 2); while there was significantly more Gracilaria on the vertical ropes than that on ropes that were placed horizontal in the water column at 3.0 m below the water surface (Figure 2).

Discussion

There are currently no commercial-scale, sea-based IMTA operations in South Africa. As part of the AquaVitae (AV) project (European Union, Horizon 2020, Research Innovation Action BG2019), partners lead by Marifeed (Pty) Ltd with support from Blue Ocean Mussels (Pty) Ltd and Rhodes University, have been developing technology for the IMTA production of mussel and macroalgae in Saldanha Bay, South Africa. In this presentation, we will show the audience how we have used these data to develop this technology and we will also show how this research initiative fits into a larger program that aims to develop IMTA value chains that span the Atlantic Ocean.

This presentation will take the audience on a journey through numerous preliminary trials in which the industry and researchers together developed technology to simultaneously produce macroalgae (*Gracilaria* sp.) and mussels on the rafts that were originally designed for mussel production only. We will discuss what worked and where improvements were needed, and we will demonstrate how these preliminary results have been used to design a final, commercial-scale test of this IMTA system, that is currently ongoing.

(Continued on next page)

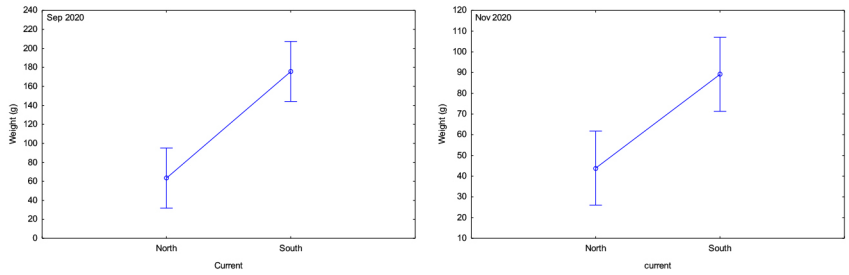


Figure 1: Mean (± standard error) weights of *Gracilaria gracilis* at each "seeding" site on the north and south side (i.e. facing the of the current) of the rafts after three months (Sep 2020; ANOVA, $F_{(1,112)}$=6.280, p=0.014) and after six months (Nov 2020; ANOVA, $F_{(1,232)}$=3.206, p=0.015).

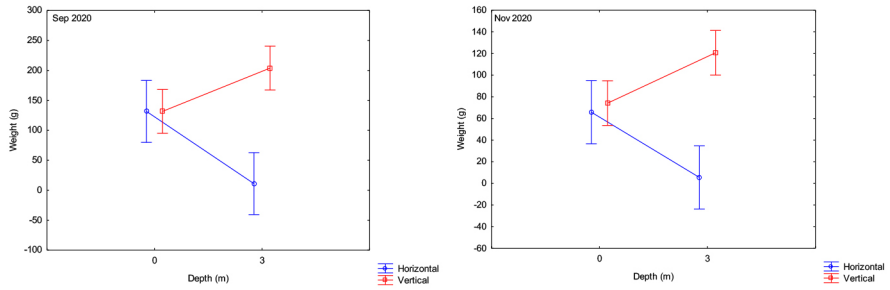


Figure 2: The interaction of water depth and the vertical/horizontal position of the twisted ropes on the rafts had on mean (± standard error) weight of algae after three months (September 2020; multifactor ANOVA, $F_{(1,112)}$=4.636, p=0.033) and six months (November 2020; multifactor ANOVA; $F_{(1,232)}$=4.469, p=0.036).

LACK OF POPULATION GENETIC STRUCTURE OF LUMPFISH ALONG THE NORWEGIAN COAST: A REAPPRAISAL BASED ON EST-STRs ANALYSES

Ó.D.B. Jónsdóttir[1], D. Gíslason[2], G. Ólafsdóttir[2], S.N. Madura[3], S.B. Hagen[3], P. Reynolds[4], S. Sveinsson[2] and A.K.D. Imsland[1,5]

[1]Akvaplan-niva, Iceland Office, Akralind 4, 201, Kópavogur. Iceland
E-mail: odj@akvaplan.niva.no
[2]Matís, Vínlandsleið 12, 113 Reykjavík, Iceland
[3]Norwegian Institute of Bioeconomy Research, Svanhovd, 9925 Svanvik, Norway
[4]GIFAS AS, Gildeskål, 8140 Inndyr, Norway
[5]Dept. of Biosciences, Univ. of Bergen, High Technology Centre, 5020, Bergen, Norway

Introduction

Cleaner fish are now used as a biological control for sea lice on farmed salmon in Europe and Canada (Treasurer 2018). As lumpfish, *Cyclopterus lumpus,* tolerate lower temperatures than wrasse species, their implementation and use has increased dramatically in recent years (> 42 m in Norway in 2019, Norwegian Directorate of Fisheries (NDF) 2019). Given the intensive use of lumpfish along the coast of Norway it is imperative that their use is done with the aim of minimizing possible genetic translocation with local populations. The prerequisite is to have reliable baseline information about the population genetic structure of lumpfish. The present study is a follow up study of the Jónsdóttir et al. (2018) study and aims to fill a knowledge gap by re-examining genetic diversity and population structure of the Norwegian lumpfish using genotypic data derived from using expressed sequence tag-short tandem repeats (EST-STRs) markers.

Material and methods

Sampling areas and protocols

A total of 291 specimens were collected at six fishing grounds from Mandal (58°N) in south Norway along the Norwegian cost up to 69°N in the north, with additional 18 samples of first-generation reared fish from a lumpfish fish farm.

DNA was isolated from all samples using the DNeasy Blood and Tissue Kit following the manufacturer's instructions (Qiagen). A total of seventeen EST microsatellite loci were genotyped. Five multiplexes PCR reactions were performed in a 10 μl volume containing 1 μl DNA, 0.8 µl of dNTP, 0.75 U Taq polymerase, 1 μl of 10x Standard Buffer, 0.08-0.3 μl of a 50:50 ratio of fluorescent dye labelled forward (100 μM) and reverse (100 μM) primer tagged on the 5'-end with a GTTTCTT PIG-tail to enhance PCR amplification. PCR reactions were performed on a Tetrad2 Peltier thermal cycler. Samples were analyzed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyped with GeneMapper v4.1.

Statistical analyses

To assess the potential numbers of genetic clusters a Bayesian cluster analysis was performed in STRUCTURE (Pritchard et al. 2000). The relative importance of geographical distance (isolation-by-distance) and genetic distance was examined using the Mantel test implemented in library ape v5.4-1 in R (Paradis and Schliep 2019).

Results

Test for population structure using both location of samples and not using the location of samples show that there is no population structure in lumpfish along the Norwegian coast (Fig. 1). In accordance with low values of differentiation observed using different statistical tests, there was no correlation between geographical and genetic distance ($R^2 = 0.0015$, $P = 0.89$) and thus no indication of Isolation-by-distance. (Fig. 2).

Discussion and conclusion

In the present study the same sampling material was analysed using functional genetic markers (EST-STRs) giving more or less the same results as found for non-functional genetic markers (g-STRs, Jónsdóttir et al. 2018). Based on the results for both g-STRs and EST-STRs analyses, the former advise of non-negative genetic effect of translocation of farmed juvenile lumpfish along the Norwegian coast is, therefore, still valid, given that wild lumpfish are used for juvenile production to prevent possible inbreeding.

(Continued on next page)

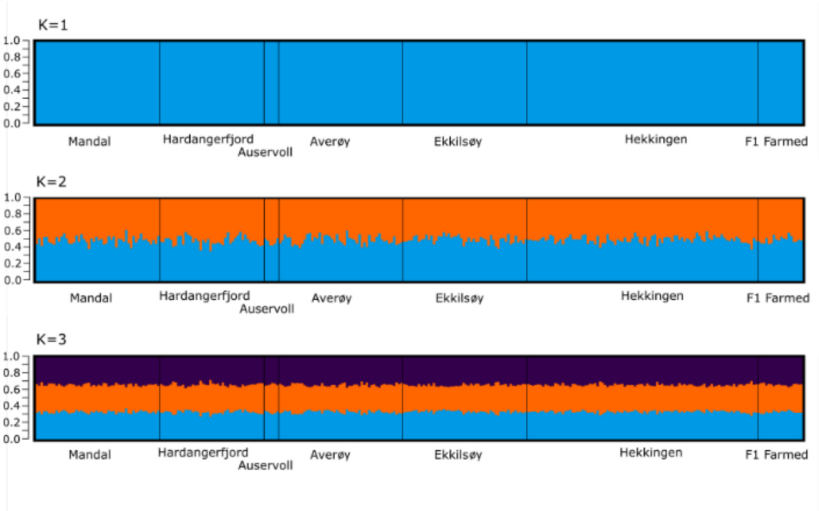


Fig. 1. Bayesian cluster analysis conducted in STRUCTURE. Shown are clustering for K = 1 to 3 for seven samples of lumpfish collected in costal Norway. Within each plot, vertical bars represent individuals while colours indicate the different clusters detected. K = 1 was the most likely number of populations.

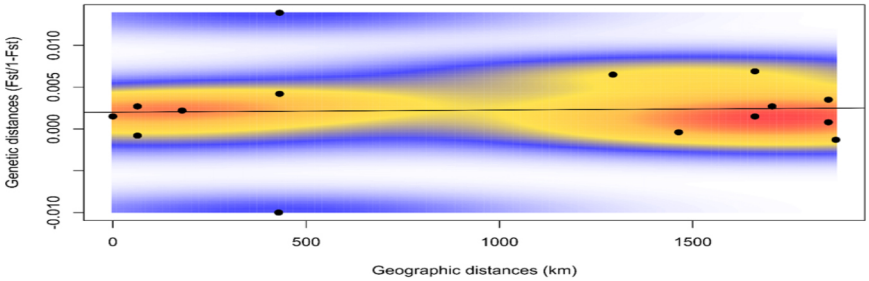


Fig. 2. Relationship between geographical (km shortest waterway) and genetic distance (estimated as $F_{ST}/(1-F_{ST})$) among pairs of lumpfish samples along the Norwegian coastline.

References

Jónsdóttir, Ó.D.B., Schregel, J., Hagen, S.B., Tobiassen, C., Aarnes, S.G., Imsland, A.K.D., 2018. Population genetic structure of lumpfish along the Norwegian coast: aquaculture implications. Aquac. Int. 26, 49–60.

NDF, 2019. https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse

Paradis, E., Schliep, K., 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526-528.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945-959.

Treasurer, J., 2018. Cleaner Fish Biology and Aquaculture Applications. (ed. Treasurer, J), 5M Publishing Ltd., Sheffield, UK, 504 pp.

ADVANCES IN HATCHERY SEMI-INTENSIVE METHODOLOGIES FOR THE REARING OF MARINE FISH LARVAE

Ricardo Jorge José[1,2*]; Carlos Andrade[1,2,3],.

[1]OOM - Oceanic Observatory of Madeira, ARDITI - Regional Agency for the Development of Research, Technology and Innovation, Funchal, Ed. Madeira Tecnopolo, 9020-105 Funchal Portugal
[2]Mariculture Center of Calheta, Dictorate for the Sea, Av.D. Manuel I, Nº7 9370-133, Calheta, Portugal
[3]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, 4450-208, Matosinhos, Portugal

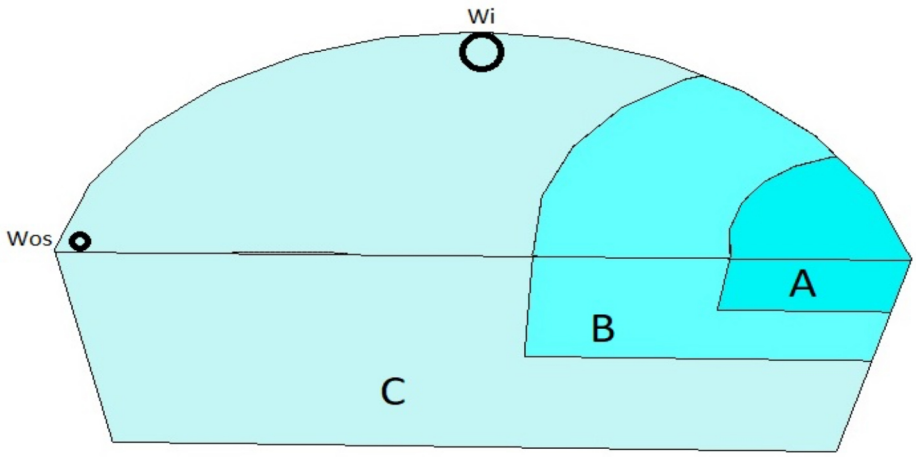
Email: ricardojjose@gmail.com

Introduction

The growth of the aquaculture industry was achieved with the improvement and development of larval rearing methodologies, as well as with the increase of the number of production facilities (Giménez and Estévez 2008). Mesocosm semi intensive methodologies has provided juveniles of high quality of well-known species, as well as being a methodology that is suited for the culture of "new" species, contributing for aquaculture diversification (Andrade, Nascimento et al. 2013). Mesocosm methodologies make use of large rearing tanks, with volumes between 30 and 100 m^3, and rearing densities between 2 and 8 individuals per litter. Most research on "new" species using this system focus on the ontogenetic development of larvae and their diet needs. Considering the size of the rearing tanks and the dramatic changes occurring during larval stages, the monitoring of larvae and water quality are difficult tasks and time consuming, which may eventually compromise the overall performance of the larvae and the rearing system (Andrade et al. 2012). In this presentation we propose a new approach to describe the spatial and temporal evolution of environmental parameters and larvae performance in mesocosm using sea bream (*Sparus aurata*) larvae as model species

Methods

Approximately 374 000 *S aurata* eggs where placed in a 40 m^3 rearing tank to hatch and rearing. Larvae were reared using standard procedures for the species and new methods are tested to evaluate daily mortality at surface and bottom of the tank. Behaviour analysis was recorded by observation of the observer standing at the edge of the tank for 5 minutes. Distribution of larvae on the surface and water column was regarding the majority of individuals as well as the first time a larvae was observed at that depth. Biotic parameters were analysed through the rearing period.



Lateral profile of the distribution of larvae in the rearing tank, regarding depth and occupation of surface through time . **A** – 0 to 20 Days after hatch (DAH), **B-** 21 to 32 DAH, **C -** 32 to 49 DAH. Wi- Water inlet; WOS – Water outlet at surface

(Continued on next page)

Results and Discussion

Sparus aurata hatching rate was high (98 ± 0.1%), and the larvae presented a high rate of swim bladder inflation (>90%). Larvae allocation patterns at the surface and different depth in the tank are described as well seven patterns of behaviour during the rearing period to reaching post-larvae stage. Mortality rates and larvae sampling were efficiently accomplished. Abiotic parameters (temperature, dissolve oxygen, light, pH and salinity) show high variability in time and space denoting changes in diet, stocking density and daily water management.

The improved methods followed in the present work provided a better understanding of the rearing tank environmental parameters, as well as of larvae behaviour, useful to enhance production quality and quantity and a baseline work for larval research on "new" species reared in mesocosm od semi-intensive methodologies.

References:

Andrade, C. A. P., Nogueira, N., Silva, P., Dinis M. T., Narciso, L. (2012). Mesocosm hatcheries using semi-intensive methodologies and species diversification in aquaculture. *Journal of Agriculture Science and Technology*, 2(4B): 428-437.

Giménez, G. and A. Estévez (2008) Effects of two culturing techniques on the growth, survival and larval quality of *Dentex dentex* Linnaeus, 1758. *Aquaculture Research* 39 (4): 354-361

Acknowledgements

Ricardo José was financially supported by the Oceanic Observatory of Madeira Project (M1420-01-0145-FEDER-000001-Observatório Oceanico da Madeira-OOM).

OPTIMUM STOCKING RATIO IN RAS DURING ADVANCED FRY REARING OF BARBEL, *Barbus barbus*

József Molnár[1*], Richárd Békési[1], Levente Várkonyi[1], Balázs Csorbai[1], Zsolt Csenki-Bakos[2], Tamás Müller[3], Béla Urbányi[1], Tamás Szabó[1]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly Str. 1., H-2100 Gödöllő, Hungary

[2]Department of Environmental Toxicology, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly Str. 1., H-2100 Gödöllő, Hungary

[3]Department of Freshwater Fish Ecology, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly Str.. 1., H-2100 Gödöllő, Hungary

*Email: Molnar.Jozsef@uni-mate.hu

Introduction

The barbel is a rheophyl fish species (Pintér K. 2002; Valló L. 2016) with great economic importance. It is a highly regarded sport fish in Europe (Pintér K. 1975). Areas required for reproduction have been greatly reduced (Szabó T. 2001; Fürész et al. 2006). Fish propagation and habitat conservation may support barbel population of natural waters (Pintér K. 1975). Several methods have been developed for the artificial propagation of barbel (Szabó 2000). Gametes can be collected during the spawning season at the spawning sites. Eggs and sperm can also be obtained after hormonal treatment. Fertilized eggs can be incubated in hatcheries. Due to the reophilic nature of the species, the offspring of the barbel cannot be effectively reared in paddy ponds typical of the Hungarian aquaculture (Pintér 1975). However, fry could be effectively raered in tanks in intensive systems.

Materials and methods

Barbus larvae required for the experiment obtained from artificial propagation of fish caught from the river Ipoly during the spawning season. Fertilization was carried out on the river-bank. The fertilized eggs were incubated at the Department of Aquaculture of the Hungarian University of Agriculture and Life Sciences in Gödöllő. The experiment was started from the day of the hatching time. The fry were reared for 21 days at a temperature of 22,4±0,5 °C and a dissolved oxigen 6,6±0,4 mg / L in Recirculating Aquacultural System (RAS). The lighting period was 12 h/day. Fish were stocked in tanks with the capacity of 12 L. The effects of two different stocking ratios (50, 100 individual/L) was examined in three repetition. The fish were fed ad libitum in three times a day. The fish were fed for the first 7 days with newly hatched Artemia larvae (*Artemia salina*). On days 8-9. fish were fed with Artemia and complete fish feed. On days 10-21. only complete fish feed was used. Wet body weight (20 individual/group; accuracy: ±0,1 mg) and total body length (25-25 individual /group, accuracy ± 0,1 mm) were measured in both group on the 0^{th} and 21^{th} days. Fish were anesthetized with 2-phenoxyethanol in dosage 0,4 ml/L before examinations. At the end of the experiment, the survival rate was also determined.

Results

On the first day of the experiment, mean body weight of the fry was 11,0±1,77 mg (*N*=20). At the end of the experiment, the mean body weights were 654±149 mg and 605±171 mg in the 50 larvae / L density group and the 100 larvae /L density group, respectively (*N*=60). The mean body weight from the three repetitions of the two treatments was 629,3±161,6 mg (*N*=120). No significant difference was observed between the two different stocking ratios.

On day 0^{th} of the experiment, total length of the fry was 13,1±0,99 mm (*N*=25). At the end of the experiment, significantly higher body length was measured in tha case of the 50 individual / L group (20±2,1 mm) compared to the 100 individual /L group (18,8±2,46 mm) (*N*=75). The body length from the three repetitions of the two treatments was 19,43±2,35 mm (*N*=150). High survival rate was recorded for the two groups (above 95%) without significant difference.

Discussion and conclusion

Pre-nursing of barbel in intensive system on three-four weeks resulted perfectly developed offspring with extremely low mortality. At the end of the experiment, body weight did not differ significantly between the two groups, but body weight was significantly lower in 50 individual / L compared to the 100 individual / L group.

(Continued on next page)

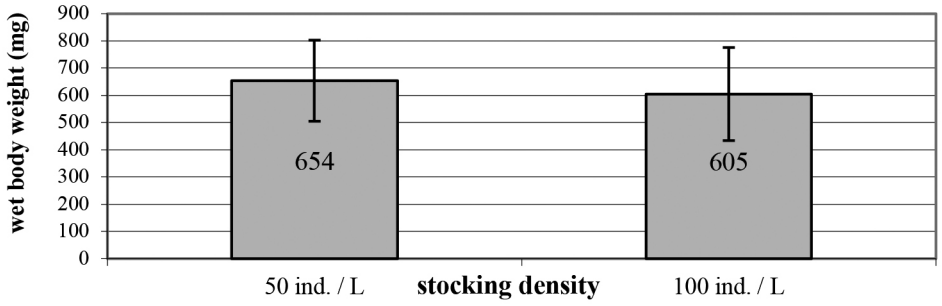


Fig. 1. Mean barbel body weight for the two experimental groups at the end of the experiment.

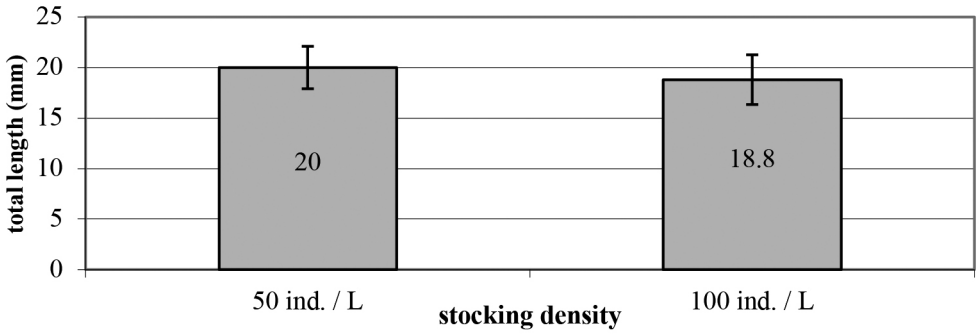


Fig. 2. Mean barbel length for the two experimental groups at the end of the experiment.

Acknowledgements

The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16).

References

Fürész Gy., Dudás T., Zellei Á. 2006. A halgazdálkodás gyakorlata. Magyar Országos Horgász Szövetség, 136. p

Szabó T. 2000. A balin, a jászkeszeg, a paduc és a márna szaporítása. Halbiológia és haltenyésztés. (university textbook) Editor: Horváth László. Mezőgazda Kiadó, Budapest, 336-341.

Szabó T. 2001. A paduc (*Chondrostoma nasus* L.) közép-európai állományának értékelése természetvédelmi szempontból. Halászat, 94(1): 11-13.

Pintér K. 1975. A márna (*Barbus barbus* L). Halászat. 21(3): supplement:1-2.

Pintér K. 2002. Magyarország halai – biológiájuk és hasznosításuk, Akadémia Kiadó, Budapest, 202 p.

Valló L. 2016**.** Kapás van! Csúcs ez a márna, Mezőgazda Kiadó.

SMART SYSTEM FOR FISH FEEDING CONTROL (SICA) IN OFFSHORE SEA CAGES

Authors: Ana Juan *[1], Rosa Martínez[1], Iván Felis[1], Hamid Er-Rachdi[1] & Anibal Gutiérrez[2]

[1]Centro Tecnológico Naval y del Mar. Carretera Lobosillo-El Estrecho Km 2, 30320, Fuente Álamo, Murcia (Spain)
[2]Camar Industrial SA. Calle Lentisco s/n, 30395, La Aparecida, Murcia (Spain)
E-mail: anajuan@ctnaval.com

Improving the efficiency of the feeding process remains one of the major challenges for the aquaculture sector. The lack of control over the fish feeding process, the considerable associated costs and the related environmental impacts are one of the main obstacles to overcome. The Marine Technological Centre (CTN) deployed its cost-effective Smart System for Feeding Control (SICA) in several offshore sea cage farms to validate this real time monitoring technology. The results obtained during these performances in salmon, Gilthead Seabream and Seabass cultures demonstrate that the SICA technology is able to detect different behaviours of fish during feeding process, anticipating human decisions and thus optimizing the feeding process. The potential contribution of SICA technology to reduce feed waste and improve the efficiency of fish production have been demonstrated.

Introduction

The control of the feeding process in aquaculture farms has traditionally been carried out by means of qualified stuff and observations of behavioural cues that indicate fish appetite while feeding (Li *et al*., 2020). Currently, although computer vision technology and camera-based systems are increasingly in use, they are limited by their dependency on illumination, water conditions, that define visibility, and their inability to monitor the entire population of fish in a net pen. Therefore, alternatives based on acoustic observation of fish behaviour have been examined.

According to different studies (Maniva, 1976; Samueloff, 2000), during the feeding process, fish make different sounds due to their own movement in the water or the splash on the surface to catch the food. In this context, SICA system (Smart System for Feeding Control), uses passive acoustics to distinguish the different sounds produced during the feeding process, without masking problems. For this, the device is based on decision-making through machine learning, a particular approach to Artificial Intelligence (AI). This makes possible to differentiate the moment when fish stop eating and thus indicate when the supply should be stopped.

The aim of this study was to validate the use of SICA technology in offshore sea cages in real production scenarios. First approaches were performed at high commercial interest species in Europe: Gilthead Seabream and Seabass (*Dicentrarchus labrax*) in Mediterranean fish farms and Salmon (*Salmo salar)* in Norwegian fish farm.

Material and methodology

SICA prototype is composed of two modules: Data Logger (DL) and Control Unit (CU). First module is deployed in the sea cage by using a mooring system, this DL acquires the signal through a hydrophone and transmits it to the CU trough wireless connection. Once the signal is received by the second module it is stored, then the acoustic records are processed and analysed automatically.

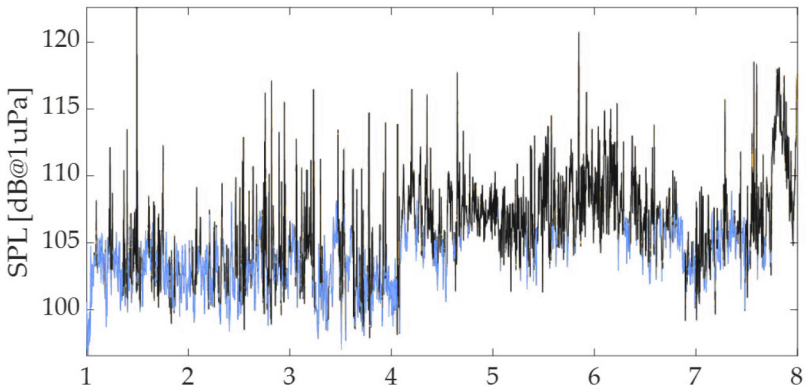


Figure 1. Sound pressure variations during one Salmon feeding register. Low feed intake events (blue colour) are differenced from the rest of feeding events (black colour).

(Continued on next page)

Information related to fish behaviour was collected during feeding process conducted according to industry standard. Appetite and feed management was registered by CTN researchers and fish farm technicians by means of underwater video camera registers and water surface fish observations.
Using acquired data by the SICA, the information collected during feeding process and a machine learning process, validation test is performed.

Results and discussion

In contrast to the traditional methodology undertaken with underwater video cameras the SICA system was found to be more efficient in detecting unusual fish behaviour since it was able to make earlier predictions during the feeding process. The accuracy in low feed intake detection was of over 84% in Seabream and Seabass cultures. In salmon cultures we find an accuracy 95% due to differences in feeding methodology and technology, an example of salmon feeding acoustic recordings are shown in Figure 1.

The SICA technology results a cost-effective and non-invasive solution in early detection of different fish behaviours during feeding process. Its ability in real time acquisition, data processing and anticipating decisions-making provides great potential to reduce the associated costs and minimizing the impact of the waste on the seabed.

Acknowledgement

The experiments are part of two projects, one of them financed by the European Maritime and Fisheries Fund of the European Commission (DEMO-BLUESMARTFEED) [project agreement number EASME/EMFF/2017/1.2.1.12/S1/05/SI2.789750] and the other financed by HORIZON^{2020} inside the research infrastructure AQUAEXCEL^{2020} (SMARTFEEDINSALMON) [project identification code AE120015].
The authors thank PLAGTON and PISCIALBA for their participation as end-users and CAMAR as partner of the project. Also, many thanks to SINTEF Ocean AS for giving its facilities and SalMar as operator.

Bibliography

Li, D., Wang, Z., Wu, S., Miao, Z., Du, L., & Duan, Y. (2020). Automatic recognition methods of fish feeding behavior in aquaculture: A review. Aquaculture, 528(May), 735508. https://doi.org/10.1016/j.aquaculture.2020.735508.

Maniva, Y., 1976. Attraction of bony Fish, Squid and Crab by Sound. Sounds Reception in Fish, Elsevier.

Samueloff, M. F. a. B., 2000. Fish Feeding Control in Aquaculture on the Basis of Sound Emitted by Fish. Tel Aviv, Patent nº WO 00/03586.

APPETITE REGULATING NEUROPEPTIDES AND THEIR DISTRIBUTION IN THE ATLANTIC SALMON (*Salmo salar*) BRAIN

Tharmini Kalananthan[1*], Floriana Lai[1], Ingvill Tolås[1], Ana S. Gomes[1], Ann-Elise O. Jordal[1], Koji Murashita[1, 2], Ivar Rønnestad[1]

[1]Department of Biological Sciences, University of Bergen, Bergen, Norway
[2]Physiological Function Division, Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Tamaki, Japan
E-mail: Tharmini.Kalananthan@uib.no

Introduction

In vertebrates, feed intake is controlled by the synergic actions of central and peripheral signals which stimulate ingestion in relation to the nutritional status of the animal. In the brain, the hypothalamus plays a pivotal role in the regulation of appetite and feeding. In mammals, the hypothalamic neuronal network comprising major distinct cell populations that express the orexigenic neuropeptides agouti-related protein (AGRP) and neuropeptide Y (NPY) and the anorexigenic peptides proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) have been described, and constitute the melanocortin system (reviewed in Lanfray and Richard, 2017). These neuropeptides act on the melanocortin-4 receptor (MC4R) in higher order neurons that control both food intake and energy expenditure. This system seems to be relatively well-conserved among vertebrates, including teleost species. However, in Atlantic salmon (*Salmo salar* L.), the salmonid-specific fourth round whole-genome duplication led to the presence of several paralog genes which may have resulted in divergent functions of the duplicated genes (Lien et al., 2016)Ss4R. In the current study, we have updated the *agrp, npy, pomc, cart* and *mc4r* gene repertoire information, including an assessment of their role in controlling appetite in Atlantic salmon. This includes their distribution in the brain regions olfactory bulb (OB), telencephalon (TEL), mid brain (MB), cerebellum (CE), hypothalamus (HYP), saccus vasculosus (SV), pituitary (PT) and brainstem (BS).

Materials and Method

In silico searches were performed to retrieve the *agrp*, *npy, pomc, cart* and *mc4r* paralogs from GenBank and Ensembl. Sequence comparisons and phylogenetic analysis were performed to confirm the identity of the newly identified sequences and qPCR assays were developed using gene specific primers. Atlantic salmon post smolt (weight ca. 200g and 250g) from two experiments each (n = 6) were randomly selected, and euthanized using an overdose of MS222, before the brain was collected and immediately transferred to RNAlater. The 8 brain regions (**Figure 1**) OB, TEL, MB, CE, HYP, SV, PT and BS were dissected using a stereo dissecting microscope and used for the gene expression analysis.

Results

In silico analysis have confirmed the presence of **2 *agrp*** (*agrp1&2*), **3 *npy*** (*npya1*, *a*2 & *b*), **3 *pomc*** (*pomca1*, *a2* & *b*), **10 *cart*** (*cart1a*, *1b1*, *1b2*, *2a*, *2b1*, *2b2*, *3a1*, *3a2*, *3b* and *4*) and **4 *mc4r*** (*mc4ra1*, *a2*, *b1* & *b2*) genes in the Atlantic salmon genome database. The Atlantic salmon Npy mature peptide shared 78-86% and Pomcs 27–37% identity with the human homologues whereas, the amino acid full length sequence of Carts shared 14-50% and Mc4rs 63-68% of identity with human homologue sequences. Brain region distribution analysis using qPCR showed a wide distribution pattern with varying range for all genes analyzed. *agrp1*, *pomca1* and *pomca2* were highly expressed in HYP while *agrp2* was high in TEL and *pomcb* in PT. The *npya1* (most abundant among *npy*), and *b* were highly expressed in TEL and *npya2* in HYP. All ten *cart* paralogs were analyzed with 7 pairs of primers for qPCR. *cart2b* is the most abundant *cart* paralog, followed by *cart3b*, *3a*, *1b*, *2a*, *4* and *cart1a*. The *cart2b* was abundant in OB and TEL; *cart3b* in BS and MB; *cart3a* in TEL, MB, and BS; *cart2a* was predominantly expressed in MB and HYP whilst *cart4* in HYP and TEL. The *cart1a* and *1b* were quite similar with major expression in MB. Among all neuropeptides *agrp1*, *pomca1*, *pomca2*, *cart2a, 2b, 3a, 3b, 4* and *npya2* showed considerable expression in HYP. The *mc4rb1* was the most abundant *mc4r* paralog, and *mc4ra1*, *a2* and *b1* showed higher expression in HYP and TEL whilst *mc4rb2* was high in HYP. (**Figure 1**).

(Continued on next page)

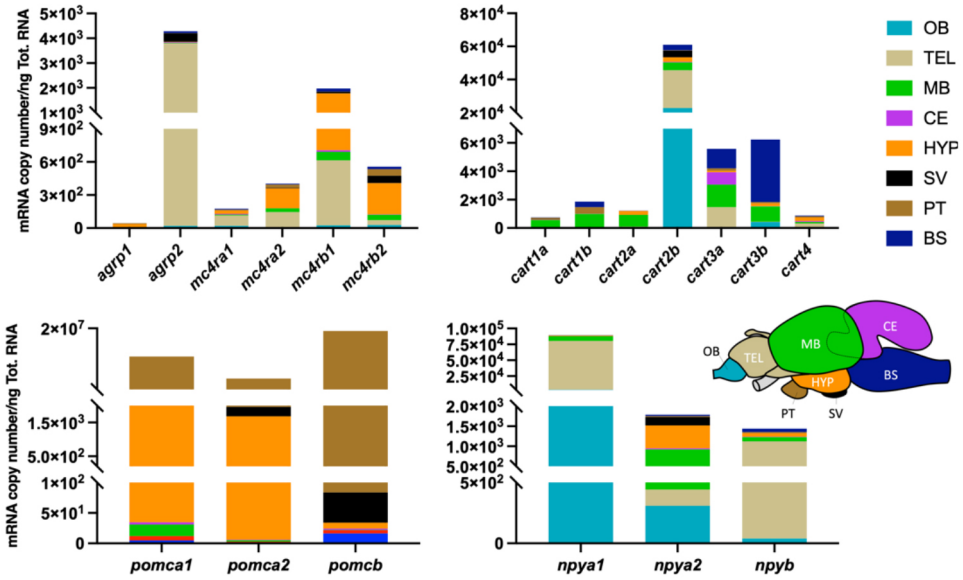


Figure 1. The mRNA expression of *agrp*, *mc4r*, *cart, pomc* and *npy* paralogs in eight brain regions (Insert: illustration of brain dissection) in Atlantic salmon. The genes were grouped according to their expression level to fit in the graphs.

Discussion

The present study updates the current knowledge of the agrp, npy, pomc, cart and mc4r gene paralogs in Atlantic salmon and shows their distribution profile and abundance in 8 brain regions. The gene expression distribution suggests that, in addition to HYP, brain regions, such as TEL, MB, BS, and PT may contribute to control of appetite, feeding and energy homeostasis. However, a remarkably diverse array of physiological functions has been reported in correlation to the site of gene expression in the teleost's brain. A recent functional analysis in grass carp (Ctenopharyngodon idellus) suggested that HYP and TEL might be involved in feeding and reproduction process, OB in immune response and reproduction, optic tectum in vision and feeding, PT in energy metabolism and medulla oblongata in auditory functions (Ye et al., 2020). Unraveling the physiological roles of these neuropeptides will provide more insight into the appetite regulation in Atlantic salmon, a global key commercial aquaculture species.

References

Lanfray, D., and Richard, D. (2017). Emerging signaling pathway in arcuate feeding-related neurons: Role of the Acbd7. *Front. Neurosci.* 11, 1–8.

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., et al. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature* 533, 200–205.

Ye, C., Xu, S., Hu, Q., Hu, M., Zhou, L., Qin, X., et al. (2020). Structure and function analysis of various brain subregions and pituitary in grass carp (Ctenopharyngodon idellus). *Comp. Biochem. Physiol. - Part D Genomics Proteomics* 33, 100653.

COMPOSITIONAL CHANGES IN LOW TROPHIC SPECIES GROWN AT A PILOT SCALE INTEGRATED MULTI-TROPHIC AQUACULTURE SITE

Joanne Casserly* [1], Frank Kane[1]

[1] Marine Institute, Rinville, Oranmore, Co. Galway, Ireland. H91 R673
Email: joanne.casserly@marine.ie

Introduction

The EU is the world's largest importer of fisheries and aquaculture products, importing 70% of the EU consumption (EUMOFA, 2018). Change in consumer attitudes has seen a parallel demand in low trophic products such as invertebrates and seaweeds (Barbier, 2019). Aquaculture continues to be a key pillar of future food production systems and there is a drive in aquaculture for sustainability and more circular economies. Strategies such as the European Green Deal, World Ocean Initiative and Sustainable Development of Aquaculture Strategy, also focuses on 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. Recapturing nutrients from aquaculture and the environment by extractive species, such as shellfish, seaweeds and invertebrates, re-circulates the wastes and by-products from fed species into feed, fertiliser and energy for others (Chopin, 2013). For this reason, culture of extractive species with fed species in the same aquaculture sites is encouraged, and this practice is shown to remove waste materials from fed species and lower the nutrient load in the water (FAO,2018). However, it is also critical to understand the value of extractive and low trophic species for the vital ecosystem services they provide in addition to their biomass as food goods (Chopin, 2013).

Methodology

IMPAQT aims to promote the eco-intensification of aquaculture by demonstrating the eco-efficiency and minimization of environmental impacts, enabling socio-economic benefits and ecosystem services, and promoting the transition towards a circular economy business model. Biometric and abiotic data from this pilot site contributed to multiple case studies to examine potential yields, crop quality, circularity, socioeconomic impacts and the interaction of farm components with the environment on the scale of an ecosystem. This study focused on monitoring of the different crop/species throughout the growing season to ascertain whether the molecular structure of the extractive species vary with enough significance to warrant a change in farming practices such as planting and harvesting, or emphasis on extractive species as environmental remediators.

Results

This IMTA implementation saw the application of two low trophic species, *Alaria esculenta* and *Pecten maximus* to a monoculture finfish facility producing Atlantic salmon. 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. To further understand the 'value' of these species, comparison of samples taken throughout the growing seasons is presented to examine the interaction of the species with the site over time and the potential benefits for planning decisions by both farmers and regulators.

References

Barbier M, Charrier B, Araujo R, Holdt SL, Jacquemin B & Rebours C. (2019) PEGASUS - PHYCOMORPH European Guidelines for a Sustainable Aquaculture of Seaweeds. COST Action FA1406, Roscoff, France. https://doi.org/10.21411/2c3w-yc73

Chopin T. (2013) Aquaculture, Integrated Multi-trophic (IMTA). In: Christou P., Savin R., Costa-Pierce B.A., Misztal I., Whitelaw C.B.A. (eds) Sustainable Food Production. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-5797-8

FAO (2018), The State of World Fisheries and Aquaculture 2018: Meeting the Sustainable Development Goals, UN, New York, https://doi.org/10.18356/8d6ea4b6-en.

World Bank. 2013. *Fish to 2030: prospects for fisheries and aquaculture (English)*. Agriculture and environmental services discussion paper; no. 3. Washington DC; World Bank Group. http://documents.worldbank.org/curated/en/458631468152376668/Fish-to-2030-prospects-for-fisheries-and-aquaculture

Acknowledgments

This work is part of the IMPAQT project, funded by the EU H2020 research and innovation programme under Grant Agreement No 774109.

IMPAQT – AN INTELLEGIENT MANAGEMENT SYSTEM FOR INTEGRATED MULTI-TROPHIC AQUACULTURE

Frank Kane* [1], Panagiotis Vlacheas[2]

[1] Marine Institute, Rinville, Oranmore, Co. Galway, Ireland. H91 R673
[1] WINGS ICT SOLUTIONS PC, 189 Siggrou Avenue, 17121 Nea Smirni, Greece
Email: frank.kane@marine.ie

Introduction
IMTA is well recognised as a promising advancement for sustainable development of aquaculture, offering a method to increase productivity while, at the same time, reducing environmental impacts. The concept of IMTA is to farm species of different trophic levels, complementary to each other, so that the wastes and by-products of one species become the feed, fertiliser and energy source for another. This practice is shown to remove waste materials from fed species and lower the nutrient load in the water. IMTA is in its infancy in Europe and commercial level IMTA is rare. Understanding the validity of the IMTA approach, the interaction of the trophic levels and. the management of IMTA in large-scale areas, remains a challenge.

Methodology
The H2020 IMPAQT project (https://impaqtproject.eu/) worked to promote the eco-intensification of aquaculture by demonstrating the eco-efficiency and minimisation of environmental impacts, the socio-economic benefits and ecosystem services, and promoting the transition towards a circular economy business model. IMPAQT has developed an intelligent management platform which featured an autonomous data acquisition and communication system, an advanced IMTA model and an intelligent management system to achieve a holistic approach addressing this complete system view.

Results
Outputs from the IMPAQT project will be presented. Technologies developed and deployed include novel sensors, data acquisition and communication systems. These data aggregators and power management tools have been developed as an integrated management platform, feeding into an intelligent management system (IMS), operating at the scale of an IMTA farm. This system comprises analysis and decision support functionalities, utilising data from various sensors, remote data and crowd sourced data. The data is used to determine environmental quality, stock welfare and assist with farm management to monitor and manage IMTA production and enable enhanced operational decisions for animal welfare, production optimisation, environmental protection and food-quality assessment. The Impaqt platform and IMTA set-ups have been validate in six pilot sites across Europe, Turkey and China.

Supporting this, an IMTA model has been utilised to show the responses and interactions between IMTA farm components and their footprint on the environment in different nutrient environments, and identified controlling processes for incorporation in future models to improve their predictive value.

The Impaqt pilot site outputs provided data to demonstrate the circularity, sustainability and eco-efficiency from IMTA as well as the socioeconomic benefits, cost effectiveness, and the ecosystem services provided by IMTA. An overview of the project out puts will be presented.

Acknowledgments
This work is part of the IMPAQT project, funded by the EU H2020 research and innovation programme under Grant Agreement No 774109.

INTRODUCING DRY FEED FROM MOUTH OPENING IN RED SEABREAM *Pagrus major* LARVAE PROMOTES LARVAE AND POST-LARVAE PERFORMANCE

Nafsika Karakatsouli[1,*], Alkisti Batzina[1], Kostas Ntomalis[2], Anemos Katelis[3], Vasiliki-Anna Alexopoulou[1], Sofia-Brinkmann Bougali[1] and Isidoros Markakis[1]

[1]Laboratory of Applied Hydrobiology, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, 118 55 Athens (Greece)
[2] BioMar Hellenic SA, 2nd Industrial Zone of Volos, Block No 6, 37500 Velestino (Greece)
[3] Hellenic Fishfarming SA, Pentelis Avenue 95C, Chalandri 152 34
E-mail: nafsika@aua.gr

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 red seabream *Pagrus major*, dry feed is progressively introduced to fish larvae not earlier than 20-22 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 red seabream. 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 (3 dph) on growth and functional development of the digestive system of red seabream 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. Four tanks of 9 m^3 were stocked with eggs of the same broodstock. In two of the tanks a commercial dry feed (Larviva Prostart, Biomar) was introduced on 3 dph (DF3), while in the other two tanks the dry feed was introduced on 22 dph (DF22) according to a common hatchery protocol. In all experimental tanks, the larvae were fed with rotifers from 3 dph up to 21 dph and with *Artemia* nauplii/metanauplii from 16 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 2 to 10 dph, and at 6-days intervals up to 40 dph to estimate larvae length and digestive function (i.e. lipase, amylase, trypsin, chymotrypsin, pepsin specific activities). On 53 dph larvae of each duplicated 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 43 dph ungraded larvae of each duplicated tank were transferred to laboratory installations. On 50 dph, six hundred fish from each duplicated Hr tank were group weighed and randomly distributed in pentaplicated tanks (120 fish per tank). Fish growth (i.e. body mass, survival, specific growth rate-SGR, thermal growth coefficient-TGC, mass variation) was monitored for six (6) weeks. All fish were fed the same commercial diet *ad libitum*. Water quality was monitored daily and fish were group weighed (app. 15-20 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

Stage 1, Hatchery rearing (Hr): After 10 dph and up to 40 dph, larvae length was significantly higher in DF3 larvae. Survival and length variability were not affected by experimental treatments, while DF3 produced a much greater percentage of Big fish (61%) than DF22 (9%). Furthermore, both the Big and Small fish of DF3 were significantly larger than those of DF22. Trypsin and chymptrypsin specific activities were higher in 3-5 dph and 16-28 dph DF3 larvae. Pepsin was firstly detected in both treatments on 22 dph, peaked on 28 dph and remained at higher levels in DF3 larvae. Amylase activity was higher on 4 dph DF3 larvae while lipase pattern was similar in both DF3 and DF22 larvae. Stage 2, Laboratory rearing (Lr): After 6 weeks of pre-growing (up to 95 dph) fish of the DF3 treatment were significantly larger than DF22 (6.7 *vs* 6.0 g respectively) with higher daily weight gain. Body weight frequency distribution were different between treatments; fish larger than 6 g consisted the 63% of DF3 fish and the 48% of DF22 fish. No differences were observed for coefficient of weight variation and survival. Furthermore, spinal cord malformations of DF3 fish were significantly lower than those observed for DF22 fish (3.8 *vs* 11.7 % respectively).

(Continued on next page)

Discussion and conclusion

Present results showed that the introduction of dry feed from mouth opening (on 3 dph), was more efficient for larvae and post-larvae performance compared to a more commonly used protocol (dry feed on 22 dph). The functional development of the digestive system was not compromised and it was similar to previously reported results for red seabream (e.g. Waqalevu et al., 2019; Khoa et al., 2020). Besides, digestive enzymes differences observed indicate an increasing number of actively feeding larvae in DF3 treatment. Present beneficial effects gained from the early introduction of dry feed are probably related to a better acclimation of larvae to dry feed particles, which once ingested offer a diet of higher nutritional value able to support the higher demands of faster larvae growth. Growth benefits obtained during larviculture were maintained during further rearing up to 95 dph. In accordance to our previous similar work on gilthead seabream larvae (Karakatsouli et al., 2019), the present markedly lower spinal cord deformities recorded at the end of the pre-growing period support the hypothesis that the ingestion and digestion of an appropriate dry feed may provide larvae with the necessary nutrients to form a healthy skeletal system at the sensitive stages of skeleton ontogenesis. Overall, the introduction of dry feed as early as on mouth opening is considered safe and feasible for red seabream providing advantages for both larval and post-larval production stages.

References

Cañavate, J.P, Fernández-Díaz, C., 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. Aquaculture 174: 255-263.

Khoa, T.N.D., Waqalevu, V., Honda, A., Shiozaki, K., Kotani, T., 2020. Comparative study on early digestive enzyme activity and expression in red sea bream (*Pagrus major*) fed on live feed and micro-diet. Aquaculture 519: 734721.

Waqalevu, V., Honda, A., Dossou, S., Khoa, T.N.D., Matsui, H., Mzengereza, K., Liu, H., Ishikawa, M., Shiozaki, K., Kotani, T., 2019. Effect of oil enrichment on *Brachionus plicatilis* rotifer and first feeding red sea bream (*Pagrus major*) and Japanese flounder (*Paralichthys olivaceus*). Aquaculture 510: 73-83.

Hardy, R.W., Barrows, F.T., 2002. Diet Formulation and Manufacture. In Fish Nutrtion, Third Edition, Elsevier Science, USA, pp. 506-601.

Karakatsouli, N., Domalis, K., Panopoulos, S., Coli, A., Anastasiadou, C., Rati, M., Bantounas, S., Batzina, A., 2019. Investigation of early introduction of dry feed on gilthead seabream *Sparus aurata* larval development and growth performance after metamorphosis. Aquaculture Europe 2019, October 7-10, 2019, Berlin (Germany).

COLD-SHOCK ANDROGENESIS IN COMMON CARP (*Cyprinus carpio*)

V. Kašpar[1]*, M. Hubálek[1], M. Pšenička[1], K. Arai[2], J. B. Taggart[3], R. Franěk[1]

[1] South Bohemian Research Center for Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Zátiší 728/II, 389 25 Vodňany, Czech Republic

[2] Laboratory of Aquaculture Genetics and Genomics, Division of Marine Life Science, Faculty and Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, 041-8611, Japan

[3] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, Scotland, United Kingdom

* Correspondence: vkaspar@frov.jcu.cz; Tel.: +420-38777-4709

Methods of induction of uniparental inheritance have been developed for model organisms as well as for species of commercial interest to fix sex-specific traits in breeding programs or to restore breeds or lines from cryopreserved sperm in aquaculture important species.

Androgenesis in common carp (*Cyprinus carpio*) has been successfully induced by egg nucleus inactivation with **γ-**, X- or UV-ray irradiation but these techniques are not widely applicable for laboratory or commercial use on practical, financial and safety grounds.

In the past decade a promising low-cost, low-tech cold-shock approach for successfully inducing androgenesis has been demonstrated for loach, Japanese flounder and zebrafish.

The aim of the current study was to develop a cold shock methodology for application in common carp, a freshwater species of high commercial interest and a widely used model species. Gametes were collected from wild-type females (dominant green phenotype) and Koi males (recessive blonde phenotype) to enable easy identification of successful induction of androgenetic haploid progeny. A combination of different temperature treatments (0, 2, 4, 6, 8 ˚C) and different cold-shock durations (15, 30, 45, 60, 75 min.) applied shortly after gamete activation (3 s after fertilization) were initially trialed.

Optimal condition for egg nucleus elimination was a cold-shock at 2 ˚C for 60 min duration where hatching rate of haploid progeny reached 35.6% and 26.26±10.19% across two attempts. Double haploid induction was then attempted with replicate 300g egg masses: a 2 ˚C cold-shock, 60 min. duration, followed by arresting first mitotic cleavage by heat shock (40 ˚C) applied 40 min after transfer to the environment of normal egg incubation temperature (20 ˚C).

These combined treatments resulted in reduced fertilization and hatching rates for all replicates and low yield of progeny (1.09-1.28% in experimental incubation, <1% in hatchery incubation*)*. A genome wide SNP analysis of a subset of progeny confirmed that they were double haploids. Cold-shock androgenesis was found to be effective in common carp, providing a new possibility of uniparental inheritance induction for isogenic line production.

POTENTIAL APPLICATION OF INDIRECT COLD ATMOSPHERIC PLASMA NOVEL TECHNOLOGY FOR THE SHELF-LIFE EXTENSION OF AQUACULTURE PRODUCTS: CASE STUDY ON SEA BREAM FILLETS

S. Chanioti[a], M. Giannoglou[a], P. Stergiou[a], D. Passaras[b], G. Kokkoris[b], E. Gogolides[b] and G. Katsaros[a*]

[a] Institute of Technology of Agricultural Products, Hellenic Agricultural Organization–DEMETER, Lykovrissi 14123, Attica, Greece
[b] Institute of Nanoscience and Nanotechnology, NCSR "Demokritos", Aghia Paraskevi 15341, Attiki, Greece
Email: gkats@chemeng.ntua.gr

Introduction

The Dietary Guidelines encourages citizens to increase the seafood consumption to improve the health of their diets. On the other hand, the seafood availability (overfishing and ocean pollution etc.) along with the growing global population will result in increased demands and constraints of seafood supplies. Seafood loss and waste reduction is prerequisite for supporting increased seafood consumption without further stressing aquatic resources.

Fish fillets are among the most consumed aquaculture products but with a very limited shelf-life due to contamination during the filleting process operations (washing, cutting and skinning) or due to mishandling during transportation and storage. These may lead to increased microbiological load, degraded quality characteristics including undesirable odors and off-flavors as well as modified texture, thus reducing their shelf life and the consumers' acceptability, contributing to seafood waste. Many strategies have been proposed for seafood waste minimization, some of them targeting to shelf-life extension applying conventional or novel non-thermal technologies (where applicable without affecting seafood freshness and quality).

Cold atmospheric plasma (CAP) has raised overwhelming attention for various food products since it can improve food products shelf-life. It could be applied either directly (plasma zone is in direct contact with the food to be treated), semi-directly (plasma generated Reactive-Oxygen-Nitrogen-Species are able to reach the food surface as the food product is positioned remotely with respect to the active plasma region) or indirectly (two-step process where a gas plasma is applied to water until enough concentration of secondary RONS is produced and then food products are treated with Activated Water in order to induce decontamination). Plasma activated water (PAW) has been proved to be a disinfection media since it is capable of inactivating microorganisms while simultaneously maintaining the quality characteristics of the treated products.

The aim of this study was to investigate the application of PAW as innovative immersion agent for the extension of shelf life of fish fillets by evaluating their microbial and quality characteristics during storage.

Materials and Methods

PAW was produced using a CAP jet (flow rate 0.5 L/min, nozzle–water surface distance 4.3 mm). The peak-to-peak voltage was 7.2 kV (100 kHz). The process time was 16 min for 20 mL water samples treatment. After CAP treatment, RONS were measured as approximately 40 mg/L for H_2O_2, 0,80 mg/L for NO^{2-} and 12 mg/L for NO^{3-}. Gilthead sea bream (*Sparus aurata*) fillets were used as the case study food product. The fillets were immersed into i) PAW (with the RONS displayed above), ii) deionized water and iii) artificially produced water with equal concentrations of PAW RONS. In all cases, the optimized fish:antimicrobial agent ratio of 1:6 and treatment time for 15 min was used. All fish fillets after the immersion time were immediately packed asseptically in transparent plastic bags and stored at 4°C.

During a 10-days storage period, quality was characterized in terms of color, texture, microbial load (total aerobic bacteria, lactic acid bacteria, yeasts/molds, *Pseudomonas*, *Enterobacteriaceae*, *Brochotrix thermosphacta* and H_2S producing microorganisms), lipids oxidation and sensory evaluation.

(Continued on next page)

Results

In general, CAP treatment appeared to significantly affect the total microbial load of fish fillets, since a 1.20 log CFU/g reduction was measured (3.60 log CFU/g for the CAP treated fillets compared to 4.78 log CFU/g for the control samples). The same trend was observed for all the microorganisms tested with variability in the reduced load. Artificially produced water with equal RON concentrations to PAW also resulted in microbial load reduction but not as much as the one obtained from PAW application (the corresponding value for the total microbial load exactly after treatment was 4.20 log CFU/g). This decrease in the microbial load at time zero was observed during the whole shelf-life period of the fillets. For the CAP treated fillets, a shelf life of 8 days was estimated (shelf-life was determined as the time to reach a total plate count value of 7.00 log CFU/g). The corresponding values for the control and the artificially produced water treated fillets were 4 and 5 days, respectively. Concerning the quality parameters, the color of all samples exhibited decrease of lightness and increase of redness vs storage time, which was more pronounced for the untreated products. The hardness of the fish fillets was slightly decreased for all samples, during shelf-life. The PAW fish fillets did not exhibited different oxidation values during storage compared to the other samples. In general, the promising results validate that PAW could act as an effective antimicrobial agent for fish decontamination, offering potential alternative options for future application to other seafood as well, increasing their shelf-life.

Conclusions

The proposed technology for seafood (especially for whole fresh fish and fish fillets) processing and shelf-life extension is very promising -considering also that other technologies cannot be applied to these products- allowing for seafood waste reduction.

Acknowledgement

Part of the results of this study were financially supported by the project NOVISH (http://novish.itap.com.gr) funded by the Hellenic Republic, Ministry of Rural Development and Food, General Secretariat of Rural Development and Food, within the framework of Maritime and Fisheries Fund 2014-2020.

EFFECT OF DIFFERENT DISSOLVED OXYGEN CONCENTRATIONS ON DIGESTIBILITY AND GASTRIC EVACUATION RATES OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND GILTHEAD SEA BREAM (*Sparus aurata*)

Lydia Katsika[1], Ioannis Papadakis[1], Panagiota Tsoukali[1], Stavros Chatzifotis[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Greece
Email: katsikalydia@gmail.com

Introduction

Low levels of dissolved oxygen in farm water and high temperatures are potential stressors parameters that affect fish growth, digestion, behavior and health (Petersen & Gamperl, 2010). Changes in oxygen levels can affect the morphology of the gut and the ability to digest nutrients (Tran-Ngoc *et al*., 2016). Under the same rearing and diets conditions, understanding the relationship between digestion and gastric evacuation can help to estimate the time of appetite return (Riche *et al*., 2004). Information of how dissolved oxygen could affect the feed digestibility and the gastric evacuation rates of fish are useful for feeding frequency estimation aiming at more efficient nutrient utilization and establishing an appropriate feeding protocol. The aim of this work was to measure feed digestibility and gastric evacuation rates of sea bream and sea bass in 3 different dissolved oxygen rages.

Materials and Methods

Two digestibility and gastric evacuation rates trials were conducted at the Aqualabs facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (Heraklion, Greece), both for European sea bass and Gilthead sea bream. Fifteen fish (250 gr initial body weight) were placed in 3 closed recirculation systems each consisting of tanks of 500 L capacity under fully controlled oxygen, light and water flow conditions. A different level of oxygen saturation in the rearing water was applied to each closed recirculation system (80-100% DO, 60-80 DO and 40-60% DO) at 26.5 ^{o}C water temperature. The fish were hand-fed a commercial diet (**Table 1**) to satiation after additing of 1% celite® as an inert marker in the feeds, three times daily. Feed and feaces proximate compositions were determined according to (AOAC, 1990). Apparent digestibility coefficients of nutrients and energy of the diet were calculated using the following formulas: ADC = 1–(F/Dx Dm/Fm); where: D=% nutrient (or MJ/kg gross energy) of diet; F=% nutrient (or MJ/kg gross energy) of feaces; Dm=% digestion marker (AIA) of diet; Fm=% digestion marker (AIA) of feaces. For the gastric evacuation rates determination, after feeding a single meal, 3 fish were killed every 3h over a period of 24 h and the digestive tract contents were collected and the dry weight were measured.

Results and Discussion

Apparent digestibility coefficient values of tested diet under different oxygen saturation regime for sea bass and sea bream are showed in **Table 2**. Apparent digestibilities of nutrients and energy for sea bass were not affected by the oxygen level (P>0.05). Crude protein and fat digestibility (86-89% and 85%, respectively) for sea bream were not significantly (P>0.05) different among three dissolved oxygen levels. Energy, Dry matter and organic matter were more digestible (P<0.05) in the group with 60-80% DO. Energy and dry matter digestibility showed the lowest values in the 80-100% DO group, whereas organic matter digestibility did not differ between the 60-80% and 40-60% DO groups (P>0.05). Digestibility coefficients values were high for all oxygen levels for both species comparing with previous studies (Ballester-Moltó *et al*., 2017). Gastric evacuation times are summarized in **Table 3**. Oxygen saturation had a significant effect on the rate of digestion of

Table 1. Proximate composition of nutrients and energy of feed for European sea bass and Gilthead sea bream

Crude protein (% DM)	44.0
Crude fat (% DM)	19.0
Gross energy (Mj/kg)	24.0
Ash (% DM)	7.7
Moisture (% DM)	6.5
Crude fiber (% DM)	2.6
Phosphorus (% DM)	1.1
Calcium (% DM)	1.35
Sodium (% DM)	0.24

(Continued on next page)

Table 2. Apparent digestibility coefficients of nutrients and energy (ADC, %) of tested diet			
	European sea bass		
	80-100% DO	60-80% DO	40-60% DO
ADC protein	91,0 ± 2,97	94,3 ± 0,54	90,5 ± 4,54
ADC fat	93,5 ± 2,18	96,2 ± 0,68	92,9 ± 3,09
ADC energy	89,0 ± 2,69	91,9 ± 0,73	88,9 ± 3,67
ADC dry matter	76,0 ± 2,94	80,8 ± 1,20	78,6 ± 4,15
ADC organic matter	85,1 ± 3,02	88,7 ± 0,76	86,2 ± 4,08
	Gilthead sea bream		
	80-100% DO	60-80% DO	40-60% DO
ADC protein	86,7 ± 0,51	88,8 ± 0,60	87,4 ± 0,61
ADC fat	85,3 ±0,83	85,9 ± 0,18	85,2 ± 1,10
ADC energy	80,9 ± 0,69 [a]	84,7 ± 0,29 [b]	82,8 ± 0,31 [c]
ADC dry matter	56,1 ± 0,38 [a]	66,5 ± 1,29 [b]	59,6 ± 2,78 [a]
ADC organic matter	72,2 ± 0,81 [a]	79,6 ± 0,97 [b]	76,6 ± 1,02 [b]

Table 3. Post-feeding evacuation time of digestive tract for sea bass and sea bream at three different oxygen saturation levels (DO)		
	European sea bass	
	50% empty	100% empty
80-100% DO	After 8 hours	After 27 hours
60-80% DO		More than 27 hours
40-60% DO		
	Gilthead sea bream	
	50% empty	100% empty
80-100% DO	After 4.5 hours	After 18 hours
60-80% DO	After 8 hours	
40-60% DO	After 13 hours	

the digestive system for both species. The evacuation rate of the digestive system, influenced by the different oxygen levels in sea bream, appeared to modify the feed transit rate and its presence in the intestine. No differences were found for sea bass ADCs under different oxygen levels in contrast to the different evacuation rates among groups. The gastric evacuation rates for the two species were significantly different ($P<0.05$), with the sea bream emptying its digestive tract completely after 18 hours while the seabass after 27 hours for the high DO group (80-100%) or more than 27 hours for 60-80% and 40-60% DO groups.

Acknowledgments

The program is co-funded by the European Maritime and Fisheries Fund in Greece (EMFF OP) and the Hellenic Republic via the Operational Programme “Fisheries and Maritime 2014-2020” (EPALTH 2014-2020). Program MIS 5030044.

References

AOAC. 1990. Official methods of analysis. Arlington, Virginia, USA.

Ballestert-Moltó, M., Follana-Berná, G., Sanchez-Jerez, P., &Aguado-Giménez, F. (2017). Total nitrogen, carbon and phosphorus digestibility in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) fed with conventional and organic commercial feeds: implications for particulate waste production. Aquaculture Research, 48(7), 3450-3463.

Petersen, L. H., & Gamperl, A. K. (2010). Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology*, *213*(5), 808-819.

Riche, M., Haley, D. I., Oetker, M., Garbrecht, S., &Garling, D. L. (2004). Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). Aquaculture, 234(1-4), 657-673.

Tran-Ngoc, K. T., Dinh, N. T., Nguyen, T. H., Roem, A. J., Schrama, J. W., &Verreth, J. A. (2016). Interaction between dissolved oxygen concentration and diet composition on growth, digestibility and intestinal health of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *462*, 101-108.

GENETIC PARAMETERS FOR CARCASS COMPOSITION OF ATLANTIC COD (*Gadus Morhua*) AFTER FOUR GENERATION OF SELECTIVE BREEDING

A. Kettunen[*1], Ø.J. Hansen[2] and V. Puvanendran[2]

[1]Nofima AS, P.O. Box 210, N-1431 Ås, Norway
[2]Nofima AS, P.O. Box 6122 Langnes, N-9291 Tromsø
E-mail: anne.kettunen@nofima.no

Introduction

The global and regional declines of wild stocks of Atlantic cod (*Gadus morhua*) prompted for the establishment of The National Cod Breeding program in Norway in 2003. The main selection criterium has been rapid growth, specifically body weight at 2+ age, after approximately 22 months of sea-rearing. It is well established that body weight is highly genetically correlated with gutted weight and filet weight, e.g., in Nile tilapia, rainbow trout and gilthead seabream (Gjerde et al. 2012; Kause et al. 2002; Navarro et al. 2009). This relationship has not been established in Atlantic cod.

Atlantic cod is a lean fish. Skeletal muscles contain only minute amounts of fat, whereas large amounts of fat can accumulate in liver. Enlarged liver has been frequently reported in practical cod aquaculture. This can be a consequence of unlimited access to feed that is not optimal or optimally utilized (Ingebrigtsen et al. 2014), but genetics may also play a role. In addition to the unfavourable distribution of ingested energy, large livers may be a welfare issue in aquaculture cod.

In this study we analysed slaughter data of Atlantic cod to get deeper insight of the genetic variation in body weight and carcass composition traits and assess the magnitude of the genetic and phenotypic interrelationships between these traits.

Material and methods

Data comprised records from 2557 individually tagged Atlantic cod from 218 full-sib families (158 sires and 134 dams) representing the fourth generation of selectively bred cod of National Cod Breeding Program. *A priori* power calculations (Kettunen and Lillehammer, 2019) were conducted to optimize the experimental design to obtain reliable estimates of heritability and genetic correlations. Fish were slaughtered at 948 days age from hatching and individual records for body weight traits (round RWT, gutted GWT, headed-and-gutted HGWT), body length (LENGTH), liver weight (LIVERWT), intestine weight (INTWT) and head weight (HEADWT) were registered. Hepatosomatic index (HSI), HEAD% and INT% were calculated as a percentage of LIVERWT, HEADWT and INTWT of RWT, respectively.

Estimates of heritability were calculated from variance components estimated with a univariate animal model using ASReml (Gilmour et al. 2015):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_A\mathbf{a} + \mathbf{Z}_C\mathbf{c} + \mathbf{e},$$

where, is the vector of the phenotypic observations; is the vector of fixed effect of sex; is the vector of random family effects; is the vector of random additive genetic effects; is the vector of random residual effects. Bivariate animal model was used for estimation of phenotypic and genetic correlations.

We also calculated conditional heritability for LENGTH, carcass weight traits (LIVERWT, INTWT, HEADWT) and HSI to examine the magnitude of genetic variation displayed in these traits independent from the genetic variation in RWT (Kause et al. 2002):

$$h^{2*}_{bodycomp} = h^2_{bodycomp} * (1 - r_g^2).$$

Results

Moderate estimates of heritability, ranging from 0.27 to 0.29 (±0.07), were obtained for RWT, GWT and HGWT from a univariate model. Slightly higher heritability was estimated for LIVERWT (0.32±0.08) and HEADWT (0.39±0.08), whereas LENGTH, INTWT, and INT% expressed relatively low heritability: 0.15-0.19 (±0.06-0.07). HSI and HEAD% were shown to be highly heritable traits (0.47±0.09 and 0.43±0.05). Proportion of family variance from phenotypic variance varied between 0.02-0.08. Conditional estimates of heritability for LENGTH, LIVERWT, INTWT and HEADWT (0.06-0.11) indicated that although a significant proportion of the genetic variation is dependent on RWT, this dependency is not complete. The conditional heritability for HSI was identical with the original estimate: 0.46.

(Continued on next page)

Genetic correlations between RWT and GWT/HGWT were close to unity (0.98). RWT was highly genetically correlated with LENGTH, LIVERWT, INTWT and HEADWT (0.75-0.87). LIVERWT, INTWT and HEADWT were strongly genetically intercorrelated (0.54-0.78). Low phenotypic (0.30±0.03) and non-significant genetic correlation (0.15±0.17) was estimated between RWT and HSI. Negative genetic interrelationships, with high standard errors of the estimates, were estimated between RWT and HEAD% (-0.29±0.10) and INT% (-0.48±0.20).

Discussion

As RWT, GWT and HGWT are genetically same trait, selection for RWT will result in feasible genetic change also in the valuable proportion of carcass in Atlantic cod. The selection for increased body weight at 2+ will increase the absolute weight for intestine, head, and liver. In contrast, selection for RWT will genetically reduce HEAD% and INT%, albeit these changes are expected to be minimal. HSI is not genetically strongly affected by selection for RWT, as indicated by non-significant genetic correlation between RWT and HSI. This observation is also supported by the high conditional heritability for HSI. Given high (moderate) heritability and moderate (high) phenotypic variation of HSI, CV=13%, (LIVERWT, CV=26%) selection for smaller liver is expected to be successful. This said, holistic understanding of the underlying biological reason(s) for frequently reported large liver in cod aquaculture is imperative before pursuing selective breeding against large liver size.

Acknowledgements

The staff of the Centre for Marine Aquaculture is warmly thanked for acquiring the data.

References

Gilmour, A. G., Gogel, B. J., Cullis, B. R., Welham, S. J., Thomson, R. 2015. ASReml User Guide Release 4.1. Structural Specification, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK www.vsni.co.uk

Gjerde, B., Mengistu, S.B., Ødegård, J., Johansen, H., Altamirano, D.S. 2012. Quantitative genetics of body weight, fillet weight and fillet yield in Nile tilapia (*Oreochromis niloticus*). Aquaculture 342-343: 117-124.

Ingebrigtsen, I.A., Berge, G.M., Ruyter, B., Kjær, M.A., Mørkøre, T., Sørensen, M., Gjøen, T. 2014. Growth and quality of Atlantic Cod (*Gadus Morhua*) fed with high and low fat diets supplemented with glutamate. Aquaculture 433: 367–76.

Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., Eskelinen, U. 2002. Coupling body weight and its composition: a quantitative genetic analysis in rainbow trout. Aquaculture 211: 65-79.

Kettunen, A., Lillehammer, M., 2019. Power calculations for optimisation of the experimental design to detect GxE: salmon experiments in FutureEUAqua. Oral presentation in Aquaculture Europe 2019, Montpellier, 9th of October, 2019.

Navarro, A., Zamorano, M.J., Hildebrandt, S., Ginés, R., Aguilera, C., Afonso, J.M. 2009. Estimates of heritability and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus* L.), under industrial conditions. Aquaculture 289: 225-230.

IN VITRO PRODUCTION OF EGGS FROM IMMATURE OVARIAN FOLLICLES OF AFRICAN CATFISH (*Clarias gariepinus*)

Nevena Kitanović, Zoran Marinović, Quyen Ngoc Nguyen, Tamás Müller, Balázs Kovács, Béla Urbányi, Gergely Bernáth and Ákos Horváth

Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1, 2100 Gödöllő (Hungary)
E-mail: nevena.n.kitanovic@gmail.com

Introduction

In vitro maturation (IVM) of oocytes is a reproductive technology that enables mature eggs to be produced *ex*. This approach is becoming a useful tool in aquaculture, for further understanding of oogenesis, refining spawning practices and providing alternative methods of egg production. Hormonal stimulation and media composition are crucial parameters to consider in IVM protocols. In fish, gonadotropins and maturation-inducing hormones, such as 17α,20β-dihydroxy-4-pregnen-3-one (DHP), are commonly used to induce maturation of isolated ovarian follicles. Addition of prostaglandins (PGs) has been shown to induce follicle rupture after steroid-induced maturation (Takahashi et al., 2018). Incorporation of protein sources, such as serums and bovine serum albumin (BSA), into the culture media was reported beneficial for cytoplasmatic maturation and maintaining the fertilizing ability of ovulated oocytes (Seki et al., 2008).

The aim of this study was to establish a culture system that would support development of fertilizable eggs from immature, postvitellogenic ovarian follicles isolated from African catfish (*Clarias gariepinus*). High growth rate and stocking density have made this species a popular choice for cultivation, especially in tropical and subtropical regions. Therefore, improvements of artificial reproduction of African catfish are of great value. In addition, its spawning in captivity relies on artificial hormonal stimulation of both males and females and does not occur spontaneously, which make it a good model for *in vitro* gametogenesis studies.

Materials and methods

Unprimed adult females of African catfish were euthanized and ovaries were immediately excised. Fully grown, postvitelogenic ovarian follicles were manually isolated and sorted in cell culture plates with maturation media consisting of 90% Leibovitz L-15 medium (pH 7.6, 285 mmol/kg), supplemented with antibiotics. Throughout treatments, the follicles were incubated at 25 °C, under gentle agitation. For *in vitro* maturation experiments, follicles were placed in media with DHP (1 µg/ml), hCG (20 IU/ml) and without hormones (control). Effect of human chorionic gonadotropin (hCG) on DHP-induced maturation was evaluated by pre-treating and co-incubating the follicles with hCG. Resumption of meiosis and maturation was evaluated by scoring the percentage of follicles that underwent germinal vesicle breakdown (GVBD) and ooplasm clearing, compared to control. For *in vitro* ovulation experiments, prostaglandins F2α ($PGF_{2\alpha}$) and E2 (PGE_2) were added to the incubation media containing DHP. Ovulation was monitored by checking the integrity of the follicular layer. Immediately after ovulation, oocytes were transferred to a clean dish and fertilized using sperm freshly extracted from testes of one male. Following insemination, eggs were incubated at 25 °C and monitored regularly until hatching. The effect of additional protein supplementation on maturation, ovulation and fertilization was assessed by adding 0.5% BSA to the treatment groups.

Results and discussion

After isolation, postvitellogenic ovarian follicles of African catfish do not mature spontaneously under *in vitro* conditions. By adding DHP to the culture medium, we were able to stimulate maturation in a time-dependent manner, with the highest percentage of GVBD (81 ± 7%) observed after 12 h. Exposure to hCG did not promote GVBD and had no discernible effect on steroid-induced maturation. Prolonged treatment with DHP did not lead to ovulation. Addition of both $PGF_{2\alpha}$ and PGE_2 caused rupture of the follicular layer in DHP-matured follicles, in a concentration-dependent manner. The highest ovulation rate was observed in treatments with 5 ug/ml $PGF_{2\alpha}$ (71 ± 8%). This is in agreement with previous reports that $PGF_{2\alpha}$ is a major mediator involved in fish ovulation (Takahashi et al., 2018). *In vitro* matured and ovulated oocytes obtained in this study maintained their developmental competence and were successfully fertilized. The fertilization rate was 39% after 24 hours, while the percentage of hatched larvae was 8%. Although the addition of 0.5% BSA to the culture medium did not influence follicle maturation, it significantly lowered the percentage of $PGF_{2\alpha}$-induced ovulation (39 ± 3%). No fertilization was achieved with oocytes that were ovulated in media supplemented with 0.5% BSA. These results demonstrate that the presence of BSA in culture medium is not necessary to improve fertilization and hatching rates.

(Continued on next page)

Conclusions

An *in vitro* maturation protocol was successfully developed for African catfish ovarian follicles. The culture media consisting of Leibovitz L-15 media, without any additional protein supplementation, maintains viability and responsiveness of follicles to hormones. Treatment of freshly isolated follicles with DHP successfully promotes their cytoplasmatic and nuclear maturation, while subsequent ovulation is achieved by adding prostaglandins to the media. This combination of optimal media composition with a two-step hormone stimulation produced mature eggs which could be artificially fertilized and give rise to developing embryos.

Acknowledgements

This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16), the EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union and the European Social Fund as well as the NKFIH FK124585 project.

References

Takahashi, T., Hagiwara, A. and Ogiwara, K., 2018. Prostaglandins in teleost ovulation: A review of the roles with a view to comparison with prostaglandins in mammalian ovulation. Molecular and cellular endocrinology, 461, pp.236-247.

Seki, S., Kouya, T., Tsuchiya, R., Valdez, D.M., Jin, B., Hara, T., Saida, N., Kasai, M. and Edashige, K., 2008. Development of a reliable in vitro maturation system for zebrafish oocytes. Reproduction, 135(3), pp.285-292.

VGLL3A ALLELES AFFECT THE BRAIN-PITUITARY-GONAD AXIS IN ATLANTIC SALMON (*Salmo salar*) VIA REGULATION OF *FSHB*

Erik Kjærner-Semb[1*], D. Crespo[1], P. Vogelsang[1], T. Furmanek[1], P. G. Fjelldal[1], T. Fraser[1], T. Hansen[1], R. B. Edvardsen[1], R. W. Schulz[1,2], A. Wargelius[1]

[1] Institute of Marine Research, Norway
[2] University of Utrecht, Netherlands
E-mail: erikkj@hi.no

Introduction

Early male maturation represents a major problem in Atlantic salmon farming, both in sea cages and in recirculation aquaculture system facilities. Problems caused by pre-harvest maturation include significantly increased disease susceptibility and osmoregulatory problems, causing higher mortalities, reduced animal welfare, and production losses. We and others have previously reported a strong association between alleles in the *vgll3a* locus and time of maturation in Atlantic salmon, however, the molecular mechanisms and roles of *vgll3a* alleles in controlling time of puberty are largely unknown. Previous studies have indicated a potential role of *vgll3a* in the salmon gonad, where expression of *vgll3a* is regulated in Sertoli cells upon entry into puberty, suggesting a possible connection between *vgll3a* and the brain-pituitary-gonad (BPG) axis. However, it is unknown which proteins link *vgll3a* alleles to the BPG axis.

Results

To search for a possible connection between *vgll3a* alleles and the BPG axis, we used an RNA-Seq approach to identify genotype-dependent gene expression in testis, pituitary and belly flap in fish stimulated to enter maturation. We uncovered several differentially expressed genes potentially involved in regulating the onset of puberty under the control of *vgll3a* alleles, including pituitary *follicle-stimulating hormone subunit beta* (*fshb*), the major hormone triggering puberty in vertebrates. Interestingly, the pituitary displayed low levels of *vgll3a* expression when measured by RNA-Seq and qPCR, prompting us to investigate if we could detect the presence of one or more endocrine factors in plasma with stimulatory or inhibitory effects on *fshb* expression. We therefore performed *ex vivo* incubations of pituitaries from immature males with plasma from immature males homozygous for the Early (EE) and Late (LL) maturation genotypes. Interestingly, we observed a significant decrease in *fshb* expression in pituitaries incubated with plasma from immature LL fish compared to pituitaries incubated with plasma from immature EE fish or negative controls (medium only). We additionally performed incubations where we boiled the plasma before the incubations to denature plasma proteins. Boiling the plasma removed the previously observed down-regulation of *fshb* expression in pituitaries incubated with LL plasma, indicating the presence of one or more protein/peptide factors in plasma from LL fish with the ability to potentially delay maturation.

LONG-TERM PERFORMANCE OF GENE EDITED, STERILE ATLANTIC SALMON – GROWTH, SMOLTIFICATION, WELFARE INDICATORS AND FILLET COMPOSITION

L. Kleppe[(1)]*, P.G. Fjelldal[(2)], E. Andersson[(1)], T. Hansen[(2)], M. Sanden[(1)], A. Bruvik[(1)], K.O Skaftnesmo[(1)], T. Furmanek[(1)], E. Kjærner-Semb[(1)], D. Crespo[(1)], S. Flavell[(2)], A.Ø. Pedersen[(2)], P. Vogelsang[(1)], A. Torsvik[(1)], S. Olausson[(3)], B. Norberg[(3)], R.W. Schulz[(1,4)], J. Bogerd[(4)], N. Santi[(5)], R.B. Edvardsen[(1)], A. Wargelius[(1)]

[(1)] Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817, Bergen, Norway
[(2)] Institute of Marine Research, Matre Aquaculture Research Station, 5984 Matredal, Norway
[(3)] Institute of Marine Research, Austevoll Research Station, NO-5392, Storebø, Norway
[(4)] Utrecht University, Science Faculty, Department Biology, Padualaan 8, NL-3584 CH Utrecht, The Netherlands
[(5)] AquaGen AS, Postboks 1240, Torgard, 7462 Trondheim, Norway
E-mail: lene.kleppe@hi.no

Introduction

Using sterile salmon in aquaculture could mitigate sustainability challenges including precocious male maturation and genetic introgression from farmed escapees to wild populations. By knocking out *dead end* (*dnd*-KO), we have created germ cell-free (GCF), sterile salmon with the potential to remain immature throughout life. While these are promising commercial traits, more knowledge is needed on potentially unwanted effects of the loss of *dnd* function. In this study, *dnd*-KO GCF and wild type (WT) Atlantic salmon were reared in an indoor common garden setup with natural light and temperature for 3 years and then terminally sampled. Fish performance in terms of growth, welfare indictors, gene expression in non-target tissues and fillet quality were evaluated.

Results

Regarding growth performance, GCF males were smaller only when compared to WT females early in life, however, this difference disappeared later. At harvest size, WT males was the largest group. Smoltification markers (mRNA levels of gill Na+/K+-ATPase [NKA] subunits) showed normal up (*nka α-1b*, *nka α3*, *nka β-1*) or downregulation (*nka α-1a*) from the freshwater to the seawater stage in both WT and GCF salmon. However, plasma stress indicators including lactate and osmolality concentrations were higher in GCF than WT plasma 24 hours after transfer to seawater, but these differences disappeared after 6 months in seawater. In postsmolts after 6 months in seawater, no differences for any plasma stress markers were detected, while in adults at harvest size we observed some differences in plasma pH (higher in WT and GCF males than in WT females), lactate (higher in WT females than in GCF females and males), Na+ and osmolality (higher in WT females than in GCF males). No differences were detected between WT and GCF transcriptomes of selected non-target tissues (muscle and pituitary) in postsmolts after 6 months in seawater. Results also showed that the prevalence of vertebra deformities was similar and within a normal range in both WT and GCF fish. No differences were found in hepatic or cardio somatic indexes (HSI/CSI) in postsmolts after 6 months in seawater, however in adults at harvest size HSI was higher in WT than in GCF fish, while CSI was unaffected. No sexual maturation was detected in GCF fish of either sex throughout the study period, in contrast to their WT counterparts. Fillets from WT and GCF salmon at harvest size showed no significant difference in proximate composition (protein, dry matter or total fat). Interestingly, the relative content of the omega-3 fatty acid DHA 22:6n-3 was higher in GCF compared to WT males despite having the lowest total amount of fatty acids.

Conclusions

GCF males lack the growth boost observed in WT males during the start of puberty, resulting in a smaller harvest size if harvested at this stage. To avoid any differences in growth it may be needed to grow GCF fish to a larger size, which is expected to be beneficial since GCF fish will not experience any growth inhibition due to sexual maturation. We also observed larger livers in WT compared to GCF salmon, which may reflect altered metabolism in line with the onset of sexual maturation, indicating that nutritional needs may differ between WT and GCF fish. Since GCF individuals were not more prone to develop deformed vertebra than their WT counterparts, GCF farmed salmon may potentially have less problems with quality downgrading losses at harvest with respect to deformities than the currently used triploid sterile salmon. Although the fillet transcriptome (postsmolts) and proximate composition (harvest size) did not differ between WT and GCF salmon, the finding of increased relative amount of DHA 22:6n-3 in GCF males compared to WT males may represent a more favorable fillet composition for consumers.

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

Adrianna Kochańska* and Michaela Aschan

UiT The Arctic University of Norway, Norwegian College of Fishery Science, N-9037 Tromsø, Norway
E-Mail: adrianna.kochanska@uit.no

Introduction

The expectations for sustainable food production in aquatic systems are high, especially of low trophic species products with a low carbon footprint. To enhance the knowledge among students, practitioners, and teachers, we are developing a massive open online course (MOOC) in sustainable aquaculture for low trophic species (SALTS). The course will contribute to the much-needed increased literacy in the field necessary for enhanced and diversified aquaculture production. In this presentation, we describe the methodological approach for MOOC development, present the course content and structure, and demonstrate a pilot module.

How to develop a MOOC

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

Course modules and the pilot

The modules are: 1) Sustainability of Low Trophic Aquaculture (LTA), 2) Governance of LTA, 3) Food nutrition and safety, and consumer perspectives 4) Business, economics, and markets, 5) Production systems, 6) Macroalgae, 7) Echinoderms, 8) Molluscs, 9) Herbivorous finfish, and 10) Future of LTA. Each module begins with a short introduction video followed by an engagement activity. The module contains 2-3 sub-topics, including videos, quizzes, reading material, advanced assessment and additional material. We aim to create educational material for online students, and for teachers who will utilise the material in a flipped classroom format. The entire course is equivalent to 5 ECTS[1] points (125-140 hours in total). Each module will take approximately 13 hours to complete. The pilot topic on Sea Urchins will be presented in more detail to exemplify the process and the outcome. We also wish to make the pilots available for testing online so that the interested attendant can provide feedback. The course will be available on the Open edX platform and disseminated through the ALTANet platform.

References

Anderson, L. W. and Krathwohl, D. R., et al (Eds..) (2001) *A Taxonomy for Learning, Teaching, and Assessing: A Revision of Bloom's Taxonomy of Educational Objectives*. Allyn & Bacon. Boston, MA (Pearson Education Group)

Biggs, J. (1996). *Enhancing teaching through constructive alignment*. Higher education, 32(3), 347-364.

Latorre-Cosculluela Cecilia, Cristina Suárez, Sonia Quiroga, Natalia Sobradiel-Sierra, Raquel Lozano-Blasco, Ana Rodríguez-Martínez (2020) *Flipped Classroom model before and during COVID-19: using technology to develop 21st century skills*. Interactive Technology and Smart Education

(Continued on next page)

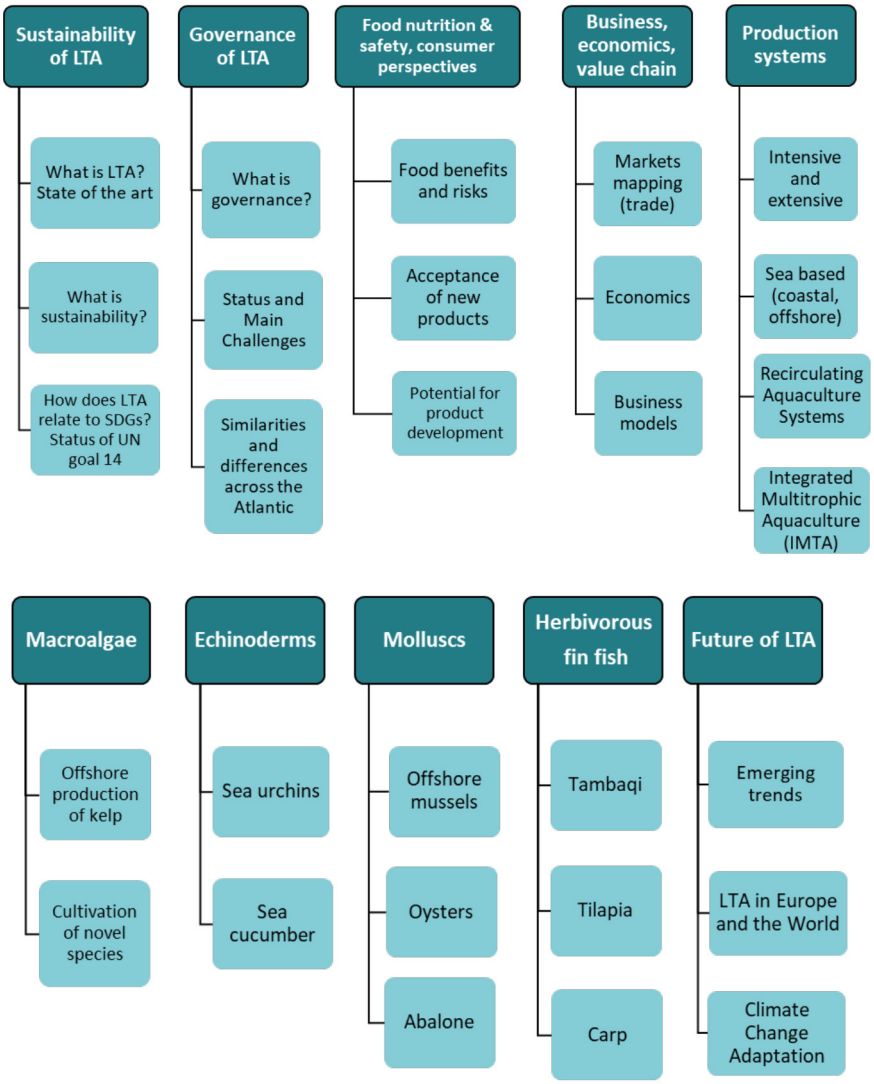


Figure 1 Modules in the MOOC on Sustainable Aquaculture for Low Trophic Species

THE WAY TO SHORTEN THE GENERATION INTERVAL IN SELECTION PROGRAM OF COMMON CARP REARED UNDER TEMPERATE CLIMATE

M. Kocour, J. Zhao, M. Prchal, Ch. Steinbach, J. Křišťan, D. Gela, H. Kocour Kroupová, O. Malinovskyi, T. Policar

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, Vodňany, Czech Republic
e-mail: kocour@frov.jcu.cz

Introduction

Selective breeding is based on genetic improvement through cumulation of genetic gain over generations. So, it is required to shorten the generation interval (GI, the age of the fish when firstly used for artificial reproduction). In common carp reared outdoor under temperate climate, the GI for females is about 5 years. However, in tropical zones, common carp females can mature in 1-2 years. It is said that common carp females need to collect 10,000-12,500 °D (°D = the sum of the average daily temperatures of the water in which the fish live) of water temperatures over 15°C to reach adulthood (Horváth, 1985). It must be also remembered that potential breeding candidates of common carp should stay before own selection challenge under the pond conditions to avoid bias of selection process. In Amur mirror carp (AM) it was found that if selection for faster growth or the proportion of edible parts were done in two-year old fish, it would have similar effect as doing the selection at market size (Prchal et al., 2018).

We therefore studied whether the GI in AM can be shortened to three years using RAS.

Material and methods

Experimental fish came from a population established in 2017 by artificial spawning using a partial factorial design of 27 dams and 29 sires of AM. The progeny was kept communally in ponds under standard stocking densities and conditions (Prchal et al., 2018). In April 2019, the following groups of fish were formed:

- A stock of i) fish selected for faster growth (group S) and ii) randomly collected fish (group A), both being kept in RAS for a year at water temperatures between 18-23 ° C and fed daily with a commercial diet for carps in a dose of 1.0-1.5% of the stock weight.
- Czech Republic (temperate climate).

From April 2019 to April 2020, ~ 6 females and 6 males from groups A and B were sampled monthly to monitor gonad development and condition. A sample of gonads was taken for histology. The gonadosomatic index (GSI) and the Fulton's condition coefficient (FC) were calculated. Water temperatures in RAS and pond were recorded at hourly intervals. As monitoring showed that group A unlike group B had reached the readiness for artificial spawning, the spawning according to the standard methodology (Kocour et al., 2005) of group S was performed in May 2020 after back adaptation of fish to pond conditions from March 2020. For the comparison, fish of the same breed in age of 6 years (group C), i.e. common broodstock, were spawned together with group S. To confirm whether the fish left in the RAS until May (group A) are still ready to be spawned even if kept all the time at higher temperatures (18-21°C), 12 fish that visually looked ready to be spawned were also included in an artificial spawning a week after spawning of group S and C. Reproductive parameters were determined in females of groups S, C and A (Tab. 1). No exact parameters were recorded in males as they were almost mature already at the age of two years.

Tab. 1. Reproductive parameters of females. **RS** – reproduction success (**NFI** – number of females included into reproduction/**NFS** – number of females successfully stripped), **WE** – weight of eggs, **NE1G** – number of eggs in 1 g, **AWF** – absolute working fecundity, **RWF** – relative working fecundity, **EY** – relative egg yield, ***** - significantly different value ($p < 0.05$) from the others within given column.

Fish group	RS (NFI/NFS)	WE (g)	NE1G	AWF (*1000)	RWF (*1000)	EY (%)
S	95.2 (60/63)	253 ± 153.3	598 ± 73.0	158 ± 91.9	43.5 ± 21.5	7.0 ± 4.06
A	50.0 (6/12)*	214 ± 73.0	-	-	-	5.1 ± 73.0
C	92.9 (13/14)	473* ± 121.7	550 ± 59.4	256* ± 58.7	90.1* ± 18.8	16.4* ± 3.51

(Continued on next page)

Results and Discussion

It was found that fish kept in RAS grew faster. Females of group A (2682 ± 791.6 g) were twice bigger in February 2020 than females of group B. In males the difference was also significant but a bit lower (2376 ± 772.0 g vs. 1307 ± 228.7 g). Also, FC was in both sexes higher in RAS. Gonad development started to be significantly different from October 2019 in females. The highest GSI value observed in group A was 13.3 ± 1.8 (February 2020) compare with 5.3 ± 2.5 in group B (December 2019). In group B, GSI increased significantly in April 2020 (8.4 ± 2.9) while in group A it decreased (9.7 ± 4.9). However, histologically the fish in group B were not fully mature unlike females of group A. In males, the GSI was almost identical in RAS and pond, and histologically males of both groups were mature. Most reproductive parameters of females in group C were better than in group S (Tab. 1). Still, the reproductive success (RS) of females out of group S was comparable with group C which is the most important for selection program. RS of group A was significantly lower. It shows that females after maturation shall be kept at temperatures below 15°C until preparation phase to artificial spawning. Fertilization and hatching rates in group S and C were comparable unlike group A. It was calculated that females of groups A and S collected 10,590 °D with daily average water temperatures over 15°C at the end of October 2019 and 12,900 °D in February 2020 while females of group B collected only 9,055 °D. Thus, it seems that information published by Horváth (1985) concerning °D needed for common carp females to reach the maturity is useful tool and that indoor RAS combined with pond culture are efficient in common carp for shortening of generation interval to three years.

Acknowledgements

This work was funded by project no. QK1910430 of NAAR (NAZV) of the Czech Republic, and project Biodiversity (CZ .02.1.01/0.0/0.0/16_025/0007370) under the Ministry of Education, Youth and Sports of the Czech Republic.

References

Horváth, L., 1985. Egg development (oogenesis) in the common carp (*C. carpio* L.) In: Muir J.F. and Roberts R.J. (Editors), Recent Advances in Aquaculture, Vol. II. Croom Helm. London and Westview Press, Boulder, CO, pp. 31-77.

Kocour, M., Gela, D., Rodina, M., Linhart, O., 2005. Testing of performance in common carp *Cyprinus carpio* L. under pond husbandery conditions I: top-crossing with Northern mirror carp. Aquaculture Research 36 (12): 1207-1215.

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M., 2018. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE 13(1): e0191624.

INTEGRATION OF TRANSCRIPTOMICS AND METABOLOMICS TO IDENTIFY POTENTIAL GENE-METABOLITE INTERACTIONS AFFECTING SIZE AT HARVEST IN FARMED ATLANTIC SALMON (*Salmo salar*)

Miyako Kodama

Center for Evolutionary Hologenomics, GLOBE institute, Faculty of Health and Medical Sciences
Email: miyako.kodama@sund.ku.dk

Introduction
One key challenge facing salmonid aquaculture is the considerable losses due to wide (largely unpredictable) variation in growth rates and hence size at harvest - with adult weight at harvest sometimes varying by an order of magnitude within a single sea pen. Although attempts to reduce such phenotypic variation have traditionally been performed under the 'phenotype = genotype*environment' model, successes are limited. Based on the recent advances in omics technologies, multi-omic approaches have been adopted to reveal genetic mechanisms underlying various commercially important traits in a wide range of species. Here, metabolome-transcriptome integration analyses were performed on farmed salmon of various sizes to identify potential gene-metabolite interactions that underlie the variation in harvest sizes, attempting to further bridge the gap between this trait and genotype.

Methods
We sampled the gut epithelium tissue and the gut content from two groups of salmon, each of which contained 180 individuals ranging from 1kg to 8.5kg. Gene expression profiles were assessed using shotgun RNA-seq data obtained from the gut tissue, and metabolomic profiles were obtained using untargeted metabolomics based on the gut content. Each layer of data was analyzed separately by 1) identifying differentially expressed genes based on the transcriptomic profiles, and 2) identifying differentially abundant metabolites in small (1 to 3kg) vs. large (6 to 8kg) groups of salmon. Subsequently, a linear modeling approach was applied to identify significantly correlated gene-metabolite pairs, assuming that co-regulation patterns reflect functionally related genes and metabolites.

Results
We identified 24 significantly differentially expressed genes (DEGs), and 113 significantly differentially abundant metabolites (DAMs). Some of these metabolites were identified as omega3 fatty acid, polyunsaturated fatty acids, as well as bile acid involved in the emulsification of fats. A fraction of DEGs and DAMs are thought to form significantly correlated gene-metabolite pairs based on the linear model.

Conclusions
The results indicate the importance of employing multi-omic approach when elucidating the genetic mechanisms underlying size at harvest in salmon. The gene-metabolite pairs identified in this study may have a role in determining the harvest size in the Atlantic salmon, and further validation may lead to the discovery of effective biomarkers for monitoring fish growth and health throughout the production cycle.

A METHOD TO ASSESS GAPING IN SPARIDAE SPECIES FILLETS

Dimitra Kogiannou*, Mado Kotsiri, Kriton Grigorakis

Sensory lab, Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Centre for Marine Research, Anavyssos (Greece)
E-mail: dkogiannou@hcmr.gr

Introduction

Numerous methods have been proposed to evaluate the degree of gaping in fish fillets, measuring either the quantity and size of slights in the fillet (Andersen, 1994) or evaluating the area covered by gaps (Kiessling et al., 2004). Automated and semi-automated methods have been also proposed for assessing fillet gaping, thus providing objective, accurate as well as re-analysable data (Ashton et al., 2010; Balaban et al., 2011; Merkin et al., 2013). However, the existing methodologies are limited to assessing gaping severity in salmonids. Sparidae species, namely gilthead sea bream (*Sparus aurata*) and red sea bream (*Pagrus major*), also suffer from gaping and consequently economic losses burden their industry. The extrapolation of methods developed for salmonids to other fish species might be inappropriate or erroneous since gaping and muscle textural characteristics are species-specific and, on the other hand, commercial fillet sizes largely differ.

The aim of this study was to develop a semi-automated method, by using digital photography and computer image analysis, for measuring gaping in Sparidae species fillets. Furthermore, the data from applying this method were used to train assessors in order to speed up the measuring process and to make the scoring procedure accessible to all commercial gilthead sea bream and/or red sea bream processing plants.

Materials and methods

Market-size (400-800 g) gilthead sea bream and red sea bream were harvested from sea cage farms during the summer period and filleted using a filleting-machine. Fillets were placed skin side down on a polypropylene surface with a convex curvature of 165 degrees of a circle with 4.5 cm diameter. A 12-megapixel camera was mounted on a retort stand and clamped 15.5 cm above the apex of the curved surface. Fillet images were taken individually and the records were digitally analysed by using ImagePro-Plus 4.5 software. The software was used to manually highlight the total surface area of the fillet as well as the number, diameter and surface area of the gaps. Due to the curvature, a percentage of inaccuracy was found in the measurement located away from the focal point of the image, which determined to be less than 5%.

Three assessors were trained to quantify gaping severity on Sparidae species fillets according to the gaping scale proposed herein. The training was considered successful only when their performance was accurate more than 95%.

The degree of gaping expressed as a % of surface covered by gaps in each of the studied fillet has been computed against the number of gaps and the maximum size of biggest gap in order to examine correlations. For method validation a $\chi 2$ method was adopted to examine a) if assessors rated in a uniform way with each other and b) to see if ratings deriving by image analysis (true) and those made by the assessors (observed) differed. A two-tail Pearson correlation was conducted to evaluate how sample scaling results correlate with gaping area percentage.

Table 1. Gaping score scale obtained by image analysis data of fillets (N=38) suitable for measuring gaping severity in Sparidae. Scale was based on the area of gaping ex-pressed as % of the total fillet area. Additional characteristics for each gaping score point are also described

Gaping score	Area (a) of gaping as % of the total fillet area	Gaping severity (Additional characteristics)
0	0	Absence/ No gaping
1	$0<\alpha<2$	Slight/Subtle gaping (up to 5 small[a] gaps)
2	$2<\alpha<4$	Mild gaping (up to 7 small gaps)
3	$4<\alpha<6$	Moderate gaping (up to 7 large[b] & few small gaps)
4	$6<\alpha<8$	Severe gaping (up to 7 large and/or many small gaps)
5	$8<\alpha$	Extreme gaping/ Non-marketable fillet (over 7 large gaps)

a: small gaps <5mm
b: large gaps >5 mm

(Continued on next page)

Results

The area of gaping (expressed as % of the total fillet), contrary to the mean number of gaps and the mean diameter of largest gap, was found to be the most suitable parameter for determining gaping severity for Sparidae species. Consequently, a six point scale (from 0 to 5), based on the fillet gaping area, was proposed (Table 1). In order to facilitate gaping classification by the assessors, additional characteristics for each gaping point were also included.

Conclusion

The developed six-point method, based on the digital photography and computer image analysis, represents a sensitive approach for evaluating gaping in Sparidae species fillets. Assessors training is a rapid and effective process and more than 95% accurate. These indicate that the proposed method for evaluating gaping severity in Sparidae species is easy to apply in practice thus allowing the scoring procedure to be accessible to all commercial gilthead sea bream and/or red sea bream farms.

Acknowledgements

PERFILLET project (EP Fisheries) is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution).

References

Andersen, U. B. (1994). Fillet gaping in farmed Atlantic salmon (Salmo salar). Norwegian journal of agricultural sciences, 8, 165-179.

Ashton, T. J., Michie, I., & Johnston, I. A. (2010). A novel tensile test method to assess texture and gaping in salmon fillets. Journal of food science, 75(4), S182-S190. doi: 10.1111/j.1750-3841.2010.01586.x.

Balaban, M. O., Ünal Şengör, G. F., Soriano, M. G., & Ruiz, E. G. (2011). Quantification of gaping, bruising, and blood spots in salmon fillets using image analysis. Journal of food science, 76(3), E291-E297. doi: 10.1111/j.1750

Kiessling, A., Espe, M., Ruohonen, K., & Mørkøre, T. (2004). Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO2 anaesthesia. Aquaculture, 236(1-4), 645-657. doi: 10.1016/j.aquaculture.2004.02.030.

Merkin, G. V., Stien, L. H., Pittman, K., & Nortvedt, R. (2013). Digital image analysis as a tool to quantify gaping and morphology in smoked salmon slices. Aquacultural engineering, 54, 64-71. doi: 10.1016/j.aquaeng.2012.11.003

FISH AS FEED: USING ECONOMIC ALLOCATION TO QUANTIFY THE FISH IN : FISH OUT RATIO OF MAJOR FED AQUACULTURE SPECIES

Björn Kok[a*], Wesley Malcorps[a], Michael F. Tlusty[b], Mahmoud M. Eltholth[a,c,d], Neil A. Auchterlonie[e], David C. Little[a], Robert Harmsen[f], Richard W. Newton[a], Simon J. Davies[g]

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA City, United Kingdom
email: bj.rn.kok@stir.ac.uk
School for the Environment, University of Massachusetts Boston, Boston, MA 02125, USA
Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt
Global Academy of Agriculture and Food Security, The Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom
IFFO, The Marine Ingredients Organisation, London SE17 3BZ, United Kingdom
Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, 3508 TC, the Netherlands
Fish Nutrition and Aquaculture Group, Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Newport TF10 8NB, United Kingdom

Introduction
Efficiency assessments of marine ingredient use in aquaculture are required to fully understand their contribution to global seafood supply and their impacts on all UN Sustainable Development Goals. Fish In: Fish Out (FIFO) ratios have become the principal metric used to ensure aquaculture does not negatively impact wild fish stocks. However, several approaches have been advocated to calculate the FIFO ratio and there have been criticisms that the different approaches employed lead to over- or under- estimates of the dependence of aquaculture on marine ingredients. Critically, FIFO does not align with Life Cycle Assessment as a measure of other environmental impacts. In this paper we present an alternative method to calculate the FIFO ratio based on the principle of economic allocation (economic Fish In: Fish Out – eFIFO) as commonly used in Life Cycle Assessments. Economic allocation acts as a proxy for the nutritional value of ingredients and places higher importance on the more limiting co-products generated and their relative demand.

Methods
The FIFO ratio represents the amount of fish used to produce 1kg of farmed fish. The amount of fish required is dependent on the amount of feed necessary to support 1 kg of growth, or the (economic) Feed Conversion Ratio (eFCR), and the fraction of feed that is fishmeal and fish oil multiplied by the embodied fish per kg of fishmeal and fish oil. In the eFIFO method the amount of embodied fish in fishmeal and fish oil was calculated based on a global average yield of 22.5 kg fishmeal and 5 kg fish oil per 100kg of fish processed. To divide or allocate the fish that is being used the economic allocation principle was used, in this method the raw material input, i.e. fish, is allocated to the co-products based on the economic revenue created. Attributing relatively more of the input to the product that creates more revenue to the producer, reflecting the socio-economic drivers for marine ingredient production and the resulting pressure on fisheries.

Results
Substitution of marine ingredients by alternate feed ingredients has significantly reduced the amount of fishmeal and fish oil in aquafeed formulations for most farmed fish species, resulting in a continually decreasing FIFO ratio. Results show that most aquaculture species groups assessed in this study are net producers of fish, while salmon and trout aquaculture are net neutral, producing as much fish biomass as is consumed, significantly lower than previous papers suggested (Figure 1). Overall, global fed-aquaculture currently produces three to four times as much fish as it consumes (figure 2).

Conclusion
Our study highlights that previous assessments of FIFO ratios can be misleading and resulting in adverse opinions in the scientific community, as well as on a retailer and consumer level. These can then in turn lead to several socio-economic and environmental implications, including failure to provide authentic information for marine resource planning, a lack of comprehension of realist goals and attainment of viable management pathways for use of commodities like fishmeal and fish oil for sustainable aquaculture practices. Marine ingredients continue to be essential in the diets of most aquaculture species, but re- search has been continuing on ways to use them more strategically in commercial diet formulations to optimize their value. Additionally, the strategic utilisation of fish by-products in feed results in a more efficient use of valuable marine resources. Therefore, it is imperative that models are based on a sound data platform and reflect accurately demand and supply to form a more robust and objective scenario for marine ingredient utilisation in aquaculture. This tool would enable policy makers and people in the industry to make well informed choices. Such a strategy contributes to the sustainable growth of the aquaculture industry and its crucial role in the global food system and nutritional security, being a valuable source of essential nutrients in the human diet.

(Continued on next page)

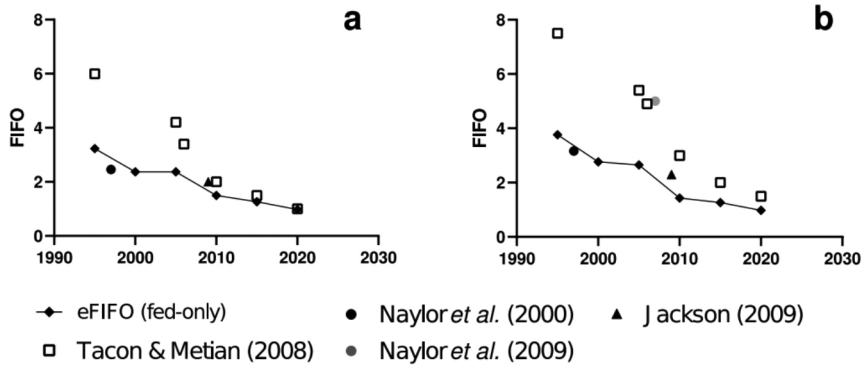


Figure 1: Comparison of FIFO ratio between our method (eFIFO) and results presented in literature for the main salmonid species groups trout (a), and salmon (b).

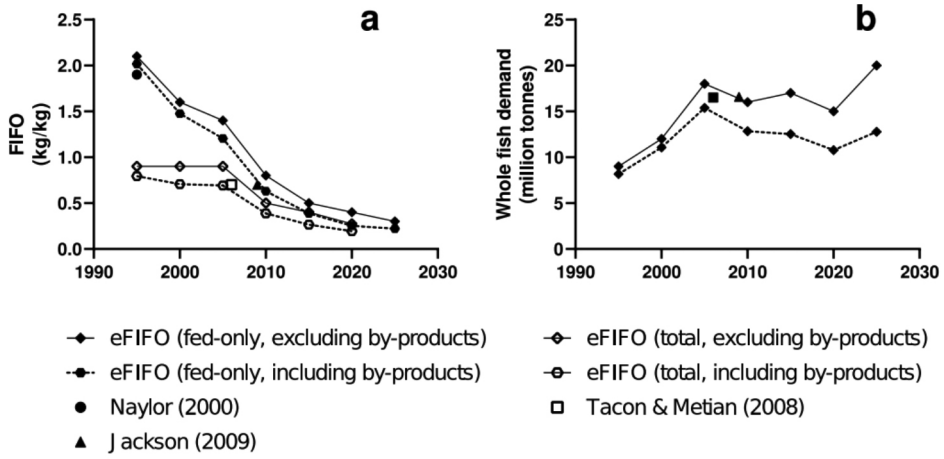


Figure 2: Comparison of FIFO (a) and total whole fish demand of aquaculture (b) with previously presented results.

COMPARISON OF LAB SCALE AND COMMERCIAL PRODUCTION RESEARCH RESULTS IN SALMON FED DIETS WITH DIFFERENT LEVELS AND TYPES OF TRACE MINERALS

M. Kokkali[1*], J-E. Dessen[2], T. Larsson[2], L. Sveen[3], E. Kvamme[4], K. Kousoulaki[1]

[1]Department of Nutrition and Feed Technology, Nofima, Norway
[2]Department of Fish Health, Nofima, Norway
[3]Alltech Inc, Summerhill Rd, Sarney, Dunboyne, Co Meath, Ireland
e-mail: marialena.kokkali@nofima.no

Introduction

Inorganic minerals are routinely added in commercial fish feeds, and there is increasing evidence that different trace mineral and phosphorous sources have different bioavailability (Maage and Sveier, 1998; Standal et al., 1999) and physiological effects (Berntssen et al., 2018). The relative levels of naturally occurring and added trace minerals in different forms will vary in diets changing from marine to plant based with unknown consequences for fish physiology. Skin ulcers and low stress tolerance are likely to be linked to sub-optimal mineral and vitamin nutrition, whereas poor smoltification and transfer performance may be linked to inadequate essential amino acid and mineral status of the fish. Fillet quality degrading conditions, as for instance gaping, liquid losses, suboptimal pigmentation, and melanin spots may also be affected by variable mineral nutrition status of the fish. There is a trend in nutrition of farmed land animals, such as poultry and swine, to replace inorganic trace mineral sources with lower amounts of bioavailable organic trace mineral sources which results in minimizing the excreted amounts of minerals, such as copper (Cu), in the environment, but also have the potential and have shown improvements in trace mineral-associated functionalities (Abdallah et.al., 2009). Organic Se sources have been shown to be assimilated more efficiently than inorganic compounds and are less toxic (Pacitti et al., 2016; Silva et al., 2019). Also, there are studies which have reported better Se apparent availability and Se retention in fillet of salmon fed organic Se diets (Sele et al., 2018; Silva et al., 2019). Reevaluation of organic minerals requirements may be necessary as studies has shown reduced levels required when organic minerals were used (Apines et al., 2003; Lin et al., 2010; Pierri Bruno da Silva et al., 2021). For example, Cu requirements for juvenile grouper, *Epinephelus malabaricus*, were reduced in half when organic Cu was included in the diets (Lin et al., 2010). Regarding mineral bioavailability, emphasis should also be placed, on the release rate of minerals to the environment when difference mineral sources are used. Russel et al. (2011) outlines the excess use of Cu and zinc (Zn) in Scottish Atlantic salmon farming, with concentrations 3-4 times more than required. In the same study, they concluded that Cu and Zn elevated levels on the sediment may cause adverse effect on the local environment around the sea farm. The scope of this study was to compare lab scale and commercial production results in salmons fed diets with either organic (OM) or inorganic (IM) trace minerals in different inclusion levels and their effects on Atlantic salmon performance, skin health and tissue mineralization.

Materials and Methods

In lab scale, 18 groups of Atlantic salmon smolt with initial mean body weight 150g were fed for 12 weeks 1 of 8 experimental diets with 3 replications. At trial end mean fish body weight was approx. 450g. The diets contained either organic or inorganic mineral premixes in 4 dietary supplementation levels (Se: 1.2-1.5ppm, Cu: 10-24ppm, Mn: 55-100ppm, Zn: 80-180ppm and Fe: 300-500ppm). The trial design included an undisturbed feeding period at start and end and 3 weekly handling stress treatments half-way the experimental period. Feeding rates, FCR, biometrics, fillet and skin technical quality, tissue mineral levels and skin histology were evaluated. In the commercial production trials we used salmon of approx. 500g body weight at start to slaughter, fed 4 different diets containing either organic or inorganic minerals at two supplementation levels (Se: 0.6-0.8ppm, Cu: 17-25ppm, Mn: 65-85ppm, Zn: 180ppm and Fe: 226-275ppm) and 2 replicate cages per treatment. All other farming operations were based on common praxis including for instance lice treatments. The mineralisation of whole body and different tissues (liver, skin, fillet, gills, and spleen) was studied at trial start, mid (when fish weighed approx. 2kg) and end (4.5 kg). Biometric and welfare measurements were taken at mid and end sampling, and liver and skin histology were evaluated at trial end. Growth and FCR at sampling points in commercial production were estimated based on collected daily farm feeding data and biomass and mean fish body weights at slaughter.

(Continued on next page)

Results and Discussion

There is an increasing interest of comparing the bioavailability of organic and inorganic minerals in fish diets, but the available data are still scarce and inconsistent (Dominguez et al. 2017; Antony Jesu Prabhu et al. 2016). Our results can fill some gaps on the bioavailability of organic and inorganic minerals and highlight the difference between mineral accumulation on tissues based on their origin (organic/inorganic) and dietary level. In lab scale, we saw significantly higher fish performance, in the organic as compared to the inorganic mineral treatments. A significantly positive correlation between dietary organic mineral level and skin Zn was also observed. Whole body mineral composition did not differ for the two mineral forms, with the exception of Se which showed a tendency for higher accumulation in the organic mineral treatments, whereas a significant negative correlation between organic Cu supplementation and whole body Cu was seen. Accordingly, in a meta-analysis by Antony Jesu Prabhu et al. (2016), it was highlighted that organic forms of Se, like SeMet and Se yeast, are more bioavailable compared to selenite; however, for other trace minerals like Zn and Mn the published results were conflicting. Comparative results with the cage trial data will be presented.

TIME- AND DOSE-DEPENDENT EFFECTS OF DIETARY DEOXYNIVALENOL (DON) IN RAINBOW TROUT (*Oncorhynchus mykiss*)

P. Koletsi*[1], G.F. Wiegertjes [1], P. Lyons[2], J.W. Schrama[1]

[1] Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[2] Alltech Biotechnology inc., Dunboyne, Republic of Ireland; plyons@alltech.com
E-mail: vivi.koletsi@wur.nl

Introduction

Lately, the aquaculture industry has shifted towards the formulation of more sustainable diets, characterized by a higher inclusion of plant-based ingredients - even in feeds for carnivorous fish species like salmonids. Because plant-based ingredients are susceptible to fungal growth, the risk of mycotoxin contamination has been increased in aquaculture. In combination with climate change, fungal growth and distribution might be affected and lead to uncertain mycotoxin profiles in feeds. A recent survey in aquafeeds highlighted deoxynivalenol (DON) as the most potent toxin for European aquaculture. The same study with a meta-analysis showed that DON reduces fish productivity due to impaired feed intake and recognized rainbow trout as the most sensitive fish species. Previous experiments in rainbow trout were performed within *ad libitum* exposure to DON, either in the natural form (coming from naturally contaminated ingredients) or pure (commercial powder). However, *ad libitum* exposure reduces the feed intake masking the effects on growth related to the toxin itself. Also, pure DON has less severe effects than the natural source, since in the last case a synergism might exist due to the co-occurrence of different toxins. Therefore, the current study aimed to elucidate the effects of two different types of DON (natural or pure) on growth performance and liver histopathology. Firstly, rainbow trout were fed restrictively and equally the experimental diets for six weeks to detect the potential effects of DON related to the toxin itself and not due to differences in feed intake. An intermediate sampling point was implemented after a week of feeding to investigate whether DON effects change over time. Finally, the experiment continued with two weeks of *ad libitum* exposure that could reveal potential DON impact on the feed intake capacity.

Material and methods

A feeding experiment was performed with rainbow trout fingerlings (8 g) to evaluate the effects of DON at realistic doses (up to 1600 µg/kg) on growth performance and liver histopathology. Thirty fish were randomly assigned to one of the five different dietary treatments with three replicates each: (1) control (CON) diet (DON < 100 µg/kg), (2) naturally DON-contaminated diet (ND1) with 700 µg/kg feed, (3) and 1200 µg/kg (ND2), (4) pure DON-contaminated diet (PD1) with 800 mg/kg, and (5) 1600 µg/kg (PD2). The feeding trial lasted eight weeks and included two feeding regimes: six weeks of restrictive feeding (12 g/kg MBW) followed by two weeks of *ad libitum* feeding. At the end of each feeding period, batch weighting was performed to calculate growth performance parameters. Initial and final crude protein (CP) and gross energy (GE) body composition at the end of the restricted period (day 40) were determined from 20 fish and five fish/tank, respectively. CP was calculated based on nitrogen x 6.25 using the Kjeldahl method, and GE measured with the adiabatic bomb calorimeter method. Two fish per tank or six per treatment (CON, ND2 and PD2) were sampled for histological assessment at two different time points of restricted feeding period (day 6 and 40). At the end of the satiation feeding period, growth and the feed intake capacity was measured, and liver samples were collected for histological assessment. Liver sections were stained with Periodic acid-Schiff's (PAS) to detect lipid-type and glycogen-type vacuolisation, and with haematoxylin and eosin (H&E) to evaluate pathological parameters based on a quantitative scoring system. The IBM Statistical Package for the Social Sciences (SPSS) program (v 23.0; New York, NY, USA) was used to perform the statistical analyses.

Results

Restrictive exposure to DON for six weeks did not affect the growth performance of trout but reduced retained protein in fish treated with ND2 and PD2. The last finding supports the hypothesis of DON inhibiting protein synthesis or increasing maintenance requirements and leads to increased protein catabolism and impaired nutrient utilisation. Liver histological assessment revealed altered nuclei characteristics and haemorrhages in the high DON-treated fish (ND2/PD2) after six days of restrictive DON exposure, but effects faded out over time (40 days). The latter may imply adaptation mechanisms in rainbow trout to recover after exposure to a certain DON amount. However, when ad libitum DON exposure was applied, hepatic damage aggravated: reduced glycogen vacuolisation, altered nuclei characteristics, necrosis and haemorrhages incidences were noted (Fig 1). Hepatic damage was more severe in PD2 than ND2 treatment, which might be explained by the higher DON content (1600 µg/kg vs 1200 µg/kg). Surprisingly, ad libitum exposure did not affect the feed intake capacity; maybe because rainbow trout had been exposed earlier to a restrictive exposure to DON for six weeks they had adapted. Finally, ad libitum exposure led to reduced body weight gain and altered feed efficiency in ND2 and PD2 treatments. The latter observation suggests the feeding regime determines the actual amount of ingested DON and the severity of the effects on rainbow trout.

(Continued on next page)

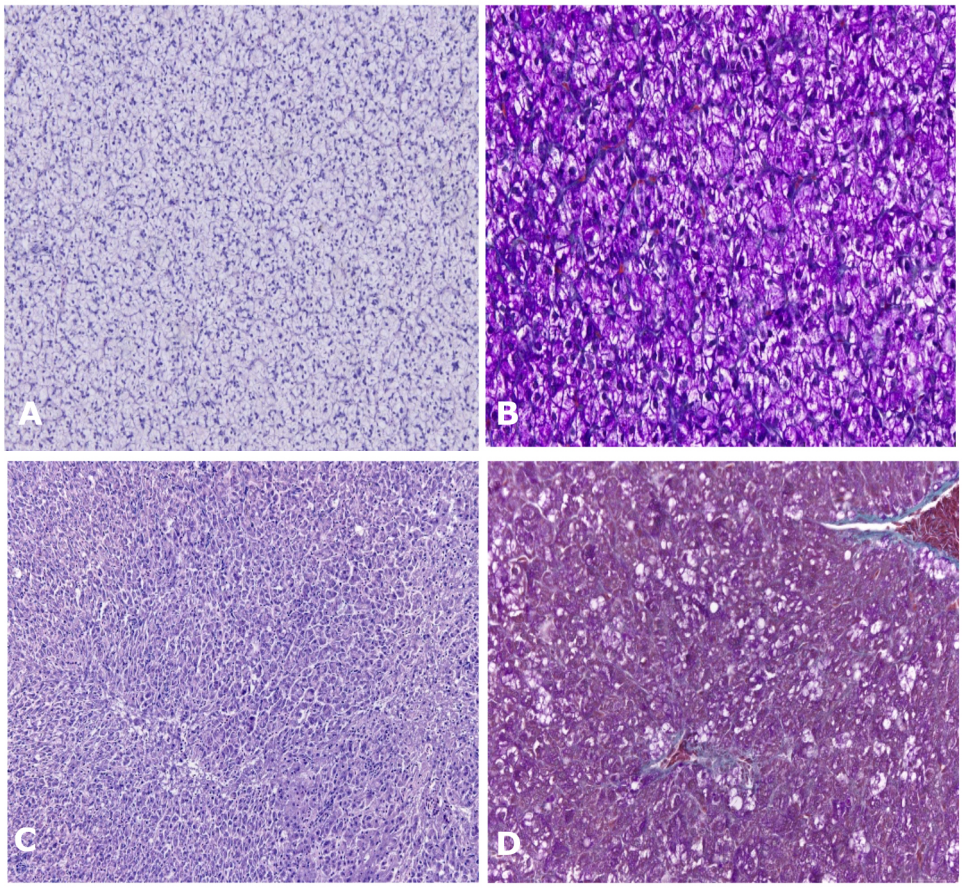


Figure 1. Histological parameters in liver of rainbow trout after feeding *ad libitum* the experimental diets; control (CON) and pure DON high (PD2). **(A-B):** Architecture of the liver in trout fed a CON diet; **(C-D):** Altered architecture of hepatocytes in trout fed PD2 diet characterised by pyknotic nuclei (C), reduced glycogen vacuolisation, necrosis and haemorrhage (D).*Pictures A and C are stained with H&E; pictures B and D are stained with PAS.

THERE IS NO SINGLE OPTIMAL PROTEIN-TO-ENERGY RATIO FOR NILE TILAPIA *Oreochromis niloticus* FEEDS

Gauthier D.P. Konnert*[1,2,3], Walter J.J. Gerrits[2], Sander W.S. Gussekloo[3], Karthik Masagounder[4], Julia Mas-Muñoz[5], Johan W. Schrama[1]

[1] Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen The Netherlands
[2] Animal Nutrition Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3] Experimental Zoology Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[4] Evonik Operations GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany
[5] De Heus B.V., P.O. Box 396, 6710 BJ Ede, The Netherlands
E-mail: gauthier.konnert@wur.nl

Introduction

Protein deposition in fish muscle tissue (i.e. fillet) is an energy-demanding process in which amino acids (i.e. absorbed protein) can act as building blocks or as energy substrates. Because protein is the most expensive macro-nutrient of commercial fish feeds, fish nutritionists intend to maximize the use of amino acids as building blocks instead of energy substrate. This is often achieved by increasing the non-protein energy content of fish feeds via inclusion of lipids and carbohydrates, thereby decreasing the dietary protein-to-energy ratio (P:E). For the sake of feed efficiency, much effort has been dedicated to defining optimal dietary P:E for farmed fish species like Nile tilapia (*Oreochromis niloticus*)[1–3]. Yet, published estimates vary largely, with optimal P:E ranging from 16 to 29 g/MJ for Nile tilapia[3,4]. Most published estimates were determined from feeding trials in which contrasts in dietary P:E were achieved by diluting dietary proteins with lipids and carbohydrates. Often, this has led to simultaneous changes in protein and energy intake, thereby making it impossible to distinguish the separate effects of protein and energy intake on growth. In addition, the emphasis placed on whole body mass gain changes with dietary P:E may have masked more meaningful underlying changes in nutrient partitioning. We conducted a meta-analysis and a dose-response trial to overcome these limitations and determine if there is a single optimal P:E for Nile tilapia feeds.

Material and methods

A systematic literature review was conducted to aggregate published feed, growth and body composition data of Nile tilapia growth trials. A total of 75 cases (i.e. dietary treatments) reported in 11 publications were selected for analysing the effects of dietary P:E on nutrient partitioning. These 75 cases provided data for Nile tilapia ranging from 6 to 250 grams and fed diets varying in crude protein (195 – 565 g/kg of dry matter) and gross energy content (17 – 24 MJ/kg of dry matter). In addition, a dose-response balance trial was conducted with all-male Nile tilapia (initial body mass = 64 g) at the Aquatic Research Facility of Wageningen University (NL) to test the separate effects of protein and non-protein energy intake on nutrient partitioning and growth. The trial consisted of a 2×8 factorial design in which duplicate 60 litres tanks of 30 fish were randomly allocated to one of 2 levels of digestible non-protein energy intake (16.0 and 22.4 kJ/day) and one of 8 levels of digestible protein intake (0.44 – 1.25 g/day). The 32 tanks were individually equipped with a settling faeces collection device and connected to a single recirculating aquaculture system kept at 28°C. Fish were hand-fed restrictively twice a day and faeces were collected overnight. Nutrient apparent digestibility was calculated via the indirect method using yttrium oxide as dietary inert marker. Initial and final body composition were determined from 20 fish and 10 fish/tank, respectively. For all analyses, crude protein was calculated by multiplying the Kjeldhal-analysed N content by 6.25. Growth, feed, faeces and body composition data collected from the literature review and obtained in our balance trial were used to calculate nutrient balances. The effects of changes in dietary P:E and digestible protein and non-protein energy intake on nutrient balances were analysed by linear regression, using SAS software package version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Protein retention efficiency (Fig. 1A) increased linearly with decreasing dietary P:E, thereby illustrating a protein-sparing effect of increasing dietary non-protein energy content. The fact that this effect was linear across a wide range of dietary P:E contradicts the concept of a single optimum in the dietary P:E of Nile tilapia. Protein gain increased close to linearly with digestible protein intake in our dose-response trial (Fig. 1B), with neither the intercept nor the slope being affected by non-protein energy intake level ($P = 0.67$ and 0.11, respectively). This indicates that protein – and not non-protein energy – intake limited protein deposition throughout the entire range. Again, this contradicts the concept of a single optimal balance between dietary protein and energy intake above and below which protein deposition would be limited by energy and protein, respectively. Instead of a single fixed value, the optimal P:E for Nile tilapia feeds is more likely to reflect situation-specific compromises between dietary protein cost and factors such as constraints on nitrogen discharge, body fat content and fillet yield.

(Continued on next page)

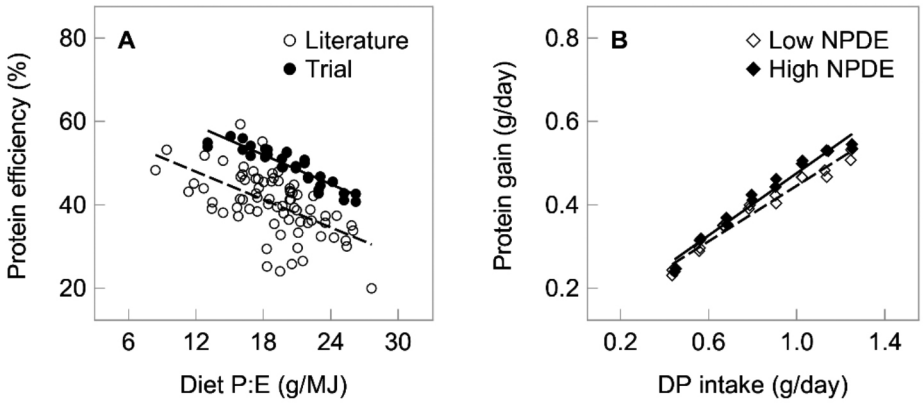


Figure 1. Effects of changes in dietary protein and energy supply on protein utilisation in Nile tilapia. **A**. Simple linear relationships between dietary crude protein-to-gross energy ratio (P:E) and protein efficiency (protein gain: protein intake) of Nile tilapia, obtained from published literature (---, n = 75, $P < 0.001$, $R^2 = 0.68$) and in our trial (—, n = 32, $P < 0.001$, $R^2 = 0.84$); **B**. Linear relationships between daily digestible protein (DP) intake and daily protein gain in Nile tilapia fed a 'Low' (---) or ' High' (—) non-protein digestible energy (NPDE) intake ($P_{slope} < 0.001$, $R^2 = 0.97$).

References

1. Kaushik, S. J., Doudet, T., Médale, F., Aguirre, P. & Blanc, D. Protein and energy needs for maintenance and growth of Nile tilapia (*Oreochromis niloticus*). *J. Appl. Ichthyol.* **11**, 290–296 (1995).
2. El-Sayed, A. F. M. & Teshima, S. I. Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. *Aquaculture* **103**, 55–63 (1992).
3. Wang, K.-W., Takeuchi, T. & Watanabe, T. Optimum protein and digestible energy levels in diets for *Tilapia nilotica*. *Bull. - Japanese Soc. Sci. Fish.* **51**, 141–146 (1985).
4. Sweilum, M. A., Abdella, M. M. & Salah El-Din, S. A. Effect of dietary protein-energy levels and fish initial sizes on growth rate, development and production of Nile tilapia, *Oreochromis niloticus* L. *Aquac. Res.* **36**, 1414–1421 (2005).

GOOD AQUACULTURE PRACTICES; THE COLLABORATIVE USE AND BENEFITS

B.Koonse

POB 1129, Harpers Ferry, WV 25425
Brettkoonse@yahoo.com

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

DIET COMPOSITION, GROWTH, GUT FUNCTION, AND GASTROINTESTINAL PATHOLOGIES OF FARMED ATLANTIC SALMON FROM SIX SITES ALONG THE NORWEGIAN COAST DURING SEAWATER GROW-OUT

Trond M. Kortner[1*], Elvis M. Chikwati[2], Erling Olaf Koppang[3], Håvard Bjørgen[3], Aleksei Krasnov[4], Violetta Aru[5], Bekzod Khakimov[5], Søren B. Engelsen[5], Gerd M. Berge[4], Øystein Sæle[6], Anusha K.S. Dhanasiri[1], Alexander Jaramillo-Torres[1] Yanxian Li[1], Paul J. Midtlyng[2], Åshild Krogdahl[1]

[1]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
[2] Aquamedic AS, Oslo, Norway
[3]Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
[4] NOFIMA AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Norway
[5]Chemometrics & Analytical Technology, Department of Food Science, University of Copenhagen, Denmark
[6] Feed and Nutrition, Institute of Marine Research, Bergen, Norway
*E-mail address: Trond.Kortner@nmbu.no

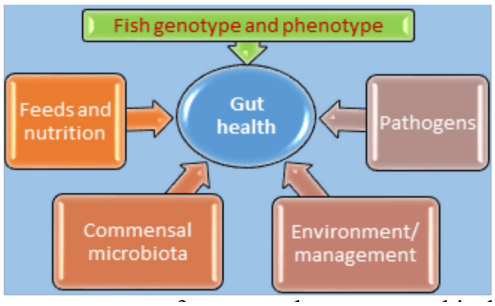
Introduction
Optimal intestinal health and digestive function are prerequisites for efficient feed utilization and for production of robust fish that will better cope with disease, stress and farming conditions. Gastrointestinal pathologies, such as inflammation and lipid malabsorption, have been underestimated challenges in farmed Norwegian salmon. Recent observations of sea-raised Atlantic salmon suggest that both conditions prevail in commercial Atlantic salmon farming. Previous research suggests that the increasing levels of plant ingredients in feeds, with their inherent antinutritional factors and fibers, can be important contributing factors. However, the significance of dietary contaminants, gut microbiota, nutritional and disease status, and developmental stage of the fish, as well as exogenous factors such as geographical location, other environmental conditions, and various management practices are not well understood. A more thorough understanding of the interplay between these factors is necessary to define causal relationships and subsequently prevent losses due to gut disease (Figure 1). A widely faceted research collaboration – the **GutMatters** project- was therefore initiated with the aim to document gut health disorders in commercial salmon farming, to further unravel the causative mechanisms, and to stimulate further control and remediation of both conditions. The project comprises extensive studies of fish from the field, and the conduct of several controlled feeding experiments. A summary of the main findings from the field study will be presented.

Materials and methods
Six marine Atlantic salmon farming sites were recruited for participation in the field study; two in the southwestern, two in the middle, and two in the northern part of the Norwegian coastline. Sampling was conducted at three timepoints following spring entry 2017: shortly following sea transfer, as well as 6-8 and 12-15 months after sea transfer. For each sampling event, complete sample sets of faeces, blood, gut and liver tissues were collected from a minimum of 12 individual fish, and used for detailed downstream nutritional, biochemical, histopathological, transcriptome, metabolome and microbiome analyses. In addition, feed samples, population data (feeding, estimated growth) and environmental variables (water temperature, dissolved oxygen, salinity) were provided by the managers of each participant site. The complete data set is currently being investigated by multivariate data analysis for capturing discriminant data acting as biomarkers of well-being or gut-disorder stress in farmed salmon.

Results and discussion
The histopathological evaluation revealed major differences between the sites and sampling time points with regards to inflammatory signs in the distal intestine, with an increase in both prevalence and intensity of inflammatory findings with time. All of the populations had fishes with moderate or marked inflammatory score in the final pre-harvest sampling. There were also clear differences between the populations with regard to lipid vacuolization of the pyloric caeca (Figure 2), indicating lipid malabsorption. Especially in the final sampling, severe vacuolization of the mucosal cells was seen in the majority of fishes from two of the populations.

(Continued on next page)

Figure 1 Causal factors affecting intestinal health



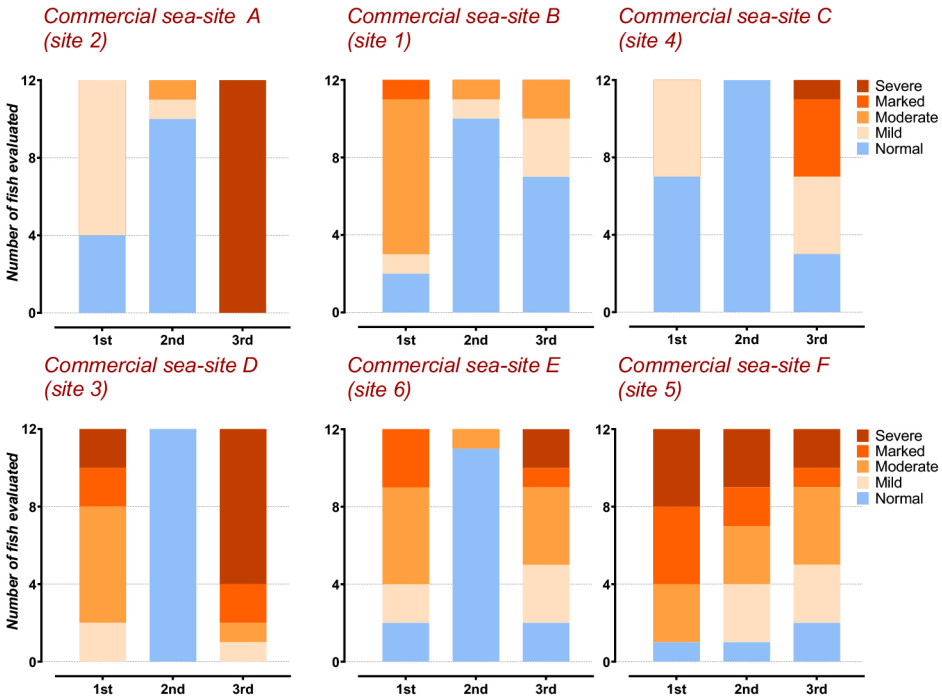


Figure 2: Prevalence and degree of lipid vacuolisation of the mucosa in pyloric caeca

Transcriptome, microbiome and metabolome profiling of gut content, tissue and blood demonstrated both site-specific signatures, and overall temporal changes that could reflect fish growth and development during production, irrespective of production site. Preliminary searches for correlation between omics data and pathology did not yet show strong relationships, but multivariate analyses of environmental, production and biological data are still ongoing. Regarding the feed analyses, the general picture indicates similar strategies among the feed companies and farmers regarding macronutrient composition of the diets throughout the production period. To conclude, the study clearly demonstrated that well-known gut health disorders are frequently found commercial Norwegian salmon farming. Holistic systematic investigations are currently ongoing to unravel causal relationships, develop non-invasive diagnostic tools and implement preventative measures for the observed gut disorders.

Funding

The experiment is one of a series conducted under the GutMatters project funded by The Norwegian Seafood Fund (FHF, project 901435).

VARIOUS MODIFIED ATMOSPHERE PACKAGING (MAP) CONDITIONS EFFECTS ON CHEMICAL SHELF-LIFE OF EUROPEAN SEA BASS

Kotsiri Mado1, Kogiannou Dimitra1, Gogou Eleni2, Grigorakis Kriton1

1Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Centre for Marine Research, Anavyssos, Greece (mkotsiri@hcmr.gr, kgrigo@hcmr.gr)
2Laboratory of Food Chemistry and Technology (School of Chemical Engineering), NTUA, Athens, Greece

Introduction

European sea bass (*Dicentrarchus labrax*) is an important marine species with major importance in the Mediterranean mariculture. Gutted sea bass is a value-added product that exhibits increasing demand in the international seafood market. Nevertheless, fresh seafood products are highly perishable and their quality deterioration could be a result of microbial activity, chemical oxidation, or autolysis. Storage temperature and packaging atmosphere influence microbial growth and thus the shelf-life of seafood (Odeyemi et al., 2018). Modified Atmosphere Packaging (MAP) can effectively alter the spoilage process and extend the shelf life of fresh fishery products (Tsironi and Taoukis, 2018).

It is based on altering the composition of gases in contact with food by replacing the air in a sealed food package with strictly controlled gaseous mixtures, containing oxygen (O2), carbon dioxide (CO2), and nitrogen (N2), or others. Their concentrations in the package headspace depend on the specific fish product and the mechanism of spoilage that limits the shelf life of the final food product (Tsironi and Taoukis, 2018).

The objective of this study is the evaluation of the active modified atmosphere packaging on the freshness and shelf life of gutted sea bass.

Materials and methods

Fresh gutted sea bass (weight: 400-600 g) was obtained in ice by a Greek aquaculture company (Selonda S.A.). Samples were transported to the Laboratory of Food Chemistry and Technology (School of Chemical Engineering, NTUA) and packed in high-density polyethylene pouches in modified atmosphere consisted of 50% CO2-50% air with two different CO2 emitters (PAD1 dimensions 300 mm × 130 mm × 40 mm and PAD2 dimensions 366 mm × 127 mm × 50 mm) (McAirlaid's Inc., Steinfurt, Germany) and under vacuum (V-PAD2) were used in order to evaluate the effect of active MAP on shelf-life extension. Samples were stored at controlled isothermal conditions (0, 2.5, 5 and 10 ˚C) in high precision incubators for 22 days. These samples were transported to the Sensory lab of HCMR for chemical freshness analysis.

Freshness assessment of the sea bass was based on ATP breakdown products analysis (*K*-values freshness index) and on free fatty acids (FFA) content. Extraction of ATP breakdown products took place from the dorsal muscle. The extraction of ATP-breakdown products and the HPLC *K*-values analysis has been previously described (Grigorakis et al., 2004)

A method for FFA detection was developed. FFA were extracted according to Bligh and Dyer (1959), separated using a NH2 SPE cartridge and esterified. The organic phase was then analyzed using gas chromatography-mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). An extract aliquot of 1 μl was injected into the GC/MS in a split mode (1:25) and separation was achieved on an Agilent DB-23 capillary column (60 m 0.25 mm, coated with a 0.25 μm film thickness).

Identification of fatty acids was carried out by comparing the retention times with the FAME standards. Nonanoic acid methyl ester was used as an internal standard to quantify the contents of the fatty acid methyl ester assuming the detector response to each fatty acid was identical. The content of each fatty acid was expressed as mg/100g of wet matter.

Results & Discussion

The *K*-values for 5ºC increased from the initial 20% on day 0 to reach 90%, respectively, on the 22th day of storage in all conditions. The acceptability limit of 60% *K*-value corresponding to the sensory shelf life of aerobically stored fresh sea bass (Grigorakis et al., 2004) was herein reached very early, in 5th day.

ATP, ADP, and AMP remained at low concentrations throughout the storage, while IMP, Ino, and Hx exhibited some differences in their patterns. The FFA contents fluctuated throughout storage without a specific pattern (Fig. 1). FFA are the main substrates for lipid oxidation and play an important role in the flavor formation of fish products.

(Continued on next page)

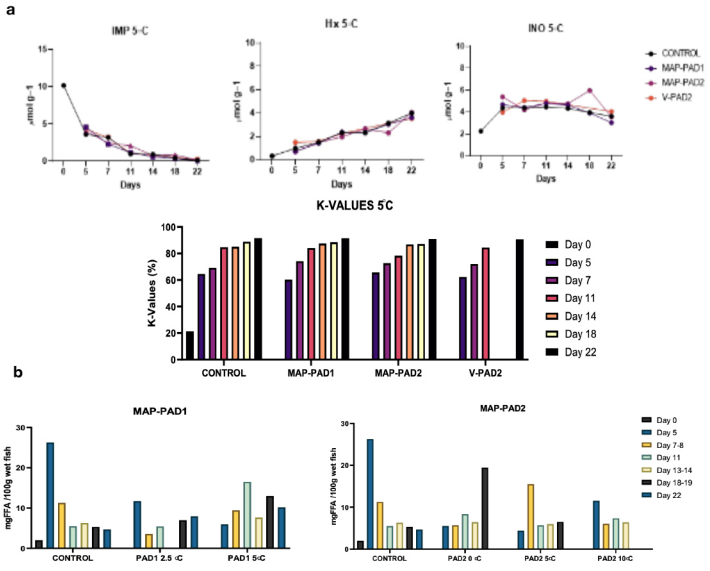


Fig.1: a. *K*-values (%) at 5ºC **b.** FFA content for the studied modified atmosphere packaging types.

Acknowledgements

This research was funded by the Greek Operational Programme for Fisheries, Priority Axis "Innovation in Aquaculture", Project title: "Application of smart and intelligent packaging for fish and development of a novel quality management and assurance tool for improved quality and extended shelf life" (2019- 2022) website: smartfish.chemeng.ntua.gr

References

Tsironi T., Taoukis P. (2018). Current practice and innovations in fish packaging. Journal of Aquatic Food Product Technology, 27, 1024-1047.

Grigorakis K, Alexis M, Gialamas I, Nikolopoulou D. 2004. Sensory, Microbiological, and Chemical Spoilage of Cultured Common Sea Bass (Dicentrarchus Labrax) Stored in Ice: A Seasonal Differentiation. European Food Research and Technology. 219: 584-87.

Odeyemi O.A, Burke C.M, Bolch C.C.J, Stanley R. (2018) Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. Int J Food Microbiol. 2; 280:87-99. doi: 10.1016/j.ijfoodmicro.2017.12.029.

EDIBLE SILK FOR PACKAGING OF HIGH ADDED-VALUE SEAFOOD PRODUCTS

Kotsiri Mado1, Pliameri Ioanna2, Georgiadou Maria2, Dedos Skarlatos3, Grigorakis Kriton1, Tsironi Theofania2

1Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Centre for Marine Research, Anavyssos, Greece (mkotsiri@hcmr.gr, kgrigo@hcmr.gr)
2Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece (ftsironi@aua.gr)
3National and Kapodistrian University of Athens, Department of Biology, Athens, Greece (sdedos@biol.uoa.gr)

Introduction

Edible coating or film is defined as a thin layer of material used for coating or wrapping food products, with the aim to extend their shelf life. Edible films are prepared separately and then applied to the food, while coatings are formed directly on the food surface (Tsironi and Taoukis, 2018). When properly formulated, both methods are considered to improve the organoleptic characteristics of packed food products. In addition, they can retard oxidation and/or delay microbial spoilage by integrating antibacterial and antioxidant agents. In order to develop edible films and coatings for food products, several materials may be used. These materials should be capable of forming a film and must be dissolved in a suitable and safe solution, that is, also compatible with the particular plasticizers, antioxidants, and/or antimicrobials. The prospective materials can be classified as hydrocolloids (e.g., polysaccharides, alginates), proteins (e.g., gelatin, casein), lipids (e.g., triglycerides, waxes), and composites (Oreopoulou and Tsironi, 2021).

Silk fibroin is an extensively investigated biomaterial for its potential in several applications, such as textile, biomedical, photonic, and electronics. Silk fibroin is a structural protein, like collagen, which has a unique feature: it is produced from the extrusion of an amino-acidic suspension by a living complex organism (while collagen is produced in the extracellular space by self-assembly of cell-produced monomers). The application of micrometre-thin silk fibroin membrane around the surface of strawberries and bananas has been reported as an effective management method of postharvest physiology of fruits (Marelli et al., 2016). The objective of the study was the investigation of the development of an edible food packaging material based on silk fibroin and the applicability on fresh shrimp for improving so the general freshness maintenance, as enzymatic post-mortem melanosis, commonly named black-spot (Goncalves & Oliveira, 2016).

Materials and methods

Cocoon was obtained from Bombyx Mori and used as fibroin source. Extraction of silk fibroin (SF) was performed using standard degumming processes (Marelli et al., 2016). The application of the silk fibroin based edible coating on the surface of whole, unpeeled shrimp was implemented by an immersion step, so as appropriate covering of the shrimp surface was achieved. Untreated (Control) and SF-coated samples were individually packed at aerobic consitions and stored at controlled isothermal conditions (0°C) in high-precision (±0.2°C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature monitoring in the incubators was based on electronic, programmable miniature dataloggers. Quality evaluation was based on microbial spoilage, instrumentally measured colour and texture, enzymatic browning (polyphenol oxidase), and sensory freshness.

Results

The application of SF edible coating resulted in a significant inhibition of microbial growth and enzymatic browning of refrigerated shrimp, leading to 4-5 days shelf life extension at 0°C compared to the untreated (Control) samples with shelf life of 7 days.

Discussion and conclusion

The results of the study show the potential of SF edible coating to preserve the quality and extend the shelf life of refrigerated seafoods. Optimized production conditions were selected based on the stability of the packaged products. This novel, edible coating might serve as the basis of an eco-friendly, active food packaging system, by the addition into the film formulation of natural antimicrobial and/or antioxidant bioactive compounds.

References

Goncalves A.A., de Oliveira A.R.M. (2016) Melanosis in crustaceans_a review. LWT - Food Science and Technology, 65: 791-799 http://dx.doi.org/10.1016/j.lwt.2015.09.011

Marelli B., Brenckle M.A., Kaplan D.L., & Omenetto F.G. (2016). Silk Fibroin as Edible Coating for Perishable Food Preservation. Scientific Reports, 6:25263.

Oreopoulou V., Tsironi T. (2021). Plant Antioxidants and Antimicrobials in Edible and Non-edible Active Packaging Films. In: Ekiert H.M., Ramawat K.G., Arora J. (eds) Plant Antioxidants and Health. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-45299-5_29-1.

Tsironi T., Taoukis P. (2018). Current practice and innovations in fish packaging. Journal of Aquatic Food Product Technology, 27, 1024-1047.

VARIABILITY OF KEY PERFORMANCE INDICATORS IN COMMERCIAL GILTHEAD SEA BREAM HATCHERIES

Ch. Kourkouta[1*], A. Tsipourlianos[2], D.M. Power[3], Z. Mamuris[2], K.A. Moutou[2], G. Koumoundouros[1]

[1] Biology Department, University of Crete, Vasilika Vouton, 70013, Heraklion, Crete, Greece
[2] Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500, Larissa, Greece
[3] Group of Comparative Endocrinology and Integrative Biology, Centro de Ciencias do Mar, University of Algarve, Campus de Gambelas, Faro, Portugal
Email: xarakourkouta@hotmail.com

Introduction

Skeletal abnormalities are an important issue for the quality of reared fishes, with either an emerging or well-established production. Skeletal abnormalities develop mainly during the larval and early juvenile stages, due to a variety of causative factors (Boglione et al. 2013), thus making important key-performance-indicators (KPI) for hatcheries. The goal of the present study was to identify and characterize the currently most important skeletal abnormalities in commercial seabream hatcheries, and to examine the variability of relevant KPIs in four seabream hatcheries in comparison with the genetic structure of the hatchery populations.

Material and Methods

From each of the four participating hatcheries, 1-3 populations per month were randomly selected during a full production year, to be monitored for their rearing parameters and examined for skeletal abnormalities. A total of 74 (17-20 per hatchery) populations were analyzed. Samples for quality control were taken on the day of fish removal from the larval-rearing tank (26-70 days post-hatching, dph; 9-19 mm mean total length, TL). A random sample of 50-100 larvae from each population was anaesthetized and fixed in phosphate buffered 5% formalin (pH=7.2).

Fish samples were stained for bone and cartilage (Walker and Kimmel, 2007) and examined for the presence of skeletal abnormalities, following the terminology of Koumoundouros (2010) and Fragkoulis et al. (2018). Besides the abnormalities frequency, examined KPIs included the frequency of fish with normally inflated swimbladder at 16 dph, specific growth rate (SGR) and heterogeneity of fish TL (coefficient of variation, CV) at quality control, survival rate (Sur) and productivity (Prd).

Four microsatellites (SauK140INRA, SaI12, Saimbb26 and Fd-78-H) were used to infer genetic structure based on validated primer sets. The Structure program was used to infer the number of Gilthead seabream (K) in the collected samples, and the phylogenetic analysis was carried out using the R package APE based on the pairwise Fst values for every locus, which was extracted using the MSA 4.05 program.

Results & Discussion

At the end of the larval rearing phase (38.6±5.8 dph), gill-cover and upper-jaw abnormalities of any severity degree were the most frequent skeletal defects observed, with a mean frequency of 11.3±17.9 % and 6.0±7.2 % respectively. At quality control, the mean fish size was 13.5±2.6 mm TL and mean CV was 11.6±2.6 (Fig. 1a, 1b). The recorded rate of normally inflated swimbladder was 92.3±7.4% (Fig. 1c). Mean survival rate was 25.9±21.0% and mean productivity was 43.2±44.5 fish per volume unit (L) of the larval-rearing tank (Fig. 1d).

Phylogenetic analysis indicated a clustering of samples according to the hatchery geographical location, while best K values, indicated K=4 for gilthead seabream. Plotting the q-values for each of the individual samples revealed admixture patterns with clear distinctions based on their geographical origin, the possible result of an extensive exchange between breeders in combination with company specific selective breeding, which could support the variability observed in different populations.

To our knowledge, this is the first study examining the variability of KPIs in commercial finfish hatcheries at a large scale (i.e. 4 hatcheries, 17-20 populations per hatchery) offering the opportunity to estimate reference values for benchmarking.

(Continued on next page)

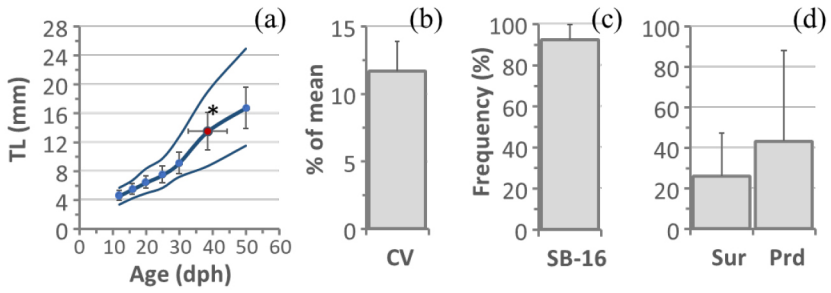


Fig 1. Mean values of the recorded KPIs. a) Average TL during the larval rearing and weaning period. Asterisk (*) indicates the mean TL and age of the fish samples at quality control. b) Coefficient of variation of fish TL at quality control. c) Mean frequency of normally inflated swimbladder at 16 dph. d) Average survival rate (Sur, in %) at 50-60 dph. Prd indicates the mean number of fish survived (50-60 dph) per volume unit (L) of the larval-rearing tank (fish/L). Error bars equal ±1SD.

PERFORMANCE OF NOVEL LOW TROPHIC RAW MATERIALS IN ATLANTIC SALMON *(Salmo salar)* DIETS

K. Kousoulaki[1*], T. Larsson[1] and L. Sveen[2]

[1]Department of Nutrition and Feed Technology, Nofima, Norway
[2]Department of Fish Health, Nofima, Norway
E-mail: katerina.kousoulaki@nofima.no

Introduction

Many different raw materials are considered as candidates for replacing fish meal (FM) and fish oil FO) in diets for salmonids, particularly focusing on locally produced low trophic level organisms with higher sustainability and circular economy potential. However, most of these novel raw materials differ from fish, containing e.g., high levels of complex carbohydrates or fully saturated triglycerides, and their nutritional value and nutrient bioavailability must be investigated before their use in commercial feeds.

Materials and Methods

In the current study, a control diet was created using high quality organic FM (25% in the diet) and FO (10% in the diet). Large parts of FM, FO or both were replaced by one of the following test ingredients: tunicate (*Ciona intestinalis*) meal, black soldier fly larvae meal (*Hermetia illucens*), cell wall disrupted phototrophic microalga *Phaeodactylum tricornutum* biomass, and spray dried heterotrophic microalga *Schizochytrium limacinum* biomass to create four test diets TM, BSFL, PT and HT, respectively. In a fifth diet (FutureEUAqua 0FM0FO) all the above test ingredients were combined at the same levels as when tested alone, and no FM or FO was used. The diets were balanced for protein, essential amino acids, EPA+DHA and n-3/n-6 ratio, phospholipids, and soluble P, using wheat and horse beans, crystalline amino acids, plant oils, lecithin, and monosodium phosphate, respectively. The experimental diets were fed to triplicate salmon smolt groups of 50 fish per tank, with initial mean fish body weight 142 and final mean body weight 433 g. Dietary effects on tissue composition, nutrient ADC, fish biometrics, skin histology and blood chemistry were evaluated.

Results and Discussion

We saw significantly higher growth rate in the control group as compared to the test groups. TGC was similar and high (approx. 4) among the single test ingredient experimental groups and higher as compared to the FutureEUAqua treatment lacking FM and FO. Growth mostly correlated with feed intake, thus FCR was similar among treatments and low FCR (Fig 1). TGC correlated significantly and positively with serum K, ASAT, ALAT and CK. ADC was significantly affected by dietary treatment for most nutrients analyzed except Cu, Zn, Se, Mn and 22:0.

Dermis was thinner for the FutureEUAqua fish, dense connective tissue (DCT) was thicker for the BSFL group than the PT group and scales areas followed a similar pattern as dermis with lowest values for the FutureEUAqua fish (Fig 2). There was no effect on epidermal area or number and area of mucous cells, and the epidermis of fish studied looked healthy.

The diets with alternative protein sources (BSFL and TM) had lower ADC of protein (86.6 and 85.3%, respectively) than the FM (91.7%) control. Their combination reduced ADC of protein further in the FutureEUAqua treatment (81.7%). Similar pictured showed the ADC of most amino acids, though their dietary levels were balanced, which may indicate suboptimal processing of these new raw materials still in development (e.g., excess heating). ADC of lipids was lowest in the HM diet, as reported in short term trials before (Kousoulaki et al., 2015) due to the high levels of tripalmitin in HM oil (Bogevik et al.,2018). HM had also slightly lower ADC of MUFA, n-6PUFA, n-3PUFA and EPA+DHA as compared to the control and test diets, except the FutureEUAqua which had surprisingly the highest levels among all the groups (Fig 3).

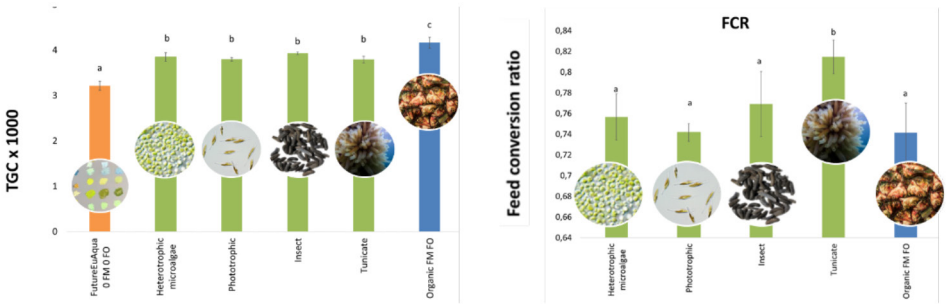


Figure 1 Growth rate (TGC; left figure) and FCR in dry matter (right figure) of fish during the experimental period.

(Continued on next page)

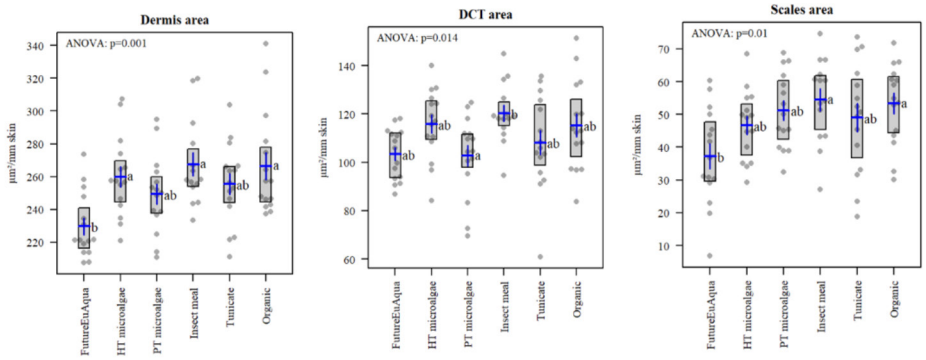


Figure 2 Dermis, DCT and scales area in fish of the different experimental treatments.

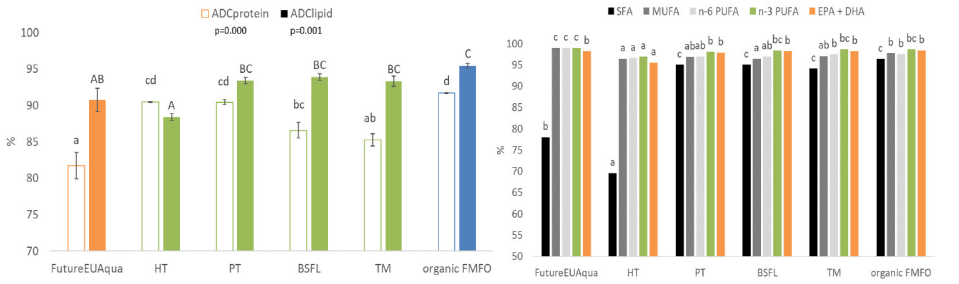


Figure 3 ADC of dietary protein and lipid (left); ADC og dietary fatty acids (right figure): saturated (black), monounsaturated (dark grey), n-6PUFA (light grey), n-3PUFA (green) and EPA+DHA (orange)

Acknowledgments

This study is part of the FutureEUAqua project which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 817737.

References

Bogevik, A.S., Nygren, H., Balle, T., Haugsgjerd, B.O., Kousoulaki, K., 2018. Enzymatic interesterification of heterotrophic microalgal oil with rapeseed oil to decrease the levels of tripalmitin. European Journal of Lipid Science and Technology, 2018, 1800063, 10 p.

Kousoulaki, K., Østbye, T-K.K., Krasnov, A., Torgersen, J.S., Mørkøre, T. and Sweetman, J., 2015. Microalgae feed for future omega-3 rich farmed fish: Fish Metabolism, Health and Fillet nutritional quality. Journal of Nutritional Science 4, e24, page 1-13.

AFRICAN CATFISH (*Clarias gariepinus*) SELECTION PROGRAMME FOR BETTER UTILISATION OF LOW FISH-MEAL FEED - PRELIMINARY RESULTS

Réka Enikő Balogh[1], Milán Varju-Katona[2], Dániel Péter[1], Adrienn Bíró[1], Szilvia Keszte[1], Julianna Kobolák[1], Bernadett Pataki[1], Nevena Kitanovic[1], Gábor Szilágyi[2], Béla Urbányi[1], Balázs Kovács*[1]

[1] Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, , Gödöllő, Hungary, Páter Károly St. 1., H-2100
[2] Győri Előre, Fisheries Cooperativ, Kisbajcs, Hungary, Arany János St. 22., H-2465
E-mail: Kovacs.Balazs@uni-mate.hu

Introduction

African catfish or sharp tooth catfish (*Clarias gariepinus*) is farmed in numerous African, Asian, and European countries, and its production has grown significantly worldwide during the last decades in intensive systems (IS). The production cost in IS is highly influenced by the feed cost, which is even more significant for species requiring high protein content. Some of the ingredients can be replaced by alternatives to reduce this cost: many experiments have been performed to replace animal protein with plant protein in several fish species, but this generally had a negative effect on the growth rate and health status (Gómez-Requeni et al. 2004, Pongmaneerat et al. 2011). However, recent research suggests that the utilisation of the feed can be improved by selection (Callet et al. 2017). Our long-term goal is to create an African catfish line selected for a low fish-meal feed, which has a similar or better growth rate than those fed with conventional feed. It could reduce production costs and provide more sustainable farming methods. Our aim is to isolate genetic markers that could be used for the prediction of phenotypic traits, and thus marker-assisted selection in the near future.

Materials and methods

African catfish (*Calrias gariepinus*) was selected and investigated on a half-industrial scale, in a flow-through system using 2 m^3 tanks at the Győri Előre Fisheries Co. site. Altogether 16 females and 18 males were selected as brooders to produce the F1 generation and offspring were fed with a widely used commercial (control) feed and an experimental feed with low fish-meal content in duplicate groups. Size selection was performed repeatedly to avoid cannibalism resulting in 3 different size groups in each experimental and control group. F2 offspring were generated by 4 multifactorial crosses of 5 male and 5 female individuals from each F1 group. Additionally, 3 groups were created from the biggest specimens fed with the low fish-meal feed to produce the positively selected line in triplicate. The body mass of 1846 and 1783 individuals from the F1 and F2 generations was measured, respectively on market-sized individuals, 8 months post-hatching, and data analysis was performed by R version 3.5.3. The effect of feed and sex on the body mass was evaluated by ANOVA analysis with a 5% significance level. Fischer's exact test was performed to investigate the association between sex and feed.

Results

Small differences were found in the sex ratios, although the association between sex and feed was non-significant. The average body mass in the F1 generation was 1461.60±490.34g for the control group and 1180.26±550.987g for the experimental groups, which is significantly lower. Additionally, a significant interaction was found between the feed and sex, suggesting that utilization of different feeds might have been affected by sex. Females had higher body mass in the experimental groups (males: 1126.05±538.45, females: 1216.36±498.65), while males had a higher average in the control groups (males: 1540.02±493.41, females: 1394.38±478.57). No difference was found in the F2 generation between the average body mass of the control (1597.05±399.49g) and the non-selected experimental (1578.34±550.98g) groups. However, the average body mass of the positively selected line (2297.41±463.78 g) showed a significant difference compared to the biggest individual in the non-selected experimental groups (2017.41±358.85g).

Discussion and conclusion

Our findings suggest that the groups fed with the low fish-meal diet had a lower growth rate compared to the control group in the F1 generation, which is in agreement with previous results (Gómez-Requeni et al. 2004, Pongmaneerat et al. 2011). However, no significant difference was found in the body mass of the control and experimental groups of the F2 generation, which might be explained by habituation. The selection had a remarkable effect on the growth rate: the selected lines grew significantly faster compared to the non-selected groups, fed with both the control and experimental feeds. The calculated average gain was around 14% in the F2 generation. In addition, feed had a significant interaction with the sex, females having higher average body mass fed with the experimental feed, suggesting that spawners might utilize plant protein more effectively than milters. In contrast, in the control groups, males had a higher growth rate, which is widely known for African catfish (Henken et al. 1987). Further research on positive selection for an African catfish line for better utilization of low fish-meal feeds and its sex interactions is recommended.

(Continued on next page)

AcknowledgmentThe work is supported by the iFishIENCi project (European Union's Horizon 2020 research and innovation programme under grant agreement No 818036) and the the EFOP-3.6.3-VEKOP-16-2017-00008 project, which is co-financed by the European Union and the European Social Fund. The work is also supported by the National Research Development and Innovation Office (NKFIH) Hungary, grant number 2017-2.3.3-TÉT-VN-2017-00004.

References

Callet T; Médale F; Larroquet L; Surget A; Aguirre P; Kerneis T; Labbé L 2017. Successful selection of rainbow trout (Oncorhynchus mykiss) on their ability to grow with a diet completely devoid of fishmeal and fish oil, and correlated changes in nutritional traits. PLoS ONE 12: 1–21.

Gómez-Requeni P; Mingarro M; Calduch-Giner J A; Médale F; Martin S A M; Houlihan D F; Kaushik S; Pérez-Sánchez J 2004. Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture 232: 493–510.

Pongmaneerat J; Watanabe T; Takeuchi T; Satoh S 2011. Use of Different Protein Meals as Partial or Total Substitution for Fish Meal in Carp Diets. Nippon Suisan Gakkaishi 59: 1249–1257.

Henken A.M., Boon J.B., Cattel B.C., Lobée H.W.J. 1987. Differences in growth rate and feed utilization between male and female African catfish, *Clarias gariepinus* (Burchell 1822), Aquaculture, 63: 221-232.

GUT HEALTH EFFECTS IN ATLANTIC SALMON (*Salmo salar* L) OF LOW DIETARY FISH MEAL LEVEL CAN BE LIMITED BY DIETARY SUPPLEMENTS

Å. Krogdahl[1*], E. Chikwati[1,2], A. Krasnov[3], A. J. Torres[1], G. M., Berge[3], P. Midtlyng[2], M. Hillestad[4], E. O. Koppang[1], Ø. Sæle[5], S. B. Engelsen[6], T. M. Kortner[1]

[1]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
[2]Aquamedic AS, Oslo, Norway
[3]Nofima AS, Ås/Sunndalsøra, Norway
[4]Biomar AS, Trondheim, Norway
[5]Institute of Marine Research, Bergen, Norway
Contact: ashild.krogdahl@nmbu.no

Introduction
A survey of gut health at three timepoints during one year after sea transfer, in six Atlantic salmon farms along the coast of Norway, conducted in the years 2018 to 2019, showed frequent signs of steatosis in the proximal intestine and inflammation in the distal intestine (Kortner et al. 2021). A controlled experiment was conducted to find if low level of fish meal in the diet might explain these findings. A second goal was to find if the symptoms might be diminished by supplementation with choline, known to be necessary for efficient lipid transport in the intestine, and nucleotides and β-glucans, claimed to improve gut immune status.

Materials and methods
Two diets series were made. Each comprised eight diets in which fish meal levels varied from 0 to 40%, replacing a mixture of plant protein sources. One series was made without supplementations (Ref), the other was supplemented with a mixture (Mix) of 3000 ppm choline chloride, 500ppm nucleotides (Lallemand®) and 500ppm β-glucans (Biorigin®). The diets were fed for 62 days to Atlantic salmon in tanks with salt water, 45 fish per tank with average start weight 186g, final weight 511g. At termination of the feeding period, from 12 fish per tank, whole body and carcass weight and body length were measured, and blood samples taken for observation of plasma biomarkers of nutritional status. These fish were divided in two groups of six fish. From one of these groups, samples of digesta and mucosa were taken for observation and analyses of weight of liver, pyloric region, mid and distal intestine, activity of digestive enzymes and concentration of bile salt. From the other group, samples were taken from the mucosa of the pyloric and distal intestine for histology and gene expression analyses, and from the digesta for metabolome and microbiota analyses.

Results
Neither dietary fish meal level nor the Mix supplementation affected growth or feed conversion ratio significantly. Fish fed the Ref diet series, showed significant effect of fish meal level on the somatic indices of the pyloric intestine (PISI) and liver (HSI) (Fig. 1, left), and the difference between the two diet-series was significant for both. The Mix decreased the indices greatly, most pronounced for the PI, and the dose-response effect of fish meal level, almost disappeared. Results of histological evaluation of the PI of the fish showed severe steatosis in fish fed low fishmeal diets without Mix (Fig. 1, right), decreasing with increasing fish meal level. Previous studies of effects of supplementation with choline alone, have shown similar effects on PISI. Our conclusion is, therefore, that the present results confirm that low fish meal diets are choline deficient. The results indicated that the level of choline in the diet with 11.4% fish meal in the supplemented series, contained sufficient choline for efficient lipid transport across the intestinal mucosa. Analyzed value of choline in this diet was 4240 ppm, a number substantially higher than the 3400 ppm estimated to be required in the study by Hansen et al (2020).

Increasing level of fish meal did not clearly affect histological signs of inflammation in the distal intestine, which in general were very mild, without or with the Mix in the diet.

Expression of genes in the mucosa of the distal intestine, showed clear correlation with fish meal level for many genes of key importance in digestive and immune functions. The effects tended to diminish with increasing fish meal level. Table 1 shows correlation coefficients (Pearson) for a number of functional groups. The results also showed that the Mix modulated these effects and reduced the effects of fish meal level.

The results of the metagenome analyses showed increasing α-diversity, i.e. number of different bacteria with increasing level of fish meal. However, supplementation with the Mix eliminated this effect and reduced α-diversity at high fish meal levels. Also, β-diversity, taking into account which bacteria were present, differed between fish fed low and high fishmeal diet, and, again, the Mix diminished this effect.

(Continued on next page)

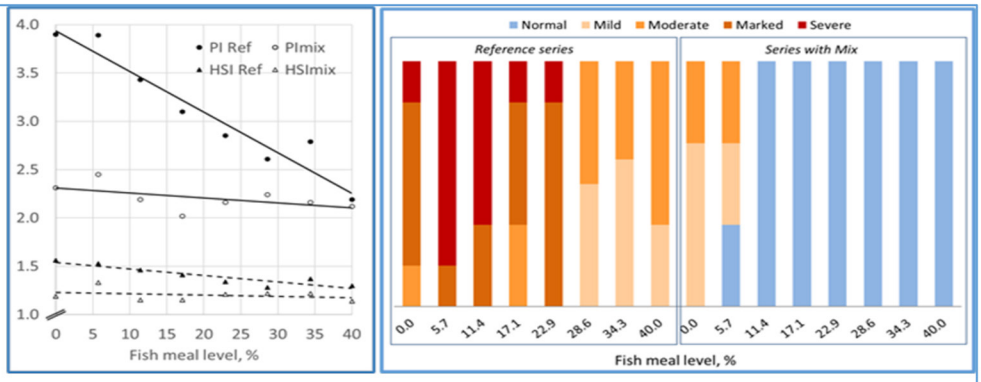


Figure 1. Effects of fish meal level and supplementation with a mix of choline, nucleotides and β-glucans on somatic index of the pyloric intestine (PISI) and liver (HIS) (left graph) and on symptoms of steatosis (lipid accumulation) in pyloric ceaca of fish fed diet with increasing levels of fish meal, without or with supplementation.

Table 1. Correlation coefficients (Pearson) for functional groups of genes showing significant relationship with FM level.

Functional group	No	Ref	Mix
Apoptosis	12	0.64	0.32
DNA replication & repair	6	-0.78	-0.32
Protein folding	30	-0.62	-0.26
Lysosome	11	0.75	0.12
Peroxisome	6	0.82	0.51
Redox	14	0.66	0.36
Chemokines	7	0.86	0.52
T cells	5	0.79	0.44
Amino acids metabolism	24	0.50	0.25
Iron heme metabolism	6	0.80	0.70
Lipid metabolism	45	0.56	0.31
Proteases	17	0.43	0.24
Protease inhibitors	11	0.47	0.34
Protein biosynthesis	28	-0.63	-0.33
Retinoid metabolism	9	0.63	0.27
Steroid, bile metabolism	14	0.55	0.34
Sugar metabolism	12	0.65	0.35
Xenobiotic metabolism	37	0.74	0.45
Collagen	13	-0.76	-0.36
Endocrine	6	0.78	0.31
Glycan	16	0.67	0.17
Growth factors	8	0.72	0.28

Literature

Hansen, A.K.G. et al. 2020. Dose-response relationship between dietary choline and lipid accumulation in pyloric enterocytes of Atlantic salmon (Salmo salar L.) in seawater. Br. J. Nutr. 28;123:1081-1093.

Kortner, T. M. et al. 2021. Diet composition, growth, gut function, and gastrointestinal pathologies of farmed atlantic salmon from six sites along the Norwegian coast during seawater grow-out. Presentation at EAS conference, Madeira.

JELLYFISH AQUACULTURE FOR HUMAN NUTRITION – PROSPECTING AND MANIPULATING LIGHT ACCESSORY PIGMENTS IN THE ENDOSYMBIOTIC JELLYFISH *Cassiopea andromeda*

Holger Kühnhold*[1], Jana Maßig[1], Andreas Kunzmann[1], Karin Springer[2]

[1]Leibniz Centre for Tropical Marine Research (ZMT), Fahrenheitstr. 6, 23859 Bremen
[2]Marine Botany, University Bremen, Bibliothekstr. 1, 28359 Bremen
*Email: holger.kuehnhold@leibniz-zmt.de

Introduction

Marine resources provide an affluence of bioactive substances that could possibly serve as novel food additives and nutraceuticals to enhance human diet and animal feed. With regards to sustainable resource exploitation, it is particularly useful to uncover the nutritious potential of highly abundant yet underutilized biomass, such as jellyfish. The endosymbiotic mangrove jellyfish *Cassiopea andromeda* might be an exceptionally promising species in this context. Next to fast growth rates, wide global distribution and robustness towards environmental change (e.g. Mammone et al., 2021), *C. andromeda* is a semi-sessile benthic species, which makes it very well suited for captivity culture (Pierce, 2005). Moreover, recent studies have revealed the presence of nutritionally valuable algae composites such as carotenoids, in other endosymbiotic jellyfish (Leone et al., 2015). As functional pigments carotenoids have strong nutraceutical properties, they can act antioxidative, anti-cancerous, anti-obese, anti-inflammatory, and cardioprotective (e.g. Van Chuyen and Eun, 2017). Hence, in this study, we investigated the pigments contained in *C. andromeda* and its endosymbiotic algae. In addition to the characterization and quantitative estimation of pigments, the potential of light spectra changes for the manipulation of the pigment composition and amount was tested.

Materials and Methods

Over a period of four weeks, *C. andromeda* specimens were exposed to four different light spectra (1. full-spectrum, 2. green-blue, 3. green-red and 4. blue-red) at a constant light intensity (250 µmol/m²/s). Over the experimental time, growth (weight and diameter) and activity (umbrella pulsation) of the jellyfish was monitored. Moreover, the photosynthetic efficiency of the endosymbiotic zooxanthellae was determined by measuring the maximum quantum yield of photosystem II (photosynthetic efficiency; Fv/Fm), using a portable pulse amplitude modulation (PAM) chlorophyll fluorometer. Pigment analyses were performed using reversed-phase high-performance liquid chromatography (HPLC).

Results

Four light accessory pigments (chlorophyll c2, β-carotene, diadinoxanthin and peridinin) were found next to chlorophyll a, in the endosymbiotic jellyfish *C. andromeda*. With a peak concentration of 480 µg/g dry weight, peridinin was the most prevalent carotenoid, followed by diadinoxanthin and β-carotene. The concentration of all pigments was generally highest, although not significantly, when the jellyfish were exposed to green-blue light. The four different light spectra did not significantly affect jellyfish growth and photosynthetic efficiency. However, jellyfish treated with green-red light conditions exhibited significantly lower umbrella pulsation rates.

Discussion and Conclusion

This study revealed that next to chlorophyll, three carotenoids, namely peridinin, diadinoxanthin and β-carotene, are the dominant light accessory pigments in the endosymbiotic jellyfish *C. andromeda*. Next to the well-known provitamin A activity of β-carotene, previous studies have identified strong anti-cancer properties of peridinin (Yoshida et al., 2017). To our knowledge, the dietary potential of diadinoxanthin has not been studied yet, however, many reports demonstrated that marine carotenoids have higher bioactivities than those from plant sources or synthetic compounds (reviewed by Van Chuyen and Eun, 2017). Hence, the carotenoid profile exhibited by *C. andromeda* can be considered of high nutritional and nutraceutical value. Although not significant, the changes of carotenoid concentrations at the different light spectra indicate promising perspectives, to enhance the production of target compounds, such as carotenoids, through the manipulation of key parameters in captivity culture. Given that the uptake of carotenoids is indispensable for animals and humans, the exploration of natural carotenoid sources is highly relevant to enhance its accessibility for human consumption and animal feed. This study provides first insights into prospecting and manipulating the *C. andromeda* holobiont for marine carotenoids. We propose to further investigate the nutritional value (e.g. antioxidants, fatty- and amino acids) of *C. andromeda* and explore its potential to counteract malnutrition, especially in countries of the global south.

(Continued on next page)

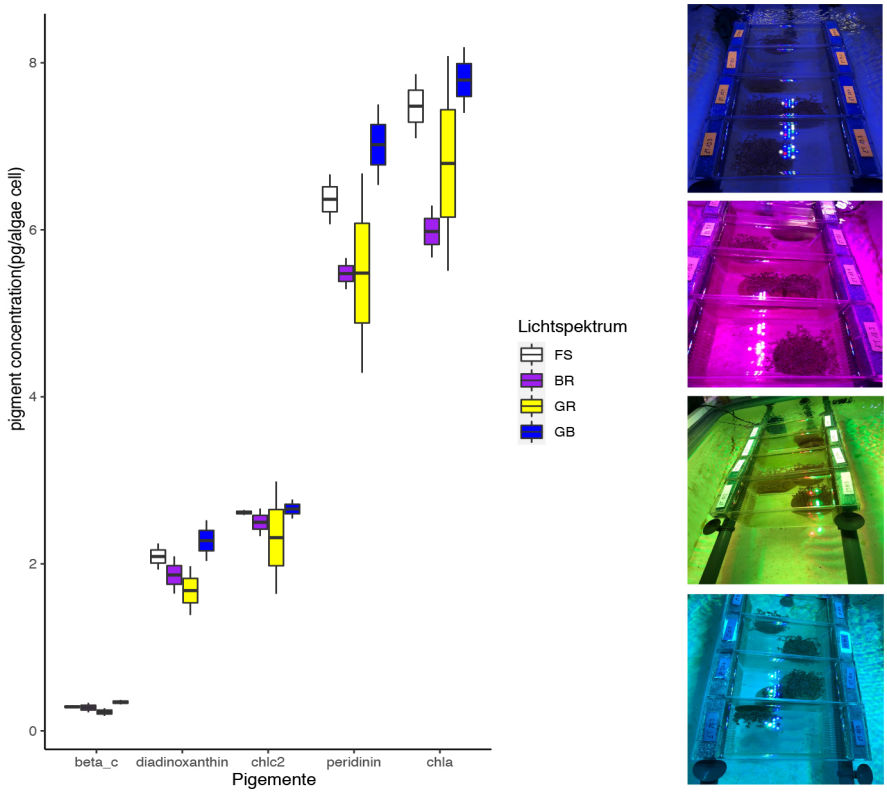


Fig.1: Photos (right) show light treatments (top down: 1. Full spectrum (FS), 2. Blue-red (BR), 3. Green-red (GR) and 4. Green-Blue (GB)). Graph (left) indicate pigment concentration (chlorophyll c2 (chlc2), β-carotene (beta_c), diadinoxanthin, peridinin and chlorophyll a (chla)) for each light spectrum.

References

Leone A et al. (2015) The Bright Side of Gelatinous Blooms: Nutraceutical Value and Antioxidant Properties of Three Mediterranean Jellyfish (Scyphozoa). Mar. Drugs 13: 4654-4681.

Mammone M et al. (2021) High photosynthetic plasticity may reinforce invasiveness of upside-down zooxanthellate jellyfish in Mediterranean coastal waters. PLoS ONE 16(3): e0248814.

Pierce J (2005) A system for mass culture of Upside-down jellyfish Cassiopea spp as a potential food item for medusivores in captivity. Int. Zoo Yb. 39: 62–69.

Van Chuyen H, Eun J B (2017) Marine carotenoids: Bioactivities and potential benefits to human health. Critical Rev. in Food Sci. and Nutrition Vol. 57, No. 12: 2600-2610.

Yoshida T et al. (2007) Halocynthiaxanthin and peridinin sensitize colon cancer cell lines to tumor necrosis factor-related apoptosis-induci.ng ligand. Mol. Cancer Res. 5: 615-625.

TOWARDS THE SETTING UP OF AN AUTOMATIC RECOGNITION FOR VERTEBRAE AND OPERCULAR ANOMALIES IN REARED GILTHEAD SEABREAM (*Sparus aurata*)

*Navdeep Kumar[a], Zachary Dellacqua[b,c], Clara Boglione[b], Arianna Martini[b], Marc Muller[a], Pierre Geurts[a], Raphael Maree[a]

[a]University of Liege, Belgium; [b]University of Rome Tor Vergata, Italy; [c]University of Las Palmas
Email Address: nkumar@uliege.be

Introduction

Gilthead seabream (*Sparus aurata*) is one of the most farmed fish in Mediterranean Europe, yielding significant economic turnover in the EU over the last few decades (FAO, 2005). However, in recent years an oversupply due to increased production efforts have led to a decline in seabream price value (Llorente et al., 2020). In order to secure future markets and value without the expansion of production efforts, farmers and businesses should focus on ameliorating the morphological quality of their current production. In fact, major economic losses are directly due to the development of skeletal disorders altering the external shape of reared seabream, i.e. opercular and vertebral column deformities. Fish with such deformities are rejected by the potential retailers or customers thereby representing a significant economic loss for the fish farmers (Verhaegen et al., 2007). Such deformities require tedious technical effort and time to manually cull out from the productive cycle; which should be done as early as possible in order to not waste resources on growing suboptimal fish. In this scenario, automatic sorting of deformed fish could represent an essential tool used to reduce the time and costs of manual selection and elimination of severely deformed fish from the reared stock, as well as be used to rapidly identify the best practices to reduce the deformation rate. Unfortunately, the present tools for the recognition of deformation are mainly based on the application of Geometric Morphometrics (Loy et al., 1997; Prestinicola et al., 2013) that require the manual selection of homologous landmark points in the form of *x* and *y* coordinates on fish' images, which again is a time consuming task that demands expertise and practice with mathematical modelling. In this scenario, the aim of this research was the setting up of an automatic recognition program developed by Geometric Morphometrics and Artificial Intelligence, in order to accurately estimate the percentage and severity of deformed gilthead seabream from digital radiographs in a sound and rapid way, without the need for manual inputs.

Materials and Methods

The methodology is based on applying Convolutional Neural Networks (CNNs), a popular deep learning procedure used in computer vision tasks such as *pose-estimation* and *hand-gesture recognition* systems. Our dataset consisted of radiograph images (digital DXS Pro X-ray, Bruker) of 875 (mean weight: 55g) randomly sampled seabream reared at the Instituto Portugues do Mar e Atmosfera in Olhão Portugal. Radiographic images were used to perform a skeletal analysis (see Prestinicola et al., 2013). According to the methodology described by Loy et al. (1997), we manually selected 19 homologous landmark points in the form of *x* and *y* coordinates on each image (FIJI, Schindelin, 2012), in order to characterize each specimen by a landmark configuration (Fig. 1). In this approach, a supervised learning algorithm is used in which labelled image data are presented to the CNNs during training to learn to automatically detect the landmark locations on the image. In particular, instead of outputting the single numbers (*x* and *y* coordinate positions per landmark), we manipulated the learning algorithm to output the probability heatmaps that signify the likely rather than exact location. For the output, we used Gaussian heatmaps for each landmark location generated by setting the location coordinates as mean (center) and 5 pixels as the standard deviation. In brief, our methodology can be considered to be a Gaussian heatmap-based regression learning in which the final output is the probability heatmap for each landmark location. To implement the learning algorithm, we use UNET (Ronneberger et al. 2015), a widely used CNN architecture in medical image analysis tasks. This architecture is based on the convolutional and deconvolutional operations used in deep learning to learn the tasks' specific features that aid the network to detect the locations of the landmarks. To implement the network architecture, we use Tensorflow and Keras libraries, and Python as the programming language.

To train the CNNs, we split the image dataset into *training*, *validation,* and *test* sets. The pre-labelled *t*raining set is presented to the CNNs for learning; the *validation* set is used for hyperparameter tuning (parameter optimization), and the *test* set is used to evaluate the performance of the CNN after it is trained. We trained our network for 1000 iterations until we optimized the CNN based on its performance in the *validation* set.

(Continued on next page)

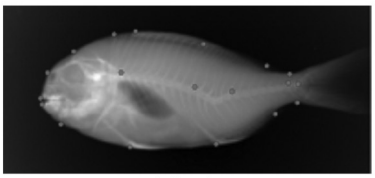


Fig 1

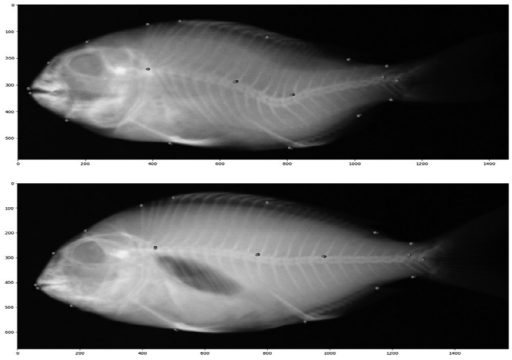


Fig 2

Results and Conclusions

The *test* set was used to measure the performance of the CNNs model. We used Mean Square Error (MSE) as the metric for measuring the squared difference (in pixels) between actual landmarks locations and predicted locations. Sample predictions from the *test* set are shown in Fig. 2. Blue dots represent the actual landmark locations whereas red dots represent the locations predicted by our model. In this way, the program is able to automatically predict the landmark locations on the radiograph images of pre-ongrowing seabream without any manual input of landmarks.

The next steps will be to couple the external shape with the inner anatomy (i.e., skeletal quality) of each fish and classify them based on different anomalies typologies. This model could be used to assist researchers in the field of aquaculture to automatically perform morphometric analyses with less time and effort, as well as aid technicians during sorting processes, thus reducing manual labor and handling stress on the fish. Furthermore, we are going to extend this approach to post-larval and juvenile stages with different image modalities (RGB, microscopic, histological). Ultimately, the plan is to make the program available online as an interactive web interface (Cytomine ,Maree et al. 2016, *Bioinformatics*) in which farmers and researchers are able to upload images and receive quick and sound data regarding the prevalence and severity of skeleton deformity types among their stock

Acknowledgement

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.

References

Llorente, I., et al. (2020) *Marine Policy*, ISSN 0308-597X (online), 117, 2020, p. 103876, JRC117066.

Loy et al., 1997; Prestinicola et al., 2013

Prestinicola, L., Boglione, C., Makridis, P., Spanò, A., Rimatori, V., Palamara, E., ... & Cataudella, S. (2013). Environmental conditioning of skeletal anomalies typology and frequency in gilthead seabream (Sparus aurata L., 1758) juveniles. *PLoS One*, *8*(2), e55736.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682.

Verhaegen, Y., Adriaens, D., De Wolf, T., Dhert, P., & Sorgeloos, P. (2007). Deformities in larval gilthead sea bream (Sparus aurata): A qualitative and quantitative analysis using geometric morphometrics. *Aquaculture*, *268*(1-4), 156-168.

Olaf Ronneberger et al. (2015). U-Net : Convolutional Neural network for Biomedical Images., *MCCAI 2015*

R. Maree et al. (2016). 'Collaborative analysis of multi-gigapixel imaging data using cytomine' *Bioinformatics*, *vol. 32, no. 9, pp. 1395–1401, 2016*

A GENETIC BASIS OF REPRODUCTIVE PERFORMANCE IN SELECTIVELY BRED ARCTIC CHARR (*Salvelinus alpinus*)

K. Kurta[1], H. Jeuthe[1,2], F. L. Pinto[1], DJ de Koning[1], C. Palaiokostas[1]

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7090, 750 07 Uppsala
[2]Aquaculture Center North, Åvägen 17, 844 61 Kälarne, Sweden
E-mail: khrystyna.kurta@slu.se

Introduction

Arctic charr *(Salvelinus alpinus)* is an economically important farmed species in Sweden. Selective breeding success for growth was achieved in the Swedish breeding program since the early 1980s. However, the farming industry of Arctic charr is relatively small (global production totaled 5000 - 6000 t per year (FAO, 2020)) and limited by the poor reproductive performance of hatchery-origin broodstock (Jeuthe et al., 2016). An in-depth study of limiting factors in fertilization success and hatching rates is needed to expand the Arctic charr production.

This study aimed to gain extensive knowledge regarding factors that affect sperm quality in selectively bred Arctic charr. The main parameters of male gametes' quality, including motility characteristics and velocity, were examined by a computer-assisted sperm analysis (CASA) system. Furthermore, we estimated inbreeding coefficients using available pedigree records. Finally, the resulted inbreeding levels were tested for the linear relationship with sperm quality using Pearson correlation coefficient.

Materials and methods

Arctic charr of year class 2017 from the national Swedish breeding program were used in this study. Sperm samples were collected from over 500 males between October and November 2020. Males with recorded data were separated into group A and B. Animals from group A comprised a test group held with higher water flow and a control group with regular water flow. Animals from group B were separated into the test group, which was exposed to water cooling (from July to November) by 3 °C lower temperature than in the control group with natural water temperature. The number of animals recorded per each group is specified in Table 1.

The evaluation of sperm motility and velocity, including average path velocity (VAP), curvilinear velocity (VCL), straight-line velocity (VSL), was performed by using a computer-assisted sperm analysis (CASA) system and SCA® Motility imaging software. Sperm density was measured using NucleoCounter® SP-100™ (Chemotech, Denmark). Basic statistics were computed for CASA-system parameters using statistical software packages in R (version .4.0.2).

Furthermore, the inbreeding coefficient for full-sibs was derived from the available pedigree recordings spanning since the 1980s using the INBUPGF90 v1.43 software from the BLUPF90 suite (Misztal et al., 2018). Finally, a total of 84 males were used to produce full-sib families (n = 127).

Results

According to the results obtained, milt density ranged between 0.003 - 13.54 $\times 10^9$ cells/ml (mean 3.27×10^9) for group A and 0.02 - 9.40 $\times 10^9$ cells/ml (mean 3.23×10^9) for group B. In group A, 10% higher density was observed in the test group exposed to higher water flow compared to the control group. However, this difference was not significant. Similarly, in group B, no significant differences were found in sperm density between the control and test groups when applying water cooling.

Table 1 Number of animals recorded per each group

	Group A			Group B			
Parameter	Test	Control	Total	Test	Control	Total	Overall
Density	219	175	394	101	66	167	561
Motility	219	172	391	97	17	114	505

(Continued on next page)

The mean sperm motility of 74% (range 5% to 99%) and 65 % (range 8% to 99%) was found for group A and B, respectively. The average VAP, VSL, and VCL for group A were equal to 52.8 μm/s, 38.5 μm/s, and 76.6 μm/s, respectively. The corresponding mean values of 47.9 μm/s (VAP), 33.3 μm/s (VSL), and 72.2 μm/s (VCL) were estimated for group B. A high positive correlation of 0.95 - 0.99 ($p < 0.05$) was observed between sperm velocity parameters (VAP, VSL, and VCL). The differences in the percentage of motility and sperm velocity between control and tested males in either group A or B were not significant.

The inbreeding coefficient amongst the studied animals ranged between 0.0 - 0.18, with a mean value of 0.07. A Pearson correlation coefficient close to zero was obtained between the inbreeding coefficient and sperm quality parameters.

Discussion and Conclusion

This study covered the males' gamete quality evaluation related to milt density, motility, and velocity characteristics. No significant differences in the sperm quality parameters were found between the control and test animal groups kept under conditions with lower temperature or higher water flow.

The inbreeding coefficient was estimated based on the available pedigree records for animals from the 1986 - 2017 year classes, which did not exceed 0.18. The correlation estimated between the inbreeding coefficient with milt quality parameters was close to zero.

A particular emphasis should be given to the application genomic technologies for identifying regions with a potential effect on gamete quality. Our prior studies have shown that genotyping using the double digest restriction-site associated DNA sequencing (ddRAD-seq) can provide essential information regarding the genetic diversity of broodstock and underline the genetic architecture of traits of interest (Palaiokostas et al., 2020). Further research will focus on applying the ddRAD-seq platform in studying the genetic factors influencing sperm quality and fertility in selectively bred Arctic charr.

References

FAO. (2020). *Fishery and Aquaculture Statistics 2018*.

Jeuthe, H., Brännäs, E., & Nilsson, J. (2016). Effects of variable egg incubation temperatures on the embryonic development in Arctic charr Salvelinus alpinus. *Aquaculture Research*, *47*(12), 3753–3764. https://doi.org/https://doi.org/10.1111/are.12825

Misztal, I., Tsuruta, S., Lourenco, D. A. L., Masuda, Y., Aguilar, I., Legarra, A., & Vitezica, Z. (2018). *Manual for BLUPF90 family of programs*. University of Georgia. http://nce.ads.uga.edu/wiki/doku.php?id=documentation

Palaiokostas, C., Clarke, S. M., Jeuthe, H., Brauning, R., Bilton, T. P., Dodds, K. G., McEwan, J. C., & de Koning, D. J. (2020). Application of low coverage genotyping by sequencing in selectively bred arctic charr (salvelinus alpinus). *G3: Genes, Genomes, Genetics*, *10*(6), 2069–2078. https://doi.org/10.1534/g3.120.401295

VIBRIO SPECIES ASSOCIATED WITH MORTALITY IN SHORT-SNOUTED SEAHORSE (*Hippocampus hippocampus*) REARED IN CAPTIVITY

Diogo Esteves[1], Diogo Teixeira[1], Miguel Correia[2], Jorge Palma[2], Cátia L. Marques[1*], Pedro Pousão-Ferreira[1], Florbela Soares[1]

1 - Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO), Av. Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
2 - CCMar, Universidade do Algarve, F.C.T., Edificio 7, Campus de Gambelas, 8005-139 Faro, Portugal
*E-mail: catia.marques@ipma.pt

Introduction

Presently, seahorse species (*Hippocampus* spp.) have become of major concern, as most of them are now globally threatened mostly due to overexploitation, incidental by-catch, habitat loss and degradation. Seahorse aquaculture has been growing rapidly in the past few decades, particularly in the hobbyist trade. As a result, efforts have been made to develop sustainable hatcheries with close attention to creating healthy and proper care of captive organisms. (Koldewey & Martin-Smith, 2010) Seahorses have also been raised in captivity to provide a venue to better understand several aspects of these species' biology and behaviour. Still considered as candidate species for aquaculture, in the 1990s seahorse aquaculture was ravaged by innumerous zootechnical problems mostly related to feeding and disease outbreaks. However, increasing attempts to breed the species was reflected in an increasing contribution of captive-bred seahorses to the aquarium trade. Currently, the majority of seahorse aquaculture involves small-scale for the home aquarium market (Curtis, 2006; González *et al*., 2006). Although, there are still considerable technical problems with diseases and species-specific problems in several species, including the *Hippocampus hippocampus* (Ofelio *et al*., 2014; Balcazar *et al*., 2014). To minimize these constrains when breeding it, and aware of the environmental threats presently faced by this species, it is of outmost importance to determine which microorganisms can co-exist in symbiosis, thus allowing a better management and protection when breeding seahorse in captivity. This information also extends to the wild populations, which presently face a risk of local extinction, independently of their symbolic status in the Ria Formosa where the EPPO is located. This assay and bacteria storage will later allow identification of potential pathologies, probiotics, development of more effective treatments and data acquisition that will optimize or inhibit the growth of these microorganisms (Kumaravel *et al*, 2012). This study will help to improve the seahorse production in captivity, facilitating potential re-stocking actions of the species in the Ria Formosa and protecting the existing natural populations.

This work aimed to investigate the bacterial presence and suitable treatments to overcome some specific case mortality observed in seahorse cultured at CCMAR.

Table 1. Biometric data of seahorses, *Hippocampus hippocampus* used in pathological analysis.

Fish number	1	2	3	4	5	6
Weight (g)	9.5	4.8	10.3	8.2	4.7	7.1
Total Lenght (cm)	10.5	12.9	9.8	14	11	10.1
Sex	Male	Female	Female	Male	Female	Female

For microbiological analysis, kidney, liver, and spleen samples were streaked in TSA (tryptone soya agar) and TCBS (Thiosulfate-Citrate-Bile-Salt Sucrose Agar), the plates were incubated at 24ºC for 24h. Successfully isolated bacteria were selected for biochemical identification. Also, an antibiogram was performed with the antibiotics, enrofloxacin, oxytetracycline, florfenicol and flumequine, using all the bacteria directly from the sample. For the bacteria identification, microscopy, biochemical tests and morphological observations were used and API 20E and API 20NE kits (bioMérieux) as a confirmation. Afterwards, using the "Vibrio2008" probability matrix of the IDENTAX software and bioMérieux kit codes it was possible to identify the isolated bacteria.

(Continued on next page)

Material and Methods

Six diseased seahorses *Hippocampus hippocampus*, reared at University of Algarve with erratic behavior, were sacrificed according to the ethical guidelines regarding animal welfare followed by the IPMA, and later analyzed to interpret the cause of mortality. The sacrificed seahorses were sampled biometrically and microbiologically. The biometrical analysis is shown in table 1.

Results & discussion

The sampled fish analysed in this study presented two clear symptoms; an erratic behaviour characterized by an almost constant swimming pattern, only addopted by seahorse species when a stress factor is present and small skin lesions (usually less than 3-4 per animal) characterized by skin discoloration and abrasion. Reared fish with visible symptoms only represented a small percentage (aprox. 5%) of the total produced fish. After running the biochemical tests, it was possible to identify three different *Vibrio* spp. species. *Aliivibrio logei*, *V. penaecida* and *V. agarivorans*. Two of these *Vibrio* spp. were present in two seahorse internal organs and one, was only identified in the skin. It's important to highlight that *A. logei* was considered as a *Vibrio* spp. until Urbanczyk *et al*., 2007 reclassification, previously described as *V. logei* and has been isolated from ocean waters, surfaces, fish, and marine mammals. Furthermore, *V. agarivorans* accommodates two agarolytic, halophilic, fermentative bacterial strains isolated from Mediterranean Sea water, described by Macián *et al*. 2001. From the successfully isolated vibrio, *V. penaecida* is a pathogenic bacterium that affect shrimp and other marine invertebrates (Lorgeril *et al*., 2005), however its effects on fish, namely seahorses, are little or not known. The antibiogram results classified all bacteria present in the samples as sensitive to the four antibiotics tested. All the bacteria described in this study have been found for the first time in seahorses, therefore further studies are recommended.

Acknowledgements

Financial support from projects SAUDE&AQUA (MAR-02.05.01-FEAMP-0009) and DIVERSIAQUA II (MAR2020-P02M01- 0656P).

References

Balcazar JL, Planas M, Pintado J. 2014. *Mycobacterium hippocampi* sp nov., a rapidly growing scotochromogenic species isolated from a seahorse with tail rot. Curr Microbiol 69(3):329-333.

Curtis, JMR. 2006. A case of mistaken identity: skin filaments are unreliable for identifying *Hippocampus guttulatus* and *Hippocampus hippocampus*. Journal of Fish Biology, 69: 1855-1859.

González, HE, Piloto, CY, Chevalier, MP, Rivero, N. 2006. Efectos de la Artemia enriquecida, sobre el crecimiento del Caballito de Mar *(Hippocampus erectus* Perry, 1810). *CIVA*, 989–995.

Koldewey HJ, Martin-Smith KM. 2010. A global review of seahorse aquaculture. Aquaculture, 302(3-4):131-152.

Kumaravel K, Ravichandran S, Balasubramanian T, Sonneschein L. 2012. Seahorses – a source of traditional medicine. Fish Shellfish Immunol, 26:2330–2334.

Lorgeril J, Saulnier D, Janech MG, Gueguen Y, Bachère E. 2005. Identification of genes that are differentially expressed in hemocytes of the Pacific blue shrimp (*Litopenaeus stylirostris*) surviving an infection with *Vibrio penaeicida*. Physiological Genomics, 21(2):174-183.

Macián MC, Ludwig W, Schleifer KH, Pujalte MJ, Garay E. 2001. *Vibrio agarivorans* sp. nov., a novel agarolytic marine bacterium. International journal of systematic and evolutionary microbiology, 51(6):2031-2036.

Ofelio C, Blanco A, Roura Á, Pintado J, Pascual S, Planas M. 2014. Isolation and molecular identification of the scuticociliate Porpostoma notata Moebius, 1888 from moribund reared Hippocampus hippocampus (L.) seahorses, by amplification of the SSU rRNA gene sequences. J Fish Dis, 37(12):1061-1065.

Urbanczyk H, Ast JC, Higgins MJ, Carson J, Dunlap PV. 2007. Reclassification of *Vibrio fischeri, Vibrio logei, Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. International Journal of Systematic and Evolutionary Microbiology, 57(12):2823-2829.

RELATIONSHIP BETWEEN EARLY OR LATE RESISTANCE TO ACUTE TEMPERATURE OR HYPOXIA STRESSES IN SIX RAINBOW TROUT ISOGENIC LINES

H. Lagarde[1], M. Prchal[1,2], S. Pouil[1], L. Goardon[3], M. Bideau[3], F. Guyvarc'h[3] L. Labbé[3], N. Dechamp[1], F. Phocas[1], M. Dupont-Nivet[1], D. Lallias[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[2] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[3] INRAE, PEIMA, 29450 Sizun, France
Email: henri.lagarde@inrae.fr

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a fish that is particularly sensitive to changes in water quality. Global warming is expected to increase the frequency and intensity of heatwaves, resulting in more common combined acute hyperthermia and hypoxia conditions in fish farms (Reid et al., 2019). Such poor thermal and oxygenation conditions induce problems including growth losses, increased mortality and pathogens pressure. However, genetic selection to breed more robust fish is an interesting option to improve fish tolerance to non-optimal water quality (Vandeputte and Prunet, 2002).

Some research studies have already highlighted the potential of genetic selection for acute hypoxia and temperature resistance traits in fish (Chen et al., 2015; Ineno et al., 2005; Prchal et al., Unpublished data). In order to improve our understanding of the genetic architecture of these two traits, we used six isogenic rainbow trout lines. Within a line, all fish have the same genotype and preliminary tests revealed contrasting levels of resistance to hypoxia and temperature between lines. Using this research model, the objectives of the study were to: i) confirm the existence of genetic variability for resistance to hypoxia and high temperature; ii) investigate the ranking stability of genotypes at different ages for hyperthermia and hypoxia resistance traits, which is an essential information to determine the stage at which fish should be phenotyped in a breeding program; iii) determinate the relationship between hyperthermia resistance and hypoxia resistance.

Materials and methods

Six heterozygous rainbow trout isogenic lines were produced at the INRAE experimental fish farm (PEIMA, Sizun, France) by crossing one homozygous isogenic line providing eggs and six homozygous isogenic lines providing sperm. Each of the six lines were reared in separate tanks, in triplicates. Half of the fish of each triplicate were PIT-tagged at 16±9 grams and all lines were then pooled into the six tanks (~50 fish of each line in each tank) for the 6 early challenges (3 temperature and 3 hypoxia). The other half stayed in separate tanks for one more year and at 236±36 grams were PIT-tagged and mixed for the 6 late challenges (3 temperature and 3 hypoxia).

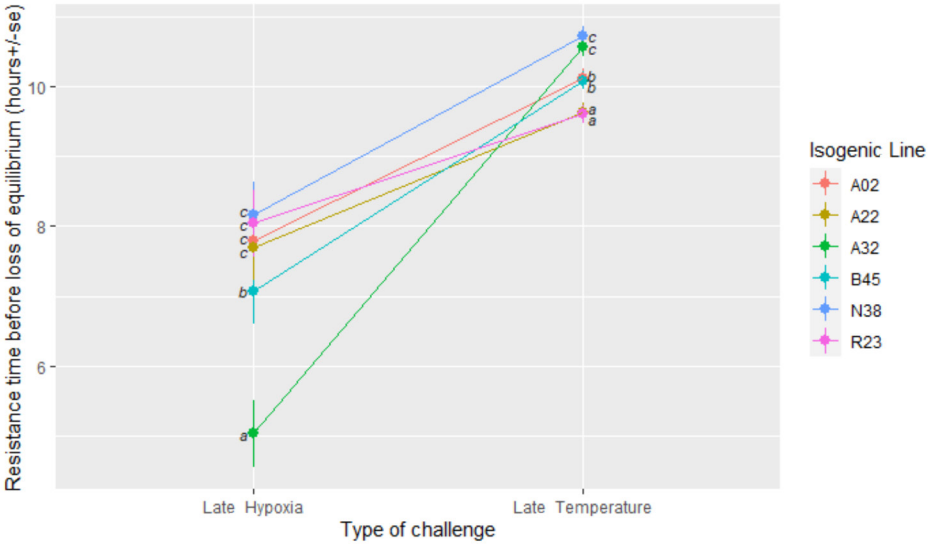


Figure 1: Relationship between late hypoxia and late temperature resistance. Means followed by a common letter are not significantly different.

(Continued on next page)

In hyperthermia challenge, temperature was gradually increased up to 27.5°C during 12 hours by adding heated water from a buffer tank. Oxygen concentration was maintained above 8mg/L. In hypoxia challenge, the level of oxygen was gradually reduced by bubbling nitrogen and reducing water renewal during 12 hours. In both challenges, fish were removed from the tank after loss of equilibrium and their PIT-tags and exact time were recorded. Fish were then individually weighted, anaesthetized and euthanized.

Resistance trait was analyzed as the time at loss of equilibrium of fish with the isogenic line as a fixed effect, the body weight as a covariate and the replicate as a random effect in a mixed linear model for each of the four challenges. When adequate, the resistance means of the different isogenic lines were compared using post-hoc Tukey tests with statistical significance set at 0.05. All analyses were completed with R software (V4.0.3).

Results and discussion

i) The fixed effect isogenic line was statistically significant in all challenges, confirming the effect of genetic variability on resistance to acute temperature or hypoxia stresses. Body weight effect was more complex with noticeable differences between early and late challenges. The interaction between isogenic line effect and body weight effect was significant in the two early challenges but not in the late challenges. Moreover, in hypoxia late challenge, body weight effect was significant while it was not in temperature late challenge. As a result, the resistance ranking of the genotypes was not stable in the early range of body weight (10 to 20 grams) because of the heterogeneity of slopes but stable in the late range of body weights (150 to 300 grams). It is therefore likely that the minimum phenotyping weight for resistance to acute temperature and hypoxia stress in rainbow trout falls between these two weight ranges.

ii) In the late challenges, i.e. once the ranking of genotypes was stabilized (with respect to weight), no clear rule appeared on the link between resistance to temperature stress and resistance to hypoxic stress (fig. 1). These results evidenced, at least partially, that resistance to hypoxia and resistance to temperature are two genetically different traits.

Acknowledgements

This study was supported by the European Maritime and Fisheries Fund and FranceAgrimer (Hypotemp project, n° P FEA470019FA1000016) and project CZ.02.2.69/0.0/0.0/18_053/0016975 - Development of the USB – International Mobilities II.

References

Chen, Z., Snow, M., Lawrence, C.S., Church, A.R., Narum, S.R., Devlin, R.H., Farrell, A.P., 2015. Selection for upper thermal tolerance in rainbow trout (Oncorhynchus mykiss Walbaum). J. Exp. Biol. 218, 803–812.

Ineno, T., Tsuchida, S., Kanda, M., Watabe, S., 2005. Thermal tolerance of a rainbow trout Oncorhynchus mykiss strain selected by high-temperature breeding. Fish. Sci. 71, 767–775.

Reid, G.K., Gurney-Smith, H.J., Flaherty, M., Garber, A.F., Forster, I., Brewer-Dalton, K., Knowler, D., Marcogliese, D.J., Chopin, T., Moccia, R.D., Smith, C.T., De Silva, S., 2019. Climate change and aquaculture: Considering adaptation potential. Aquac. Environ. Interact. 11, 603–624.

Vandeputte, M., Prunet, P., 2002. Genetics of adaptation in fish: Effects of domestication, stress resistance and adaptation to the environment. Prod. Anim. 15, 365–371.

MICROALGAE EXTRACTS AS A DIETARY SUPPLEMENT TO IMPROVE SKELETAL STATUS IN ZEBRAFISH *Danio Rerio* AND GILTHEAD SEABREAM *Sparus aurata*

J. T. Rosa[1], A. Carletti[1], K. Pes[1], I. Borges[1], S. Engrola[1], V. Serra[1], R. Colen[1], M.L. Cancela[1,2,3], P.J. Gavaia[1,2], V. Laizé[1,*]

[1] Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal
[2] Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve, Faro, Portugal
[3] Algarve Biomedical Center (ABC), University of Algarve, Faro, Portugal
* E-mail: vlaize@ualg.pt

Introduction

Skeletal deformities observed in teleosts – e.g. vertebral malformations such as kyphosis, lordosis, scoliosis, and vertebrae fusion – negatively impact fish health and welfare, and, in the case of commercial species, cause production losses and reduce fish market value. The factors triggering these skeletal deformities are mostly related to nutritional, environmental, and genetic factors. Among the solutions proposed to improve the skeletal status of farmed fish species, the nutritional supplementation of fish feeds with marine compounds that stimulate skeletogenesis has been seen as a sustainable and competitive manner to address the problem. In this regard, we have used zebrafish (*Danio rerio*) *in vivo* systems to identify microalgae extracts with osteogenic properties and used them as dietary supplements to improve fish skeletal status. We have gathered consistent results regarding the ethanolic extracts of two microalgae species, produce and commercially available in Portugal. Importantly, microalgae extracts containing osteoactive compounds can be produced in rather considerable quantities and used to supplement large batches of feed. In this work, the effect of the ethanolic extracts as diet supplements was first tested in zebrafish, to evaluate performance indicators, skeletal parameters, and dietary toxicity, and to refine working concentrations. A proof-of-concept experiment was then performed using gilthead seabream (*Sparus aurata*), an aquaculture species of great economic value and whose skeletal deformities are well documented.

Materials and Methods

Extracts were prepared through the maceration of freeze-dried biomass of *Skeletonema* sp. and *Tetraselmis* sp. (Necton S.A.) with 96% ethanol. Ethanol extracts were coated on fish commercial diets (Sparos, Lda) at 0.5% and 2.5% for zebrafish and 0.5% for seabream. Zebrafish larvae at 5 days post-fertilization (dpf) were maintained in 2.5-L tanks in static conditions (60 larvae/L) and co-fed 3 times/day with the supplemented diets and rotifers, that were gradually reduced to address the weaning from live feeds. At 20 dpf, fish were moved to 3-L tanks in recirculating system (23 larvae/L) and fed 3 times/day with supplement diets until 50 dpf. Mortality and toxicity parameters were monitored throughout the entire experiment. At the end of the experiment fish were given a lethal anaesthesia and sample to assess performance indicators, calcium and phosphorus content, type, severity and number of skeletal deformities, and the expression of several marker genes of bone development and oxidative stress. Seabream larvae of 30 days after hatching (dah) were fed *at libitum* 3 to 4 times a day until 60 dah with supplemented diets. Larvae were maintained in cylindroconical 100-L tanks in a semi-closed RAS with an initial density of 52 larvae/L and a photoperiod of 10h light:14h dark. Environmental parameters and mortality were monitored daily. At the end of the trial larvae were euthanized and sampled to assess biometric parameters, oxidative status, digestive capacity, calcium and phosphorous levels, skeletal deformities, and the expression levels of marker genes for bone formation and oxidative stress.

Results and Discussion

Zebrafish is a well-established animal model for a wide range of research areas and has recently emerged as a good model fish for research in aquaculture, where it is expected to provide applicable results in the areas of husbandry and survival, immune response, nutrition and growth. In this regard, zebrafish exhibits several technical advantages over aquaculture fishes, e.g. it is eased to handle throughout breeding and experimentation, has short generation times and large numbers of eggs per breeding event, and the costs associated to its maintenance are lower, thus it allows to perform trials with a high number of specimens and to test different parameters. We have used this model to first test two different concentrations of each extract in the supplemented diets and evaluate their effect on fish survival rates and performance indicators, as well as to monitor the possible toxic effects of the extracts, prior evaluation of the feds using seabream larvae. We show that the extracts, independently of the concentration tested, did not impact on zebrafish survival rate, with inclusively, a slight decrease in mortality for groups treated with *Tetraselmis* sp. Also, an increase in fish length, despite only significant for fish treated with *Skeletonema* sp. at 2.5%, was reported for all the treatments in comparison to control fish. None of the diets

(Continued on next page)

triggered toxic effects. We then tested the supplemented diets, at the lowest concentration, on seabream larvae, and saw a similar response in terms of survival rates, with no significant alterations upon treatment. Though, the condition factor for specimens treated with *Tetraselmis sp*. significantly increased as well as the relative growth rate between 30 and 60 dah, indicating a positive effect of this diet on fish biometric parameters. For both species, the impact of the supplementation of the extracts on calcium and phosphorous content did not reveal significant changes, with the optimal ratios maintained. The skeletal deformities observed for both zebrafish and seabream are mostly associated with the caudal vertebrae and caudal fin complex, independently of the treatments, however, they appear to be less severe for fish treated with *Skeletonema* sp. The expression of marker genes for oxidative stress were also shown to be upregulated (e.g. *cat*, *sod1, gsr*) suggesting a protective effect of the treatments by the activation of antioxidant mechanisms. Several marker genes of bone formation were also differentially expressed (e.g. *oc1, mmp9, sp7, col1a1*) indicating a direct impact of the extracts on bone regulatory mechanisms in both fish species. The data gathered in this study indicates that the use of microalgae osteogenic extracts as fish meal supplements could be a cost-effective solution to improve fish skeletal status, without compromising their growth and welfare, and acknowledges the use of zebrafish as a primary platform for nutrition research directed to aquaculture species.

Funding

This study was funded by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020 and by the European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 and project OSTEOMAR MAR-02.01.01-FEAMP-0057.

IMPACT OF TEMPERATURE REGIME DURING EMBRYONIC DEVELOPMENT ON GENOMEWIDE PATTERNS OF DNA METHYLATION IN LIVER SAMPLES OF JUVENILE RAINBOW TROUT

D. Lallias[1]*, A. Aubert-Frambourg[2,3], A. Chaulot-Talmon[2,3], L. Jouneau[2,3], L. Labbé[4], L. Goardon[4], M. Bideau[4], J.-M. Le Calvez[4], E. Quillet[1], H. Jammes[2,3], H. Kiefer[2,3] and M. Dupont-Nivet[1]

[1]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France
[2]Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France
[3]Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France
[4]INRAE, PEIMA, 29450, Sizun, France
E-mail: delphine.lallias@inrae.fr

Introduction

Epigenetic mechanisms are involved in the long-term persistence of physiological effects resulting from events that occurred earlier in the life of an animal. We aim to investigate the potential role of epigenetic marks in the expression of phenotypes and their variability in fish, in particular to study whether the epigenetic marks established in response to an environmental stress depend on the genetic background. In this context, rainbow trout isogenic lines (Quillet et al. 2007) are the material of choice. Within each line, all fish have the same genome i.e. there is no genetic variability. This allows the comparison of epigenetic marks among several individuals with the same genotype. The environmental stress chosen here is temperature, a known induction factor of epigenetic marks in fish. A recent study has shown that temperature experienced during development has prolonged effects on DNA methylation levels throughout the genome of threespine stickleback (Metzger and Schulte 2017). We have demonstrated that thermal history during embryonic development alters genome-wide patterns of DNA methylation at eyed-stage, but to a greater or lesser extent depending on the genetic background (Lallias et al. 2020). The objective of this study was to investigate the persistence in time of genome-wide patterns of DNA methylation established in response to early temperature regime in rainbow trout isogenic lines.

Material and methods

Eight rainbow trout isogenic lines were produced at INRAE PEIMA. For each line, half of the eggs were incubated at standard temperature (11°C) and the other half at high temperature (16°C), from eyed-stage to hatching. Just before hatching and for the rest of the rearing, all batches were reared at 11°C. Liver samples (central organ for intermediary metabolism) were collected on 4 month-old juvenile fish, snap frozen in liquid nitrogen and kept at -80°C until DNA extraction.

Global methylation levels were quantified using LUminometric Methylation Assay (LUMA) for 80 juvenile fish (8 lines x 2 temperature regimes x 5 fish per condition). Statistical analyses were performed using non-parametric tests suited for small samples (permutation tests for two/K independent samples with Monte-Carlo sampling; coin plug-in in RCommander).

Genomewide patterns of DNA methylation were analysed by Reduced Representation Bisulfite Sequencing (RRBS) on 40 juvenile fish (4 lines x 2 temperature regimes x 5 fish per condition). RRBS libraries were prepared on the same DNA extracts used for LUMA and then sequenced on an Illumina NovaSeq6000 sequencer to produce 100 bp paired-end reads (Integragen SA, France). Trimmed reads were aligned to the current reference genome with the bisulfite mapping tool Bismark. Differential methylation analyses were performed using methylKit. Identified DMCs (Differentially Methylated Cytosines) and DMRs (Differentially Methylated Regions) were finally annotated. All steps are monitored using a homemade pipeline previously described (Perrier et al. 2018).

Results

As for results obtained at eyed-stage, there was no overall effect of temperature regime (11°C vs 16°C) experienced during embryonic development on global DNA methylation of liver samples of 4 month-old juvenile fish ($z = 1.5705$; $p=0.116$) but significant differences between lines at 11°C (chi-squared = 15.224; $p=0.033$) and 16°C (chi-squared = 18.586; $p=0.010$).

An average per individual of 41 million paired-end reads were obtained (lowest: 23 million; highest: 77 million). Bisulfite conversion rates were very high (>99%). Total mapping efficiency of the RRBS reads to the reference genome ranged between 74 and 79%, but only 50% on average mapped uniquely and were used for subsequent analysis. Preliminary results reveal that the numbers of DMCs and DMRs are relatively low but vary greatly depending on the line. Further analysis is ongoing.

(Continued on next page)

Discussion

Rainbow trout isogenic lines are a unique and powerful biological model to study whether the methylation marks established in response to an environmental stimulus (temperature here) depend on the genetic background, persist in time (several months after exposure) and can impact the response to later challenges. Our results suggest some level of persistence in time of methylation marks established in response to an early temperature stress.

Acknowledgements

This study was carried out within AQUAEXCEL^{2020} funded by European Union's Horizon 2020 research and innovation programme under grant agreement No 652831. We are grateful to the Genotoul bioinformatics platform Toulouse Occitanie (Bioinfo Genotoul, doi: 10.15454/1.5572369328961167E12) for providing computing and storage resources.

References

Lallias, D., Bernard, M., Ciobotaru, C., Labbé, L., Goardon, L., Le Calvez, J.-M., Bideau, M., Fricot, A., Prézelin, A., Charles, M., Moroldo, M., Cousin, X., Bouchez, O., Roulet, A., Quillet, E., Dupont-Nivet, M. 2020. Sources of variation of DNA methylation in rainbow trout: combined effects of temperature and genetic background. Epigenetics, doi: 10.1080/15592294.2020.1834924

Metzger, D.C.H., Schulte, P.M. 2017. Persistent and plastic effects of temperature on DNA methylation across the genome of threespine stickleback (*Gasterosteus aculeatus*). Proceedings of the Royal Society B: Biological Sciences, 284:20171667.

Perrier, J.-P., Sellem, E., Prézelin, A., Gasselin, M., Jouneau, L., Piumi, F., Al Adhami, H., Weber, M., Fritz, S., Boichard, D., Le Danvic, C., Schibler, L., Jammes, H., Kiefer, H. 2018. A multi-scale analysis of bull sperm methylome revealed both species peculiarities and conserved tissue-specific features. BMC Genomics, 19:404.

Quillet, E., Dorson, M., Le Guillou, S., Benmansour, A., Boudinot, P. 2007. Wide range of susceptibility to rhabdoviruses in homozygous clones of rainbow trout. Fish and Shellfish Immunology, 22: 510–519.

SPONTANEOUS SPAWNING OF CAPTIVE GREATER AMBERJACK (*Seriola dumerilii*) REARED IN SEA CAGES AND TRANSFERED TO LARGE TANKS DURING THE SPAWNING SEASON

S. Lancerotto*, I. Fakriadis, I. Sigelaki, and C.C. Mylonas

Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research
P.O. Box 2214, Iraklion, Crete 71003, Greece.
Email: s.lancerotto@hcmr.gr

Introduction

Up to date, the most effective method to obtain good quality eggs from Mediterranean greater amberjack (*Seriola dumerili*) has been to (a) maintain breeders in sea cages during the year -where they undergo full gametogenesis- and then (b) move them to land-based tanks after gonadotropin-releasing hormone agonist (GnRHa) induction of spawning (Fakriadis et al., 2019, 2020). In order to reduce the reliance of the industry on the usage of hormonal treatments for spawning induction, and potentially also improve egg quality parameters, we examined the hypothesis that using large diameter tanks for spawning, would allow spontaneous maturation and spawning of fully vitellogenic females and spermiating males.

Materials and Methods

Reproductively mature breeders were maintained in a sea cage over the year and were transferred to two 75-m^3 tanks supplied with filtered surface seawater for the spawning period. Fish were selected based on evaluation of ovarian biopsies and sperm production. In the first tank, fish were given (GnRHa) in a controlled release implant (Induced, six males of mean ± SD weight of 22.1 ± 1.6 kg, and four females of 25.0 ± 2.03 kg). In the second tank (Spontaneous, five males of 19.0 ± 0.56 kg and four females of 29.7 ± 0.92 kg), fish did not receive any hormonal treatment. Reproductive success was evaluated through fecundity and fertilization success. Computer-assisted sperm analysis (CASA) was used to assess sperm quality of the selected males. Statistical analyses were done using t-test or two-way ANOVA ($P \leq 0.05$).

Results

The mean oocyte diameter of the largest vitellogenic oocytes was 675 ± 38 and 645 ± 16 for the Induced and Spontaneous group respectively. Some females in each tank were in early oocyte maturation (lipid droplet coalescences and germinal vesicle migration). These females presumably were the ones that spawned the next day. Females treated with GnRHa spawned 12 times, out of which 8 were the following days after hormonal therapy (Fig. 1A). On the contrary, the Spontaneous group spawned less frequently for a total of 6 times (Fig. 1A). The mean spawning batch relative fecundity (t-test, $P = 0.009$) (Fig. 1B) and fertilization success (t-test, $P = 0.001$) (Fig. 1C) of the Spontaneous group were higher than the Induced group. Additionally, embryo survival of eggs from the Spontaneous group reached 99 ± 0.3% and was also significantly different than from the GnRHa-induced group (t-test, $P = 0.009$) (Fig. 1D). Sperm quality parameters diminished after 24 days in the spawning tanks, in both GnRHa-treated and non-treated males (two-way ANOVA, $P \leq 0.05$).

Discussion

Inappropriate environmental conditions in captivity may lead fish to exhibit reproductive dysfunctions (Mylonas et al., 2010). Providing greater amberjack with a large-volume tank, allowed spontaneous and consistent spawning with excellent fecundity, fertilization and embryo survival, which were higher than the values obtained from GnRHa-induced breeders. Sperm quality parameters after 24 days were similar between GnRHa-treated and non-treated males, indicating also in the males, that allowing greater amberjack to spawn in large tanks is supportive of reproductive performance.

(Continued on next page)

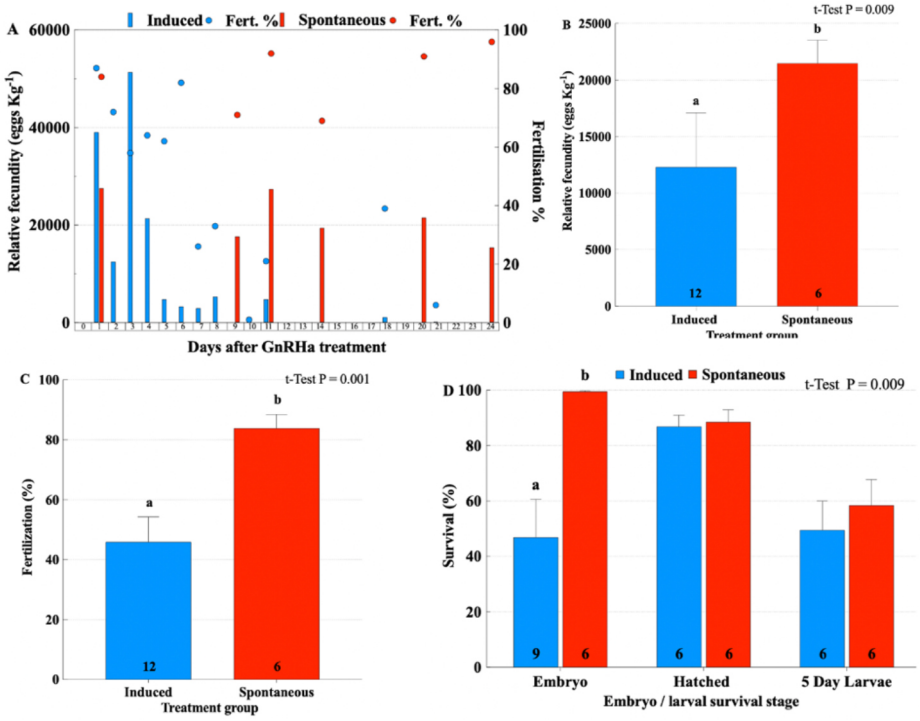


Fig 1. Reproductive parameters in greater amberjack (*Seriola dumerilii*) induced to spawn with GnRHa or allowed to spawn spontaneously. A) Daily relative fecundity (bar, eggs Kg^{-1} female) and fertilization success (circle, %). B) Mean (±SEM) total relative fecundity (eggs Kg^{-1} female) and C) Mean (±SEM) fertilization success (%). D) Mean (±SEM) percentage of survival of embryos and larvae. The numbers inside the bars indicate the number of spawns considered for the mean. Different lowercase letters above means indicate significant differences between treatment groups.

Acknowledgements

Financial support has been provided by the European Union's Horizon 2020 program under grant agreement No 862658, NEWTECHAQUA).

References

Fakriadis, I., Lisi, F., Sigelaki, I., Papadaki, M., Mylonas, C.C., 2019. Spawning kinetics and egg/larval quality of greater amberjack (Seriola dumerili)in response to multiple GnRHa injections or implants. Gen. Comp. Endocrinol. 279, 78–87. https://doi.org/10.1016/j.ygcen.2018.12.007

Fakriadis, I., Sigelaki, I., Papadaki, M., Papandroulakis, N., 2020. Control of reproduction of greater amberjack Seriola dumerili reared in aquaculture facilities. Aquaculture 519, 734880. https://doi.org/10.1016/j.aquaculture.2019.734880

Mylonas, C.C., Fostier, A., Zanuy, S., 2010. General and Comparative Endocrinology Broodstock management and hormonal manipulations of fish reproduction. Gen. Comp. Endocrinol. 165, 516–534. https://doi.org/10.1016/j.ygcen.2009.03.007

ARTIFICIAL PROPAGATION AND LARVAE REARING IN RECIRCULATION AQUACULTURE SYSTEM (RAS) OF THE HUNGARIAN CARP LANDRACE (*Cyprinus carpio morpha accuminatus*)

Levente Zete Láng [1*], Zoltán Bokor[1], Gergely Bernáth[1], Balázs Csorbai[1], Borbála Nagy[1], Tamás Bartucz[1], Tibor Izsák[1], Zsolt Csenki-Bakos[1], Ferenc Fodor[2], Zsolt Szári[2], Béla Urbányi[1], Levente Várkonyi[1]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1., H-2100 Gödöllő/H-2484 Agárd, Hungary
[2]Balaton Fish Management Non-Profit Ltd, Horgony u. 1., H-8600 Siófok, Hungary
Email: lang.zete98@gmail.com

Introduction

An increasing tendency was observed in the angling interest at lake Balaton since 5 years ago. The growing angling demand directly affects the natural populations of the common gamefish (e.g. common carp). The angling management belongs to the Balaton Fish Management Non-Profit Ltd who is responsible for the annual reintroduction of the natural populations in the same time. Recirculating aquaculture system (RAS) allows a controlled propagation and a larvae rearing in a constant safe environment.

Materials and methods

In our experiments, stripped egg batch (332 g) from 1 female was fertilized using pooled sperm (7,5 mL) from 5 males. Hatched larvae were reared at a "Rack" system in a RAS. The infrastructure was performed using 10 L plastic tanks. The water quality was maintained with a programmable logic controller (PLC system) as well as using UV, mechanical and biological filtration. Larvae were reared at a density 50 individual L^{-1}. Feeding was carried out 4 times per day (*ad libitum*) with freshly hatched *Artemia salina* nauplii. Standard length (mm, *N*=20), average body weight (mg, *N*=20) and larvae malformation (*N*=10, curved body, deformed tail development: eye deformity, yolk-sac deformation, craniofacial malformation, edema, somites deformation) was recorded at 3 developmental stages (1. hatched, 2. non-feeding: 3 days post hatching and 3. feeding: 7 days post non-feeding).

Results

Results showed a slight increment in the ichthyologycal parameters between the hatched (standard length: 4.4±1 mm, average bodyweight: 1.0±0.3 mg) and non-feeding larvae stage (standard length: 5.5±0.5 mm, average bodyweight: 1.5±0.1 mg). A notable increasing tendency was recorded at the feeding larvae stage in standard length (10.5±0.7 mm) and average bodyweight (12.1±1.7 mg). A negligible prevalence of malformations was observed at the 3 different larvae stages. A high survival rate (94±2%) was recorded at the end of the experiment (10 days post hatching).

Discussion and conclusion

The results can contribute: 1. to the efficiency of the hatchery process in the mentioned carp landrace; 2. to maintain the natural population at Lake Balaton; 3. to satisfy the increasing angling demand.

Acknowledgements

Our experiments were supported by the GINOP-2.3.2-15-2016-00004: "Establishing the sustainable angling-aimed management of Lake Balaton. This research was also supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12). The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

UNRAVELLING *Rhodomonas salina*

C. Latsos*, J. van Houcke

HZ University of Applied Sciences, P.O. Box 364, 4380 AJ Vlissingen (The Netherlands)
Email:christos.latsos@hz.nl

Introduction

The microalgae species *Rhodomonas salina* is known to be an excellent diet for many aquaculture species. It has been shown that *R. salina* contributes to egg production, growth, survival, reproduction and lipid content of copepods, brine shrimps and scallops (Seixas et al. 2009; Guevara et al. 2011; Arndt and Sommer 2014)fish and cephalopod species. The improvements of both Artemia growth and its biochemical composition are key issues for the suitable use of Artemia biomass in these rearing processes. In this study we evaluated the growth and survival rates of Artemia fed with the cryptophyte Rhodomonas lens in comparison with different microalgal species commonly used in aquaculture: the prasinophyte Tetraselmis suecica, the prymnesiophyte Isochrysis galbana Parke, and the eustigmatophyte Nannochloropsis gaditana. Microalgae were cultured semi-continuously in nutrient saturated conditions and with a daily renewal rate of 30% of the volume of cultures, to obtain biomass of controlled and optimized composition. Considerable differences in Artemia growth were observed, as well as in the survival rate. At day 8 of rearing, Artemia fed R. lens had the highest length (4.9 ±0.6 mm, $P < 0.001$ and as specialty diet in the refinement of oysters (van Houcke et al. 2017). The beneficial aspects of using *R. salina* as feed are attributed to their favourable content of polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for the growth, survival and pigmentation of the aquaculture species mentioned above (Caramujo et al. 2008). In addition, *R. salina* contains, besides the common pigments such as chlorophyll a/c and several carotenoids, the water-soluble pigment Cr-phycoerythrin 545 (PE) that harvests light in the green wavelength ($\lambda max = 545$ nm) (Doust et al. 2006). PE is also used in food colouring and cosmetics (Sudhakar et al. 2015) and linked to anti-parasitic and anti-tumour activities in studies using the marine mollusc *Aplysia californica* (Coelho et al. 1998). Optimization of *R. salina*. cultivation has been conducted in aspects of biomass production rate and biochemical composition, but no commercially stable cultivation plan has been applied in aquaculture yet (Seixas et al. 2009; Oostlander et al. 2020; Yamamoto et al. 2020) fish and cephalopod species. The improvements of both Artemia growth and its biochemical composition are key issues for the suitable use of Artemia biomass in these rearing processes. In this study we evaluated the growth and survival rates of Artemia fed with the cryptophyte Rhodomonas lens in comparison with different microalgal species commonly used in aquaculture: the prasinophyte Tetraselmis suecica, the prymnesiophyte Isochrysis galbana Parke, and the eustigmatophyte Nannochloropsis gaditana. Microalgae were cultured semi-continuously in nutrient saturated conditions and with a daily renewal rate of 30% of the volume of cultures, to obtain biomass of controlled and optimized composition. Considerable differences in Artemia growth were observed, as well as in the survival rate. At day 8 of rearing, Artemia fed R. lens had the highest length (4.9 ±0.6 mm, $P < 0.001$. The goal of our research was to optimize the biomass, fatty acid and PE production, by altering the nitrogen availability and the light quality.

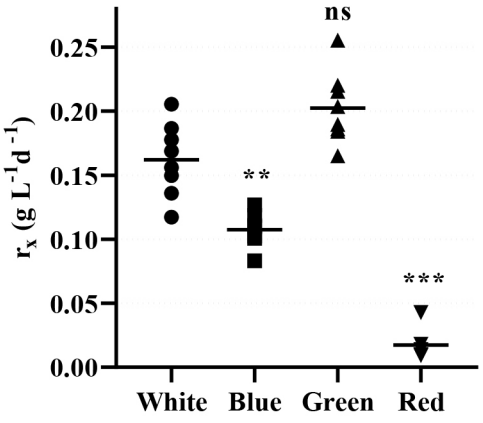


Figure 1. The effect of different wavelengths (blue, green, red, white) on volumetric productivity r_x (g L^{-1} d^{-1}) of R. salina. (indicates the significant difference after ANOVA test and t test of pairwise comparison between the groups ns $p>0.05$, *$p<0.05$, **$p<0.01$, **$p<0.0001$)*

(Continued on next page)

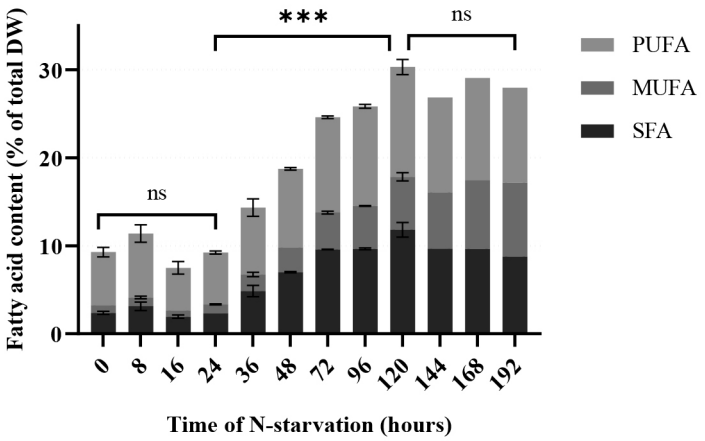


*Figure 2. Fatty acid concentration of R. salina during a 192 h N-starvation period. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids and PUFA: Polyunsaturated fatty acids. Data are expressed as the average of three replicates _ SD. *Indicates the significant difference after ANOVA post hoc test. ns P > 0.05, ***P < 0.001.*

Methodology

Two experiments were conducted in order to optimize *R. salina* production: 1. Effect of light wavelength on growth rate and biomass composition of *R. salina*. 2. Effect of nitrogen starvation on the lipid profile of *R. salina*. In both experiments *R. salina* was cultivated in 400 ml photobioreactors (Algaemist-S), where temperature was stable at 22 °C and pH at 7.5, regulated by CO_2 flow.

In the first experiment *R. salina* cultures were exposed to 50 $\mu mol_{photons}\ m^{-2}\ s^{-1}$ of four different light wavelengths, blue (380-520 nm), green (520-600 nm), red (600-700 nm), and warm light as reference. The photobioreactors were maintained in turbidostat mode with outgoing light of 15 $\mu mol_{photons}\ m^{-2}\ s^{-1}$.

The second experiment was divided into two stages. In the first stage *R. salina* was cultivated under optimal conditions in turbidostat mode, which reached a dilution rate of 1.3 d^{-1}. The turbidostat was maintained for more than a week to reach a steady culture. Samples were taken every 24 hours. In the next stage, the biomass was washed and transferred in a reactor filled with N^- depleted medium. During nitrogen starvation, samples were taken over a period of 8 days. Samples were taken for biomass concentration, cell size, PE, and fatty acid composition.

Results

The results of the first experiment demonstrate that the highest productivity in volumetric biomass (0.20 $g_{dry\ weight}\ L^{-1}\ day^{-1}$) was observed under green light conditions. Blue and red light illumination resulted in lower productivities, 0.11 $g_{dry\ weight}\ L^{-1}\ day^{-1}$ and 0.02 $g_{dry\ weight}\ L^{-1}\ day^{-1}$ respectively. The differences in production could be ascribed to increased absorption of green and blue wavelength by phycoerythrin, chlorophyll and carotenoids, causing higher photosynthetically usable radiation (PUR) from equal photosynthetically absorbed irradiance (PAR). Moreover, phycoerythrin concentration (281.16 mg $g_{dry\ weight}^{-1}$) was stimulated under red light illumination. Because photosystemII (PSII) absorbs poorly red light, the algae had to induce more pigments in order to negate the lower absorption per unit pigment of the incident available photons. The results of this study indicate that green light can be used in the initial growth of *R. salina* to produce more biomass and, at a later stage, red light could be implemented to stimulate the synthesis of PE.

The results of the nitrogen starvation experiment demonstrate that the lipid content of biomass increased significantly from t=0h to t=120h from 6% to 28.2% of dry weight respectively. Furthermore, the highest increase within 120h was illustrated for C16:0, C:18:1, C18:2, C18:3. However, the maximum EPA and DHA concentration was observed after 48 hours of stress, while the maximum DHA to EPA ratio was detected at the end of the starvation.

(Continued on next page)

References

Arndt C, Sommer U (2014) Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, Tisbe sp. and Tachidius discipes, and a discussion about their suitability for marine fish larvae. Aquac Nutr 20:44–59. https://doi.org/10.1111/anu.12051

Caramujo MJ, Boschker HTS, Admiraal W (2008) Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshw Biol 53:77–90. https://doi.org/10.1111/j.1365-2427.2007.01868.x

Coelho L, Prince J, Nolen T (1998) Processing of defensive pigment in Aplysia californica: acquisition, modification and mobilization of the red algal pigment, r-phycoerythrin by the digestive gland. J Exp Biol 201:425–42538

Doust AB, Wilk KE, Curmi PMG, Scholes GD (2006) The photophysics of cryptophyte light-harvesting. J Photochem Photobiol A Chem 184:1–17. https://doi.org/10.1016/j.jphotochem.2006.06.006

Guevara M, Bastardo L, Cortez R, et al (2011) Rhodomonas salina (Cryptophyta) pastes as feed for Brachionus plicatilis (Rotifera). Rev Biol Trop 59:1503–1515. https://doi.org/10.15517/rbt.v59i4.3415

Oostlander PC, Latsos C, van Houcke J, et al (2020) Production of Rhodomonas sp. at pilot scale under sunlight conditions. Algal Res 48:4–9. https://doi.org/10.1016/j.algal.2020.101934

Seixas P, Coutinho P, Ferreira M, Otero A (2009) Nutritional value of the cryptophyte Rhodomonas lens for Artemia sp. J Exp Mar Bio Ecol 381:1–9. https://doi.org/10.1016/j.jembe.2009.09.007

Sudhakar MP, Jagatheesan A, Perumal K, Arunkumar K (2015) Methods of phycobiliprotein extraction from gracilaria crassa and its applications in food colourants. Algal Res 8:115–120. https://doi.org/10.1016/j.algal.2015.01.011

van Houcke J, Medina I, Maehre HK, et al (2017) The effect of algae diets (Skeletonema costatum and Rhodomonas baltica) on the biochemical composition and sensory characteristics of Pacific cupped oysters (Crassostrea gigas) during land-based refinement. Food Res Int 100:151–160. https://doi.org/10.1016/j.foodres.2017.06.041

Yamamoto S, Bossier P, Yoshimatsu T (2020) Biochemical characterization of Rhodomonas sp. Hf-1 strain (cryptophyte) under nitrogen starvation. Aquaculture 516:734648. https://doi.org/10.1016/j.aquaculture.2019.734648

DISINFECTION, BIOSECURITY AND FISH HEALTH IN ATLANTIC SALMON RAS

Carlo C. Lazado[1*], Danilo Carletto[2], Kevin T. Stiller[1], Vasco Mota[1], João Osório[3], Gerhardus C. Verstege[1], Jelena Kolarevic[14], Lena Hovda Aas[3], Britt-Kristin M. Reiten[1], Roy-Inge Hansen[1], Chris Good[5], Lill-Heidi Johansen[1]

[1]Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, 9019 Tromsø, Norway
[2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 S Agata-Messina, Italy
[3]CIISA, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal
[4]The Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, N-9037 Tromsø, Norway
[5]The Conservation Fund Freshwater Institute, Shepherdstown, WV 25443, USA
Email: carlo.lazado@nofima.no

Introduction

Recirculating aquaculture systems (RAS) provide possibilities to rear fish in a highly controlled environment. Some of the advantages of RAS-based farming include minimum water use, improved biosecurity, efficient control of production parameters, better protection from challenging environmental conditions, among many others. All these contribute to ensuring an ideal environment that fosters health, welfare and performance. Disinfection strategies play a crucial part in maintaining optimal water quality and preventing disease outbreaks. These protocols are adapted to different levels of farm operations – from materials and ancillary equipment used daily, to intake and RAS loop water and to system-wide disinfection after each production cycle. This paper summarises different aspects of disinfection in both freshwater and brackish water RAS of Atlantic salmon aquaculture. This study discusses the effects of disinfection on both the fish and the RAS environment.

Materials and methods

Four studies are included in this paper, each covering different aspects of disinfection in RAS. ***Sub-study 1*** surveyed different salmon RAS farms in Norway and North America to benchmark the current disinfection protocols. ***Sub-study 2*** established the thresholds and biological consequences in post-smolt of the use of ozone in brackish water RAS. ***Sub-study 3*** explored the health and welfare aspects of using peracetic acid as a routine disinfectant in RAS. Lastly, ***Sub-study 4*** explored biosecurity breach in RAS and system disinfection following an outbreak. *Yersinia ruckeri* was used as a model pathogen.

Results

Sub-study 1: Twenty-five (25) salmon RAS farms participated in total. The survey highlighted that: 1) despite having disinfection protocols in-house, majority of which were not experimentally verified; 2) Norway and North America differed on the disinfectants commonly used; 3) efficacy and safety were the common criteria for selecting the disinfectant in both regions.

Sub-study 2: A 10-day exposure trial identified that the range 300-350 mV was the safe range for ozone use in salmon brackish water. Higher than 350mV resulted in substantial mortality and health issues. The long-term trial using the identified safe dose revealed that ozone use had minor effects on survival, external welfare and production performance. It had a favourable impact on gill health. Ozone exposure did not alter the ability of salmon to respond to the secondary stressor.

Sub-study 3: In the brackish water experiment, we found that daily PAA dosing triggered a slight local and systemic oxidative stress. Pathological alterations were predominant in the gills, where cases of epithelial lifting, hypertrophy and clubbing were prevalent. Lastly, oxidant exposure did not alter the ability of salmon to mount robust physiological stress responses to a secondary stressor. In the freshwater trial, continuous and pulse application resulted in the modulation of antioxidant defence genes in the mucosal organs. Histology revealed minimal changes in the key structures of the skin, gills and olfactory organ.

(Continued on next page)

Sub-study 4: The biosecurity breach simulation studies found that the breach through intake water resulted in higher mortality than introducing an infected fish to the system. Mortality was similar whether biosecurity was breached via the intake water once or for 3 successive days. Diseased fish developed classical external signs of yersiniosis, including skin darkening, exophthalmia, and haemorrhaging. Disinfection of the system using pH manipulation following a *Yersinia* outbreak was effective.

Conclusions

Disinfection is an integral component of a RAS facility. This series of studies revealed that disinfection strategies varied between regions, disinfection of the RAS loop water was crucial to maintain optimal water quality but could impact fish health and welfare when not appropriately managed, and biosecurity breach simulations offered insights into the dynamics of infection in RAS. These results are expected to contribute to developing a system-wide disinfection protocol in salmon RAS.

FISH CELLS CAN "SMELL" ROTTEN EGGS: MOLECULAR INSIGHTS INTO THE RESPONSES OF ATLANTIC SALMON TO EXOGENOUS HYDROGEN SULPHIDE

Carlo C. Lazado[1*], Gerrit Timmerhaus[1], Vibeke Voldvik[1], Nikko Alvin Cabillon[2], Christian R. Karlsen[1], Øivind Andersen[1]

[1]Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, 1433 Ås, Norway
[2]Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
Email: carlo.lazado@nofima.no

Introduction

Hydrogen sulphide (H_2S) is a naturally occurring compound generated either endogenously or exogenously and is common in many physical and biological systems. The endogenously generated H_2S is a known gasotransmitter and redox-active sulphur species that acts as an antioxidant and signalling molecule to support cellular functions. On the other hand, the exogenous form produced by anaerobic bacterial decomposition of protein and other sulphur-containing organic matter is a water-soluble and colourless gas with the distinct odour of rotten eggs. In recent years, H_2S-related mortality is prevalent in Atlantic salmon reared in recirculating aquaculture systems. Significant advances have been made in understanding its formation in these systems, however, the biological consequences of H_2S are largely unknown. Its toxicity is known since it can cause sudden mass mortality, but the physiological processes leading to this endpoint are yet to be unravelled. In this study, we investigated the underlying molecular mechanisms behind salmon-H_2S interactions. We used two *in vitro* model systems: first, the nasal leukocyte model to explore immune functions of H_2S, particularly at the mucosa that serves as a point of interaction; and second, hepatocytes (liver cells), a traditional model for xenobiotics research in fish.

Materials and methods

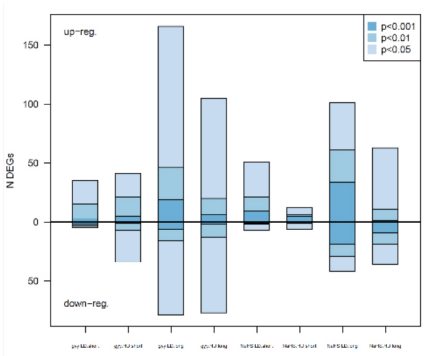
In this study, we used two kinds of sulphide donors: the salt form, *sodium hydrosulfide* (NaHS) and the synthetic analogue, *morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate* (GYY4137). NaHS releases H_2S instantaneously into an aqueous solution, while GYY4137 is a slow-releasing H_2S donor.

The cells were isolated from salmon smolts weighing around 500-700 g. Primary cultures of nasal leukocytes and hepatocytes were prepared using enzymatic digestion and cells were cultured in the laboratory for two days before H_2S exposure. For the leukocytes, cells were exposed to three concentrations (1, 10, 100 μM) of either of the two sulphide donors NaHS and GYY4137 for 24 hrs. Thereafter, the cells were collected for molecular analysis. For the hepatocytes, the cells were exposed to either low (20μg/ml) or high (100μg/ml) dose of the two hydrogen sulphide donors. Two exposure durations were tested, transient (for 1 hr) and prolonged (for 24 hrs). Samples for microarray were collected 24 hrs after the addition of the H_2S donors to the cell cultures. The viability of the cells was likewise checked after exposure. The cells were subjected to microarray analysis using the Nofima 15K array and annotated through the bioinformatics pipeline STARS.

Results and Methods

Responses of nasal leukocytes to H_2S. Cellular viability was minimally affected by the exposure to two H_2S donors, nonetheless, GYY4137-exposed cells exhibited reduced viability compared with the NaHS group at the highest dose. There were apparent concentration-dependent H_2S-induced transcriptomic changes in the nasal leukocytes regardless of the kind of sulphide donors. A larger number of differentially expressed genes (DEGs) were identified in the NaHS-exposed versus GYY4137-exposed groups across concentrations. In all comparisons, at least 53% of the DEGs identified were significantly upregulated. Gene ontology (GO) terms enriched in the lists of upregulated DEGs at higher concentrations included ferric iron binding. A comparison of the two sulphide donors showed a clear grouping of different GO terms relative to concentrations. Pathway enrichment analysis revealed a significant influence in VEGF ligand-receptor interactions, oxidative stress, innate and adaptive immunity, and interleukin signalling, especially at higher concentrations. Congruence analysis demonstrated that there were 16 GO terms overlapping; of these, 12 were upregulated by both sulphide donors, including several involving iron-binding and transport.

(Continued on next page)



Number of DEGs. The four bars to the left show numbers for GYY4137 and the four bars to the right, numbers for NaHS. The four bars represent the four treatment groups (short or long treatment, high or low dose). Each bar was split for three p-value levels, represented by shades of blue.

Responses of hepatocytes to H_2S. Prolonged exposure reduced cell viability. These changes were significantly affected by the type of sulphide donors and duration of exposure, however, these alterations were not dependent on H_2S concentration. A similar tendency was identified in the proliferative potential of the hepatocytes. Further, it was determined that there were significant interactions between sulphide donors and concentrations, as well as sulphide donors and exposure duration. Transcriptomic analysis revealed that GYY4137 caused a slightly more substantial effect than NaHS. However, the impact of both treatments was very different from each other. Since both sulphide donors demonstrated different response profiles, particularly the number of DEGs and expression dynamics, similarities in each set were analysed separately from each other. Of the two factors studied (i.e. concentration and duration), the duration had a more substantial effect, and this was exhibited by the two sulphide donors. Overall, the number of DEGs in NaHS was lower compared with the GYY4137-exposed group.

Conclusions

The two *in vitro* models provide the first insights into the molecular processes governing how exogenous H_2S affect salmon. To our knowledge, this is also the first report to provide this level of resolution for this interaction in teleost fish in general. The two cell models demonstrated different transcriptional responses to H_2S. NaHS-exposed leukocytes showed a higher number of DEGs than GY4137; an opposite trend was observed in the hepatocytes. Responses to the sulphide donors showed a clear concentration-dependence in the nasal leukocytes, but the hepatocytes did not exhibit this feature. In the latter, duration of exposure and sulphide donors elicited more robust responses. Molecular pathways affected by H_2S were clearly identified in the leukocytes, but no clear predictions can be made from the hepatic transcriptome. The H_2S-responsive molecules identified can be explored further as potential biomarkers for environmental H_2S.

Acknowledgement

This study was funded by the Research Council of Norway under grant number 300825.

NEW METHOD FOR FISH STERILIZATION – EVALUATION OF GERM CELL ABLATION ON THE ACTIVATION OF THE BRAIN-PITUITARY-GONADAL AXIS IN PRECOCIOUS ATLANTIC SALMON MALES

Carlo C. Lazado[1*], Øivind Andersen[1], Kristian Karlsen[2], Krasimir Slanchev[3], Tina Thesslund[1], Gunhild Johansson[1], Dhivya Borra Thiyagarajan[2], Helge Tveiten[2]

[1]Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, 9291Tromsø, Norway
[2]Department of Fishery Science, University of Tromsø, 9037 Tromsø, Norway
[3]AquaGen AS, Sluppen, P.O. Box 1240, 7462, Trondheim, Norway
Email: helge.tveiten@uit.no

Introduction

The genetic impact of escaped farmed Atlantic salmon on wild populations has been identified as being the largest current threat to wild salmon populations. Further, sexual maturation is a major economic and welfare problem for the industry due to negative impacts on somatic growth, flesh quality, hypoosmoregulatory ability and immune function. Also, control of intellectual property is difficult for the salmon breeding companies since the eggs/fish will be able to reproduce. Farming of sterile fish should provide a solution to several of these challenges. However, triploid salmon perform poorly under suboptimal environmental conditions, and gene edited sterile salmon in aquaculture production raises serious ethical and legal concerns. We have developed an alternative strategy for producing sterile salmon by ablation of the primordial germ cells (PGC) using antisense oligos for mRNA degradation of the key PGC factor Deadend (DnD). Here we examine pubertal changes of the brain-pituitary-gonad (BPG) axis in sterile and intact fertile salmon males by inducing sexual maturation using an out-of-season photoperiod/temperature regime.

Materials and methods

Germ cell ablation was achieved by microinjection of *dnd* Gapmers in fertilized Atlantic salmon eggs delivered by AquaGen breeding company. The *dnd*-morphants and intact fertile control fish were held under continuous light at 6-10ºC until august 2018 at the Aquaculture research station at Kårvika, Tromsø. Totally 360 fish were Pit-tagged and transferred into six 500-L freshwater tanks for triplicate analysis. Smoltification was induced by exposing the fish to a photoperiod of daily 6 hours light (6L:18D) and water temperature of 5-6ºC for eight weeks followed by continuous light and 10ºC throughout the experiment. 10-12 fish were randomly sampled from each tank at three time points T1 (24.10), T2 (14.11) and T3 (05.12). Blood was sampled from caudal vessels, and body weight and length were recorded before the fish were opened for dissection of brain, pituitary and gonads. Tissues were stored in RNAlater until gene expression analyses using real-time quantitative PCR (qPCR).

Results

Microinjection of *dnd*-antisense oligos resulted in undetectable *dnd* mRNA levels and complete loss of gametes in the string-like gonads of almost all (92 %) males and females about two years after the treatment. This contrasted with the high *dnd* expression in the intact fertile gonads containing differentiating gametes. Precocious maturation was induced in about one-third of the sterile males and in almost two-thirds of the fertile males based on the bimodal frequency distribution of several hallmark indicators of puberty onset. Both maturing sterile and fertile males showed upregulated expression of pituitary *fshβ* concomitant with elevated plasma androgen levels together with reciprocal levels of testicular *igf3* and *amh* mRNAs.

Conclusions

This study shows that the germ cells are not required for the activation of the BPG axis and for the production of gonadal sex steroids at least not at the freshwater stage. The highly reliable sterilization method supports ethical and legal guidelines for animal breeding to achieve long-term environmental sustainability of our seafood supplies. However, an efficient antisense delivery strategy is needed for large-scale production of sterile salmon.

Acknowledgement

This study was funded by the Norwegian Seafood Research Fund (FHF) under grant number 901459.

USING DIGITAL TWIN FOR DECISION SUPPORT IN RAS FEEDING PROCESSES

F. Le Gall[*], J. DePrisco[a], SG. Prescott[a], S. Budaev [b], L. Ebbesson[c], I. Rønnestad[b]

[*] EGM, 444 Route des Dolines – 06560 Valbonne France
franck.le-gall@egm.io
[a] AquaBioTech Ltd, Central Complex, Naggar Street, Targa Gap, Mosta, Malta
[b] Department of Biological Sciences, University of Bergen, Pb 7803, 5020 Bergen, Norway
[c] NORCE, P.O.B 22 Nygårdstangen, NO-5838 Bergen

Introduction

For aquaculture to grow to meet world food demands, more intensive and sustainable farming practices must be implemented. This requires developing more effective ways to monitor and control the factors that influence fish health and welfare and to identify more efficient and sustainable ways of feeding fish. This reduces pressure on source ingredients, such as agricultural crops and wild-caught fish. More intensive aquaculture production requires greater control and monitoring to reduce mortality. This can be achieved through integrating a) optimisation of feeding regimes, b) observation of fish behaviour, and c) expansion of the use of sensory technology to measure water quality. Farmers will have greater and more precise information when it comes to decision making, which will reduce stress and increase overall fish health and welfare. The ambition of iFishIENCi project in which this research is taking place, is to develop and demonstrate disruptive IoT/AI based innovations, considering the feeding value chain and addressing commercially important species, with fish quality as focus. Through close collaborations between engineers and fish biologists, the smart feeding and monitoring systems will be more elaborate and precise. New value chains will be identified for the valorisation of specific waste from different production systems. This cutting-edge research is being combined with a holistic understanding of how new technologies interact with society and stakeholders in terms of economy, politics, social welfare, animal welfare and ethics under an RRI framework.

iBOSS platform

Information about fish behaviour, physiology and their environmental conditions can be collected in real-time from a variety of fish farms, and then securely transferred to a digital representation of a real fish. The digital fish will then send a message back to the monitoring system, which can then either inform the farmer, or make an automated change. The core of the iFishIENCi platform, is named iBOSS, is based on the NGSI-LD specification produced by ETSI and made open-source within the FIWARE ecosystem. From a technical perspective, FIWARE brings a curated framework of open-source software components which can be assembled together and combined with other third-party platform components to build platforms easing the development of smart solutions and smart organizations in multiple application domains: cities, manufacturing, utilities, agrifood, etc. The Digital Twin data representation is built based on information gathered from many different sources, including sensors, cameras, farm information systems and farmers observation. It is constantly maintained and accessible in near real-time. Applications constantly process and analyze this data (not only current values but also the history generated over time) in order to automate certain tasks or bring support to smart decisions by end users.

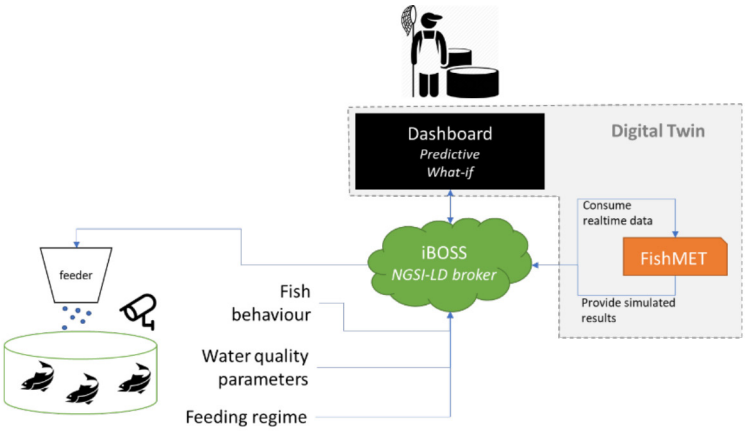


Figure 1: Overall schematic of digital twin integration in a fish farming system.

(Continued on next page)

Digital Twin purpose

The digital twin model is based on understanding the whole fish organism as an adaptive agent, robust, testable biological theory that is implemented in the computer code. This means that the model not only describes a specific aspect of fish nutrition, energetics, growth or behaviour in the form of an equation or system of equations. Instead, the digital twin aims to work as a digital organism simulating the most crucial aspects of physiology, neurobiology and behaviour in a digital environment. The agent can therefore act autonomously, make decisions in response to the internal and external environment with continuous feedbacks at multiple biological levels. This means, for example, that the simulation system aims to predict voluntary behaviour and food intake of the fish. This digital twin for feeding will enable running various scenarios and predict the response including unplanned, emergent and stochastic effects. Such a capacity will provide an indispensable tool for decision support and operational optimization and can be run in the AI-controlled precision fish farm environment of iBOSS.

FishMet model

The fish digital twin model accepts the basic biological parameters of the fish as input, such as body size, energetic characteristics, and is built on experiments and literature-based parameters of fish physiology. Environmental inputs include the physical characteristics of the feed, feeding schedule, ambient temperature, oxygen level. The model will then output fish appetite, food intake, feed conversion efficiency, waste production and growth. The autonomous agent type of the model also allows to collect additional outputs, such as behavioural patterns, and predict the internal state of the fish, e.g., motivation and stress level, responses to unexpected change of the feed, stochastic environmental perturbations etc. The modular organization of the model allows to simulate a single fish or a large group in an individual-based model. Water quality, environmental data, fish physiology and behaviour data are collected and made available to the FishMET model in real time through the NGSI-LD data broker of the iBOSS. FishMet is a digital simulation of feeding, fish behaviour, physiology, and metabolism, which consists of differential equations that define feed intake, stomach filling, digestion, absorption, retention of nutrients and will be developed to include feeding behaviour and waste production. The FishMet model include new knowledge about physiological factors that affect appetite and feed intake. Intestinal passage and postabsorptive processing are principal elements. A central part of the model focuses on the changes that occur due to variations in feed type, feeding amount and frequency as well as how environmental factors including temperature, oxygen availability and stress (e.g. handling) will affect feeding behaviour and processes leading to growth. The software engineering work aims to produce a highly modular and customizable system that can run as a separate application and be embedded into the larger decision-support and intelligent fish farm control system iBOSS (Figure 1). Specific experiments that will provide appropriate datasets to the modelling activities are currently being performed. In addition to environmental data, individual and shoal behaviour, such as swimming speed and direction, will be monitored with cameras detecting individually marked fish before, during, and after feeding. The fish will also be fed a diet containing a marker to allow correlations between gut transit and feeding behaviour.

FUNCTIONAL NUTRITION MITIGATES THE IMPACT OF REPETITIVE NON-MEDICINAL INTERVENTIONS IN SEAWATER ATLANTIC SALMON

Leclercq, E.[a]*; Rawling, M.[b], Valdenegro, V.[c], Aasum, E.[c], Vera, L.M.[d]., Castex, M.[a], Migaud, H.[d]

[a] Lallemand SAS, 19 rue des briquetiers, 31702 Blagnac, France
[b] School of Biological, Plymouth University, PL4 8AA Plymouth, UK,
[c] BioMar, Havnegata 9, 7010 Trondheim, Norway
[d] Institute of Aquaculture, University of Stirling, FK9 4LA Stirling, Scotland, UK
* eleclercq@lallemand.com

Introduction

Non-medicinal interventions against parasitic infections have unequivocal benefits but impose repetitive exposure to external stressors and associated handling with the potential of severely compromise fish health and performance. Strategies that bolster mucosal robustness and mitigate physiological stress are therefore critical to support current de-medication effort. The study applied a repetitive-stress model mimicking current commercial practices against ectoparasites to evaluate the putative benefits of a blend of functional feed ingredients on the fish stress, oxidative, immune and mucosal status with the view to mitigate the impact of such practices. The blend consisted of a multi-strain yeast fraction product (MsYF; Rawling et al.; 2021) along with a Melon Pulp Concentrate (MPC) rich in the primary antioxidant superoxide dismutase (SOD; Carillon et al., 2013) previously shown to stimulate skin SOD level in Atlantic salmon (Barbé et al., 2018).

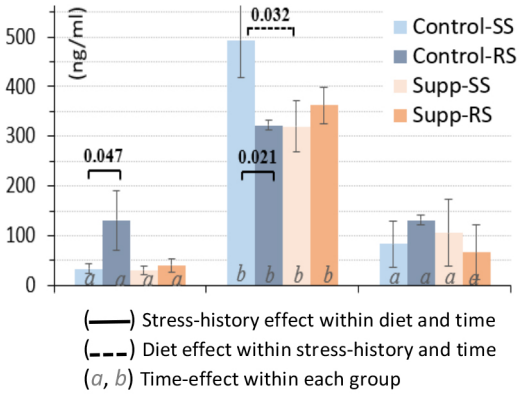
Materials and Methods

The 9-week trial tested 4 groups (2 diets x 2 stressors schedule) in triplicate using 500 g Atlantic salmon (37 fish / tank; 12°C, 36 ppt). The test diets consisted of a baseline diet non-supplemented (**Control diet**; BioMar) or supplemented (**Supp diet**) pre-extrusion with MsYF and MPC (800 g/t feed and 50 g/t respectively; Lallemand SAS, France); fed ad-libitum over the trial duration. The stressors schedule consisted of exposure to either a **single-stressor** (**SS**) or to **repeated-stressors** (**RS**) as follow: after a 5-week preparation phase, the RS group was crowded and netted-out 3 times at weekly intervals for immersion into 2 freshwater bath (1h) and a hydrogen peroxide bath (H_2O_2; 30 min; 1500 mg/L) then given a 2-week recovery period. In comparison, the SS group was exposed to the H_2O_2 bath only (no prior handling). Sampling was performed immediately prior (T1), 1 h (T1+1h), 48 h (T1+48h) and 2-week after (Tend) H_2O_2 exposure. Sampling documented the primary stress response, antioxidant status, mucosal integrity, innate immunity, plasma biochemistry and fish performance over time. Data were analysed by a Linear model ($P < 0.05$) and presented as mean ± SEM.

Results and Discussion

In the control-diet, the RS-schedule significantly increased baseline cortisol level (T1) and reduced the acute response to an immediate stressor (H_2O_2-bath, T1+1h) (**Fig 1**). This characterizes a state of chronic-stress and a subsequent exhaustion of the primary stress-axis which were not observed in the Supp diet. This apparent state of chronic stress was associated with a statistically higher skin goblet cell coverage (GCC, RS compared to SS at T1 in Control diet, **Fig 2**) without further increases upon H_20_2 exposure (T1+48h) suggesting an exhaustion of the mucosal response. Indeed, in the Supp diet; there was no apparent effect of stress-history on baseline skin GCC (SS compared to RS at T1) but a significant increase upon exposure to H_20_2 (T1+48h) indicating the persistence of a functional mucosal response to H_20_2-exposure. Exposure to RS also impacted intestinal architecture which was partly mitigated by the Supp diet (not shown).

Fig 1: Plasma cortisol level



(Continued on next page)

Fig 2: Skin goblet cell coverage

(——) Stress-history effect within diet, time
(– – –) Diet effect within stress-history, time
(*a*, *b*) Time-effect within each group.

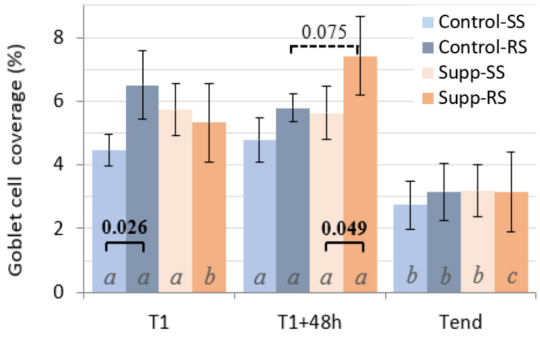


Fig 3: Skin SOD level

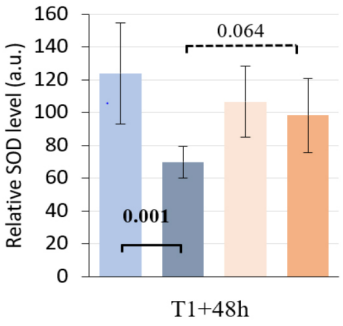
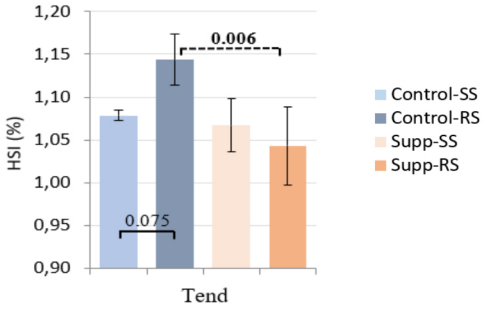


Fig 4 Hepatosomatic Index



Similar to serum values (not shown); skin SOD was depleted under RS compared SS in the Control but not in the Supp diet (**Fig 3**) shortly after H_20_2 exposure (T1+48h). The Supp diet therefore appeared to prevent low SOD levels induced by RS exposure.

There was no effect of the diet or stress-schedule on growth performance. However, RS was associated with a significant increase in Hepatosomatic Index (HSI) in the Control but not in the Supp diet (**Fig 4**), suggesting a contribution of the test against the tertiary impact of chronic stress on resources allocation and animal energetics.

Conclusion

By applying a commercially relevant repetitive-stress model, the study documented the negative effect of repetitive-stress exposure on the physiological and mucosal health of SW Atlantic salmon. Dietary supplementation with a blend of primary antioxidant and yeast-based ingredient was able to mitigate the pernicious effects of repetitive-handling and associated losses of mucosal robustness, responsiveness and recovery capacity. This is expected to have clear benefits at animal and farm level over the production cycle.

Funding The authors are grateful for the support received from SAIC (UK; SF_2018_01)

References

Barbé, F., Leclercq, E., Carillon, J., Kiron, V., Castex, M. Stimulation of superoxide dismutase in the skin of Atlantic salmon receiving SOD-rich melon pulp concentrate dietary supplement and upon H202 exposure. European Aquaculture Society; 25-29 August 2018; Montpellier, France.

Carillon, J., Rouanet, J-M., Cristol, J-P., Brion, R., 2013. Superoxide dismutase administration, a potential therapy against oxidative stress related diseases: several routes of supplementation and proposal of an original mechanism of action. Phramaceutical Research 30, 2718-28.

Rawling, M.; Leclercq, E.; Foey, A., Castex, M., Merrifield, D., 2021. A novel dietary multi-strain yeast fraction modulates intestinal toll-like-receptor signalling and mucosal responses of rainbow trout (*Oncorhynchus mykiss*). PLoS One 16, e0245021.

YEAST-BASED INGREDIENTS AS EARLY PROMOTERS OF MECHANICAL SKIN WOUND HEALING - A COMPARATIVE STUDY USING A ZEBRAFISH MODEL

Leclercq, E.[a*], Edirisinghe, S.L.[b], Nikapitiya, C.[b], Kim, C.H.[b], Castex, M.[a], De Zoysa, M.[b]

[a] Lallemand SAS, 19 rue des Briquetiers, 31700, Blagnac, France
[b] Chungnam National University, Yuseong-gu, Daejeon34134, Republic of Korea.
* eleclercq@lallemand.com

Introduction

Mechanical disruption of the skin barrier function can quickly lead to morbidity and secondary infections with the potential of significant biological and market value losses in aquaculture. Yeast β-glucans are known modulators of skin wound healing acting both indirectly via their immune functionalities as well as directly by promoting re-epithelization (Majtan and Jesenak; 2018). More commonly addressed for topical application in mammals, they are seldom studied as skin health ingredients in aquafeed. The study compared the effect of a standard a yeast β-glucans to a multi-strain yeast-fraction (MsYF) ingredient on full-thickness skin wound healing using an adult zebrafish model. The MsYF and its mucosal immune properties are further described in Rawling et al. (2021) raising promising prospects as skin wound healing promoter.

Materials and Methods

Wild-type adult zebrafish (20 L tanks; 28°C; 25 fish/tank) were fed one of three test diets for 7 weeks prior to being inflicted a full-thickness dermal wound (2 mm Ø biopsy punch). Four experimental groups were tested in quadruplicate: 1) negative control (not wounded, basal diet), 2) positive control (wounded, basal diet), 3) β-glucans (wounded; basal diet + 315 g/t feed of β-glucans) and 4) MsYF (wounded; basal diet + 1.5 kg/t feed of MsYF).
Wound-healing was assessed over time by computer-assisted measurement of gross wound-surface area on individual fish, (quantitative) histopathology, transcriptional and immunoblotting analysis at the wound-site. Datasets were analysed by ANOVA with Bonferroni's post-hoc test or unpaired two-tailed t-test where significance occurred ($p < 0.05$) and are presented as mean ± SEM.

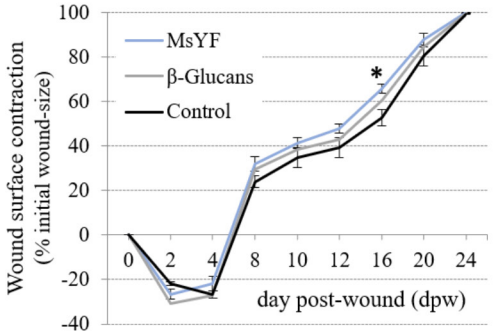
Results and Discussion

Wound surface contraction first occurred from 2 to 4 dpw in 18%, 78% and 72% of the Control, β-glucans and MsYF fish, respectively, such that mean wound-size decreased in the test groups but further increased in the Control (**Fig. 1**). Subsequently, gross healing remained superior in the MsYF compared to the Control (significant at 16 dpw with MsYF = 66 ± 4% and Control = 53 ± 4% contraction).

Histopathological diagnostic of the wound margins revealed a higher prevalence of inflammatory cells at 1 dpw followed by a greater scab clearance at 4 dpw in the β-glucans and MsYF group; as well as a thicker granulation tissue in the MsYF group. At the wound-bed, the thickness and transversal surface area of the granulation tissue (new connective tissue) was significantly higher in the MsYF while differences between the β-glucan and Control groups were observed (**Fig. 2**).

Transcriptional and immunoblot analysis at the wound-site supported the observed promotion of early-stage wound-healing by the test compounds. The apparent advantage of MsYF was associated with a significant up-regulation of matrix metalloproteinase genes (**Fig. 3a**) known to be involved in extra-cellular matrix remodelling during skin tissue regeneration; as well as with a more balanced (anti)-inflammatory process at an early healing stage compared to the control and β-glucans (e.g. hsp90 and il-10; **Fig. 3b**).

Fig. 1: Gross wound-healing profile



(Continued on next page)

Fig. 2 Granulation tissue **a.** Image analysis, **b.** thickness, and **c.** surface area at 4 dpw. * denotes significant differences between groups

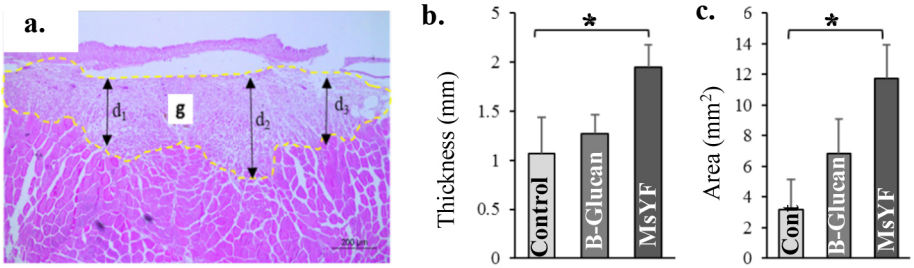


Fig. 3a. Transcriptional profile of target genes; **b.** immunoblot levels of selected proteins at the wound-site. Data expressed relative to negative control; shown in bold or marked ***u*** when significantly upregulated; * denote significant differences between ingredients.

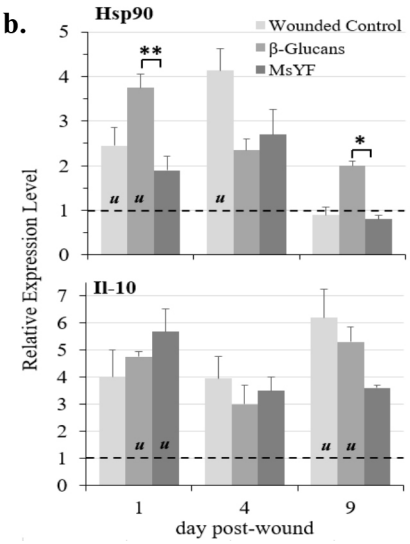
a.

	dpw	Wounded Control	β-Glucans	MsYF
mmp9	1	**51.6**	**40.3**	**113.4*** **
	4	**9.0**	1.6	3,0
	9	**19.1**	**9.3**	6.4
mmp13	1	**10.9**	**5.9**	**67.6*** **
	4	**7.4**	1.6	1.5
	9	1.5	0.3	0.6

Fold range	
> 5.00	
2.00 - 4.99	Up-regulated
1.00 - 1.99	
0.50 - 0.99	Down-regulated
< 0.49	

(*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

b.



Conclusion

The study establishes in-feed yeast-based ingredients as promoters of fish skin wound healing. The MsYF ingredient had a clear benefit over established yeast-β-glucans by promoting the early formation of new connective tissue filling the wound bed, i.e. wound closure, and holds the benefit of being suited for continuous in-feed application. This comparative study deciphers the distinct properties and modes of action of different yeast-based ingredients opening the way to further optimizing their potency as skin health promotors in aquaculture.

References

Majtan and Jesenak; 2018. β-Glucans: multi-functional modulator of wound healing. Molecules 23,806.

Rawling, M.; Leclercq, E.; Foey, A., Castex, M., Merrifield, D., 2021. A novel dietary multi-strain yeast fraction modulates intestinal toll-like-receptor signalling and mucosal responses of rainbow trout (*Oncorhynchus mykiss*). PLoS One 16, e0245021.

UNLOCKING THE WILD POTENTIAL: INTEGRATION OF GEOGRAPHIC DIFFERENTIATION IN DOMESTICATION PROCESSES TO FACILITATE FISH AQUACULTURE DIVERSIFICATION

T. Lecocq*, L. Toomey and P. Fontaine

University of Lorraine, INRAE, UR Animal and Functionality of Animal Products, Team Domestication in Inland Aquaculture, Nancy, France

*Presenting author: thomas.lecocq@univ-lorraine.fr

Context and aims

One way to promote the aquaculture sector sustainability relies on the production diversification, notably through the domestication of new species. However, domesticating new species remains a long and challenging process often resulting in unfruitful attempts because of technical issues, socio-economic limitations, or biological features of species (Teletchea and Fontaine 2014). Most incipient domestication programs often consider species as a unity, disregarding a part of the biodiversity: the wild intraspecific geographic differentiation. Yet, this differentiation can shape local genetic, phenotypic, and ecologic specificities, affecting the domestication predisposition or the socio-economic attractiveness of a particular population (Toomey et al. 2020)disregarding a part of the biodiversity: the wild intraspecific geographic differentiation. Yet, this differentiation can shape local specificities, which could lead to different domestication predisposition or socioeconomic attractiveness between populations. Therefore, considering this population- specific potential could facilitate domestication and subsequent production of new candidate species. Here, we propose a three-step integrative approach to standardise and facilitate new domestication attempts by taking advantage of wild geographic differentiation. Step 1 consists of classifying the wild biodiversity to identify prospective units (i.e. groups of differentiated allopatric populations. Therefore, choosing wild populations exhibiting a high potential for aquaculture (i.e. presenting interesting expression of key traits) could facilitate the domestication and production of new species. Here, we (i) introduce a new method to integrate geographic differentiation in domestication programs and (ii) apply it on a species of interest for inland aquaculture diversification, the European perch (*Perca fluviatilis*).

A 3-step integrative approach to integrate geographic differentiation in domestication programs

We propose a 3-step integrative approach to standardize and facilitate new domestication attempts by taking advantage of wild geographic differentiation (Toomey et al. 2020)disregarding a part of the biodiversity: the wild intraspecific geographic differentiation. Yet, this differentiation can shape local specificities, which could lead to different domestication predisposition or socioeconomic attractiveness between populations. Therefore, considering this population- specific potential could facilitate domestication and subsequent production of new candidate species. Here, we propose a three-step integrative approach to standardise and facilitate new domestication attempts by taking advantage of wild geographic differentiation. Step 1 consists of classifying the wild biodiversity to identify prospective units (i.e. groups of differentiated allopatric populations. Step 1 consists of classifying the wild biodiversity to identify prospective units (i.e. groups of genetically differentiated allopatric populations), which are likely divergent for key traits. Step 2 aims at comparing performances of these units in standardized conditions (i.e. rearing system) through a multi-function and multi-trait assessment. Finally, step 3 highlights units with highest potential for aquaculture through the calculation of an aquaculture potential score (Toomey et al. 2021).

The European perch case study

The 3-step integrative approach has been applied to compare the aquaculture potential among prospective units of the European perch (Toomey et al. 2021). More specifically, we aimed at finding units with the best performance in larviculture, a critical stage in the *P. fluviatilis* production. The step 1 allowed identifying five prospective units across 84 West-Palearctic sampling sites using mitochondrial and microsatellite markers: the European Plain, Danube, Northern and Eastern Fennoscandia, Eastern Europe, and the Balkans units. At the step 2, we compared performances of key traits for fish larviculture for three of these prospective units in standardized recirculated aquaculture system. A geographic differentiation was highlighted for six important traits for perch larviculture: survival rate, swim bladder inflation rate, deformity rate, length at hatching, mean of interindividual distances, and change in activity following a stress event. Along with fish farmer-advice-based weighting of the traits, the calculation of an aquaculture potential score allowed identifying, during the step 3, the populations from the Danube region as the most interesting to potentially overcome current bottlenecks in European perch larviculture.

(Continued on next page)

Prospects

Overall, the early applications of the 3-step approach support that it could allow facilitating domestication of new species or species at incipient stages of domestication which face major bottlenecks. So far applied to fish culture for human consumption, the approach also aims at being extended to other taxa (e.g. crustaceans, mollusks) and other production goals. Nevertheless, the implementation of the approach is still challenging due to some method limitations, pragmatic concerns, and legal regulation constraints needing future improvements to further facilitate aquaculture development.

Key references

Teletchea F, Fontaine P (2014) Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish Fish 15:181–195. https://doi.org/10.1111/faf.12006

Toomey L, Fontaine P, Lecocq T (2020) Unlocking the intraspecific aquaculture potential from the wild biodiversity to facilitate aquaculture development. Rev Aquac 12:2212–2227. https://doi.org/10.1111/raq.12430

Toomey L, Lecocq T, Pasquet A, Fontaine P (2021) Finding a rare gem: Identification of a wild biological unit with high potential for European perch larviculture. Aquaculture 530:735807. https://doi.org/10.1016/j.aquaculture.2020.735807

JELLYFISH FROM EUROPEAN SEAS AS VALUABLE SOURCE OF BIOACTIVE AND HEALTH PROMOTING COMPOUNDS WITH NUTRACEUTICAL VALUE

De Domenico S*[1,2], De Rinaldis G*[1], Albano C[1], Bleve G[1], Gallo A[1], Javidpour J[3], Mammone M[2], Ramires FA[1], Piraino S[2,4], Leone A[1,4],

[1]National Research Council, Institute of Science of Food Production (CNR, ISPA –Lecce), Via Prov.le Lecce – Monteroni, 73100 – Lecce, Italy
[2]DiSTeBA, University of Salento, Via Prov.le Lecce – Monteroni, 73100 – Lecce, Italy
[3]University of Southern Denmark, Campusvej 55, DK-5230 Odense M
[4]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), P.le Flaminio, 9, 00196 Rome
*These authors contributed equally.

***Introduction**:*
The massive blooms of jellyfish (JF), that more and more frequently explode in European seas, usually negatively impact human health and activities in coastal waters, representing a nuisance or damage for marine and maritime activities. As an alternative, these marine gelatinous organisms, should be viewed through a more positive perspective as a new important bio-resource. Indeed, known for their nutritional and medical value in the Chinese pharmacopeia, increasing attention has been pointed to jellyfish as an unexploited source of essential nutrients, novel bioactive metabolites, and lead compounds.

Within the H2020 European GoJelly project, JF and their biodiversity, were analysed as an available and abundant source of new natural health-promoting compounds. Different species, *Rhizostoma pulmo* (Macrì, 1778) that undergoes recurrent outbreaks in the Mediterranean coastal waters, and the zooxanthellatae JF species, as *Cotylorhiza tuberculate and Cassiopea andromeda,* this last one not native of Mediterranean Sea), were considered as source of new bioactive compounds, both proteinaceous (such as collagen) and non-proteinaceous compounds, as the hydroalcoholic-soluble extracts.

R. pulmo peptides were analysed for their antioxidant activity in vitro and in HEKa cell cultures, and the potential immunomodulatory activity of low molecular weight peptides were investigated in monocytes-macrophages couture system (De Domenico et al., 2019). Hydroalcoholic extracts from *C. tuberculata* and *C. andromeda* moreover, were assayed for their cytotoxicity effects on cancer cells and for their ability to modulate Gap Junction Intercellular Communication (GJIC) (De Rinaldis et al., in preparation).

Material & Methods:
From 2017-2020, target jellyfish species *R. pulmo and C. andromeda* were collected at Marina di Ginosa (Taranto, Italy) and in two different locations of Palermo (Sicily, Italy) harbour. Samples were individually frozen in liquid nitrogen, stored at -80 °C, and lyophilized. *R. pulmo* lyophilized tissues were subjected to aqueous protein extraction by phosphate-buffered saline (PBS) to separate the hydro-soluble proteins from the insoluble ones, which were exposed to a two-step sequential enzymatic hydrolysis, as described in Figure 1 (Leone et al., 2013-2015). Soluble proteins (SP), Hydrolysed by Pepsin JF peptides (HPJp) and Hydrolysed JF Collagen peptides (HJCp) were molecular weight (MW)-fractionated by membrane filtration and each fraction was analysed for antioxidant activity in vitro and for their effect on cultures of Human Epidermal Keratinocyte, adult (HEKa) cells. MTS assay was used to analysed HEKa cells vitality, when exposed to different peptides concentrations and under oxidative stress conditions (H_2O_2 0.1 mM). Calf skin collagen (Vertebrate Collagen, VC) was used as control. Zooxanthellatae species were lyophilized and subjected to aqueous and/or hydroalcoholic extraction (80% ethanol) and further to the same sequential enzymatic digestions. Extracts were essayed on cancer and non-cancer human cell cultures.

***Results**:*
Jellyfish proteins and peptides obtained from enzymatic hydrolysis showed significant antioxidant activity. In particular, collagenase-hydrolysed collagen resulted in peptides with different range of molecular weight (MW lower than 3 kDa, ranging 3–10 kDa or 10–30 kDa), with antioxidant activity inversely proportional to MW. No cytotoxic effect was detected on cultured human keratinocytes (HEKa) except for soluble proteins higher than 30 kDa, likely containing the jellyfish venom compounds, which were easily neutralized by mild thermal treatment. Furthermore, hydrolyzed jellyfish collagen peptides showed a significantly AA and provided a protective effect against oxidative stress in HEKa cells (Figue 2). Some of the jellyfish hydrolized proteins, showed also anti-inflammatory activity on appropriate cell culture.

Finally, non-proteinaceous extracts from zooxanthellatae jellyfish showed a noticeable anti-proliferative effect on cancer cell culture (MCF7).

(Continued on next page)

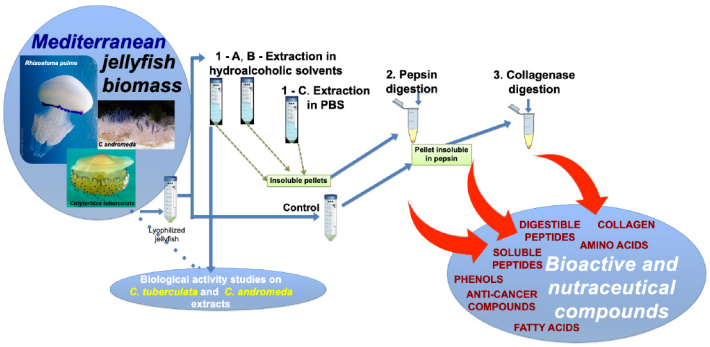


Figure 1: Schematic representation of the procedures used for the extraction of bioactive compounds from Mediterranean jellyfish species.

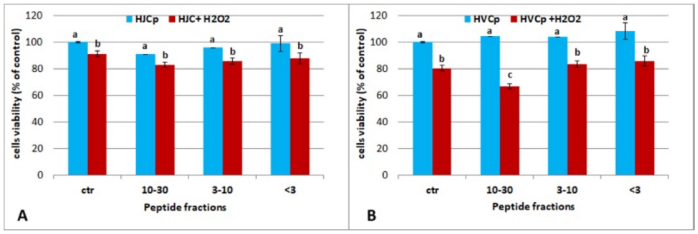


Figure 2: Effect of oxidative stress on HEKa cells of different concentration of jellyfish (A) and calf skin (B) collagen hydrolyzed peptides with different MW

Discussion & Conclusion:

Due to a high biodiversity and reproductive potential, jellyfish may represent a potential socioeconomic opportunity as a source of natural bioactive compounds, with far-reaching beneficial implications. Eventually, improvements in processing technology will promote the use of untapped marine biomasses in nutraceutical, cosmeceutical, and pharmaceutical fields, turning marine management problems into a more positive perspective.

Literature

De Domenico, S.; De Rinaldis, G.; Paulmery, M.; Piraino, S. & Leone, A. Barrel Jellyfish (*Rhizostoma pulmo*) as Source of Antioxidant Peptides. Mar. Drugs, (2019), 17, 134. https://doi.org/10.3390/md17020134

Leone A, Piraino S. 2015. Jellyfish: old Eastern food becomes the Western novel food. Shaping the Future of Food Safety, Together: Proceedings of the 2nd EFSA Scientific Conference. Milan, Italy, 14–16 October 2015. EFSA Journal, 2015;13(10):s1310, 137 pp. doi:10.2903/j.efsa.2015.s1310

Leone A, Lecci RM, Durante M, Meli F, Piraino S. 2015. The bright side of gelatinous blooms: nutraceutical value and antioxidant properties of three Mediterranean jellyfish (Scyphozoa). Marine Drugs, 13, 4654-4681; Special Issue "Marine Functional Food" doi:10.3390/md13084654

TRANSCRIPTOMIC APPROACH TO ENVIRONMENTALLY FRIENDLY FARMING OF EUROPEAN SEA BASS IN THE ADRIATIC SEA, USING ALTERNATIVE PROTEIN SOURCES

I. Bušelić[a]*, I. Lepen-Pleić[a], T. Šegvić-Bubić[a], E. Kaitetzidou[b], E. Tibaldi[c], L. Grubišić[a] and E. Sarropoulou[b]

[a]Laboratory of Aquaculture, Institute of Oceanography and Fisheries, Setaliste Ivana Mestrovica 63, 21000 Split, Croatia
[b]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research Crete, Thalassocosmos, Gournes Pediados 2214, 71003 Heraklion, Greece
[c]Department of Agri-Food, Environmental and Animal Science, University of Udine, Via delle Scienze 206, 33100 Udine, Italy
*E-mail: buselic@izor.hr

Introduction

European aquaculture is facing the challenge of satisfying the growing seafood demands and reducing the pressure on fishing areas. Aquaculture feeds use 70 percent of the world's fishmeal and fish oil, obtained from overexploited small pelagic fish (Froehlich et al., 2018). By 2040, the demand for fishmeal and fish oil will exceed the supply, emphasizing the need for novel feed ingredients to enhance production. Recently, new protein sources include fish by-products, poultry by-products, insects, and algae, displaying promising results towards optimal fish growth (Beheshti Foroutani et al., 2018).

The ongoing Interreg AdriAquaNet project is dedicated to enhancing the innovation and sustainability of Adriatic aquaculture. Within the project, new feeds were designed and tested at a laboratory scale on sub-adult European sea bass. For the present study, three of the five tested diet formulations were selected for a comprehensive exploration and comparative transcriptomics of European sea bass intestines after performed feeding trial.

Material and methods

Selected formulations included two control diets, one rich in fish-derived ingredients (CF) contained 85 % and 66 % of fish-derived protein and lipid, and one rich in plant-derived ingredients (CV) contained 85 % and 66 % of plant-based protein and lipids. Test diet (VH10P30) contained the same vegetable: fish lipid ratio as the CV diet, replacing crude proteins from the plant-based sources with 10 % of crude proteins from a commercial defatted *Hermetia illucens* pupae meal and 30 % of poultry by-product meal. The experimental diets were formulated to be iso-proteic (45%), iso-lipidic (20%), isoenergetic (20.3 MJ kg^{-1}) and to meet the dietary requirements of sub-adult European sea bass.

Based on quality, 24 samples of total RNA were selected for cDNA library preparation, comprising 4 biological replicates per feeding treatment of two selected intestinal parts, pyloric caeca, and distal intestine. One biological replicate corresponded to a single fish, meaning pyloric caeca and distal intestine were paired as subsamples. Single-end 3'UTR sequencing was performed using NextSeq 500 System (Illumina, San Diego, CA, USA).

Results and discussion

Two analyses were performed for differential expression analysis, comparing the VH10P30 test treatment to CF treatment as positive control, and comparing the VH10P30 test treatment to CV treatment as negative control. In total, 1,963 (915 up and 1048 down) differentially expressed (DE) genes were found in the distal intestine of European sea bass in the VH10P30 treatment vs. CF treatment. Using CV treatment as the negative control, there were 711 (439 up and 272 down) DE genes in the distal intestine of European sea bass in the VH10P30 treatment. In contrast to the distal intestine, no clear differences were detected between the diet treatments in the pyloric caeca of the experimentally fed European sea bass. Previously performed gene expression profiling revealed functional specialization along the intestinal tract of European sea bass. Molecular and cellular functions related to feed digestion and nutrient absorption and transport were over-represented in the anterior and middle part of the intestinal tract, while the initiation and establishment of immune defense mechanisms became especially relevant in the distal intestine (Calduch-Giner et al., 2016). Although this could offer an explanation why there were such pronounced differences in this study in the distal part of the intestine between treatments, and at the same time no differences detected in the pyloric caeca, it is also possible that 24 h starvation prior to fish sampling affected obtained results. While the digestion was still active in the distal intestine at the time of the sampling, the active part of the digestion in the pyloric caeca was probably finished. This is also supported by functional analyses of DE genes in this

(Continued on next page)

study, which revealed over-representation of gene ontology terms in signal transduction, transport and various metabolic processes (for example cellular nitrogen compound, small molecule and lipid metabolic process) in the distal intestine, to name a few, while the immune response was generally absent, for both comparisons (using CF and CV as control, respectively). Taking into account growth performance and overall fitness of the European sea bass in the VH10P30 treatment, *H. illucens* larval meal and poultry by-product meal demonstrated great potential as alternative protein sources for European sea bass aquaculture.

Acknowledgments

The authors are thankful to all members of the Laboratory of Aquaculture in Split for their help during the feeding trial and sampling. Financial support for this study has been provided by Interreg AdriAquaNet (Project ID 10045161) and Joint EuroMarine-EMBRC 2020 grant to I. Bušelić.

References

Beheshti Foroutani, M., Parrish, C.C., Wells, J., Taylor, R.G., Rise, M.L., Shahidi, F., 2018. Minimizing marine ingredients in diets of farmed Atlantic salmon (*Salmo salar*): Effects on growth performance and muscle lipid and fatty acid composition. PLoS One 13, 1–14.

Calduch-Giner, J.A., Sitjà-Bobadilla, A., Pérez-Sánchez, J., 2016. Gene expression profiling reveals functional specialization along the intestinal tract of a carnivorous teleostean fish (*Dicentrarchus labrax*). Front. Physiol. 7, 1–17.

Froehlich, H.E., Jacobsen, N.S., Essington, T.E., Clavelle, T., Halpern, B.S., 2018. Avoiding the ecological limits of forage fish for fed aquaculture. Nat. Sustain. 1, 298–303.

SYSTEM DYNAMICS APPLIED TO OYSTER FARMING TO ASSESS DEVELOPMENT SCENARIOS WITH STAKEHOLDERS IN THE CHARENTE COASTAL ZONE

Jean-Marie Lescot[1*], Benoit Othoniel[1], Jean Prou[2], Charlotte Rhone[3], Françoise Vernier[1]

[1]INRAE Environment, Territories and Infrastructure Research Unit ; 50, Avenue de Verdun BP 3; 33612 Cestas, France
E-mail: jean-marie.lescot@inrae.fr
[2] Conchy Consulting ; 72 rue docteur Emile Roux; 17420 St Palais, France
[3] Comité Régional de la Conchyliculture, 89 Quai du Ponant, 17000 La Rochelle, France

Introduction

The rationale of the EU Horizon 2020 funded COASTAL (Collaborative Land-Sea Integration Platform) project is to improve land-sea synergy. The project uses a participatory modelling approach divided into several steps: a multi actor analysis with mind mapping, system modelling with data collection, definition of scenarios and transition pathways, and finally business road mapping for policy solutions, focusing on marine spatial planning and environmental protection. The approach is applied to six different European case studies called Multi Actor Labs (MAL). The French MAL consists of the Charente river basin and adjoining Pertuis sea coastal zone in the South-West of France, where oyster farming plays an important role in the local economy.

Modeling the oyster farming system in System Dynamics

One objective of the approach is to foster dialogue among all stakeholders of the land- sea system, and use system dynamics modeling to simulate the evolution of the regional system and its components over time. System dynamics is a methodology and mathematical modeling technique to frame, understand, and discuss complex issues and problems using computer simulations. We used a visual programming language (Vensim PLE) that was particularly well suited to stakeholder interactions. It provides a graphical modeling interface with stock and flow and causal loop diagrams, on top of a text-based system of equations in a declarative programming language.

In the first round of sectoral workshops, stakeholders from the aquaculture sector identified system components and processes they believed played a role in the functioning of the aquaculture sector within the land-sea system, and gave perspectives of shellfish farming in synergy with other activities. Currently, more than 75% of the oysters sold under the regional label are grown in other regions or abroad during a part of their life, notably in the Northern Sea where water quality is the most suitable. While the capture of spats is currently at an acceptable level, it may be affected in the future if coastal water quality continues to worsen. Like all bivalve shellfish, oysters are highly sensitive to the quality of water in the marine environment.

In the Charente coastal zone and hinterland, there is growing concern about water supplies (increased domestic water uses caused by residential population and waves of tourists in summer, and water for irrigation). Over the past few years, water shortages in summer have become more and more frequent. Downstream shellfish production needs fresh water from streams - particularly the Charente River - to maintain water with a desirable level of salinity. Other concerns include reduced water quality in coastal zones caused by high concentrations of pesticides, originating mainly from agricultural sources upstream, and bacteriological contamination caused by seasonal overloading of waste water treatment plants. This pollution affects downstream oyster production with bans on sales, and has a direct impact on juvenile mortality.

The water quality factors considered in the oyster model are water salinity and concentration in trophic resources (phytoplankton) in coastal zones depending of the discharge of the Charente river. Other factors considered are the density of oyster production (number of oysters grown per bag) and the type of bags (on tables or floating) affecting the quantity of nourishment oysters have access and ingest over their lifetime.

(Continued on next page)

According to our stakeholders, there are two complementary techniques that can help to completely relocate oyster production: improving the quality of locally-grown oysters and maintaining current spat catch levels. On one hand, given the downstream location of oyster parks and spat catching sites, changes in upstream activities (more sustainable agriculture and better treatment of wastewater) could have a positive effect on water quality in the estuary and thus on oysters farming. On the other hand, technical solutions (such as rearing oysters in floating bags instead of on tables, or reducing the number of oysters in bags) could provide better-quality oysters. To assess scenarios, the oyster farming model simulates spat capture, oyster flesh content (depending on water quality), rearing techniques, and the proportion of production carried out locally.

As part of our integrated land-sea model, this allows us to assess how changes in farming practices and other upstream activities, along with market demand, may impact overall oyster production. Key indicators (model outputs) are tracked over time to respond to the needs of the shellfish farming sector: quality index (oyster flesh weight to total weight ratio), spat capture and purchase, weight of oysters produced, and gross margin. The objective of increasing the quality index responds to market demand, with higher sale prices. The quality of oysters depends predominantly on the quantity of trophic resources assimilated during their life time. To meet production targets, oyster farmers seek to collect as many spats as possible, because these spats can be labelled. In the model, the capture of spats depends on coastal salinity and concentration in trophic resources. In case of insufficient catches or mortality, spats can also be purchased in nurseries.

The proportion of local oysters represents the percentage of oysters produced only in the local area, without any transfer to other regions. With the objective to relocate oyster production, this share should be as high as possible. However, relocation will be possible only if yields can generate sufficient profits. Availability of labor is also considered in the shellfish model.

Given that most oyster parks are located downstream, most of the changes in activities upstream will have an effect on the estuary's environmental conditions, thus affecting oyster growth. In the overall land-sea model, oyster production is impacted by several decision variables of other submodels linked with the oyster submodel, such as changes in the cropping pattern determining the quantity of irrigation water use, population determining domestic water use, or performance of wastewater treatment facilities.

Overall, our model helps simulate the roadmap and scenarios designed by the actors, and results are further discussed with them to decide collectively on the course of action that will be preferable for all. First results of scenarios for shellfish sector with interactions with other sectors are presented.

Conclusion

Developed in estuaries and coastal zones, shellfish farming is dependent on the quality of marine coastal waters which in turn depend of the impact on these marine environments of other economic sectors on the coast and upstream. To achieve sustainable development in these areas while maintaining other economic activities, there needs to be a consensus between all stakeholders, underpinned by research and evidence-based solutions. The proposed approach and the model developed in system dynamics could be replicated in other places where aquaculture plays an important role in the local economy.

WHAT DRIVES THE UNCERTAINTY IN MAPPING OFFSHORE AQUACULTURE POTENTIAL?

Matt Lewis [1], Peter Robins [1] David Christie[1] Jonathan Demmer[1] and Simon Neill[1]

[1] School of Ocean Sciences, Bangor University, UK

m.j.lewis@bangor.ac.uk

Uncertainty of offshore aquaculture resilience to physical conditions is a barrier to mapping locations and assessing the economic potential of offshore aquaculture. Quantifying oceanographic conditions requires long time-series of high resolution data, yet coarse ocean models and simplified variables are used to map offshore aquaculture potential in a variety of methods. Here, we hypothesise uncertainties within oceanographic data is greater than uncertainty of resilience thresholds (e.g. sites of waves < 4 m and currents < 1 m/s); and we apply a sensitivity test to explore the impact on mapping sea-space and location. Using data from a range of ocean models, the impact of spatial and temporal resolution to mapping based on extreme event intensity is determined: 1km to 10km spatial resolution ROMS and AMM15 tidal models; and wave data from ERA5, at hourly to daily mean values, resolving annual and inter-annual variability. Furthermore, the persistence of an extreme event is also calculated (number of hours above resilience threshold), and spatial differences in the maps compared. Differences in the mapping (km^2) of potential offshore aquaculture sites, based on data not physical condition thresholds, was found to be larger than differences in sea-space when changing resilience thresholds by 50% - consistent with spatial variability at such location (e.g. <2m or <6m in Hs, versus <0.5m/s to <1.5m/s). We therefore find a need for a common framework for physical ocean condition assessment, which could then be used to inform future generations of resilience offshore aquaculture – as is done in other marine industries (such as oil and gas).

REPRODUCIBLE CHANGES IN THE INTESTINAL MICROBIOTA OF ATLANTIC SALMON FED DIETS CONTAINING BLACK SOLDIER FLY LARVAE MEAL

Yanxian Li[1*], Karina Gajardo[1], Alexander Jaramillo-Torres[1], Ikram Belghit[2], Erik-Jan Lock[2], Trond M. Kortner[1], Åshild Krogdahl[1]

[1]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, NO-1432 Ås, Norway
[2] Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway
Email: yanxianl@nmbu.no

Introduction

Limited availability of sustainable feed ingredients is a major obstacle in the continuous growth of salmon aquaculture. Being part of the natural diet of salmonids, insects are a good source of nutrients for salmon. Insects possess an outstanding capacity to upgrade low-quality organic materials, require minimal water and cultivable land, and emit little greenhouse gases (Van Huis, 2013). Among the insect species suitable as feed ingredients, black soldier fly (*Hermetia illucens*) has been produced at an industrial scale for its good nutritional value (Barroso et al., 2014). In recent years, extensive research efforts have been directed to investigate its potential as a sustainable feed ingredient for various fish species including Atlantic salmon (*Salmo salar*). However, less is known regarding its influence on the intestinal microbiota. Herein, we report the response of intestinal microbiota in pre-smolt Atlantic salmon fed an insect meal diet containing 60% black soldier fly larvae meal for 8 weeks.

Materials and methods

Atlantic salmon with a mean initial body weight of 49 g (1.5 g SEM) were randomly assigned into 8 fiberglass tanks (450 L, 100 fish per tank). Quadruplicate tanks of fish were fed either a commercially relevant reference diet (REF), or an insect meal diet (IM) wherein black soldier fly larvae meal accounted for 60% of total ingredients. At the end of the feeding trial, digesta and mucosa samples from different intestinal compartments (proximal and distal) were collected in addition to feed and water samples from both dietary groups. Samples were processed along with positive and negative controls. Microbial communities were profiled by sequencing V1-2 regions of the 16S rRNA gene.

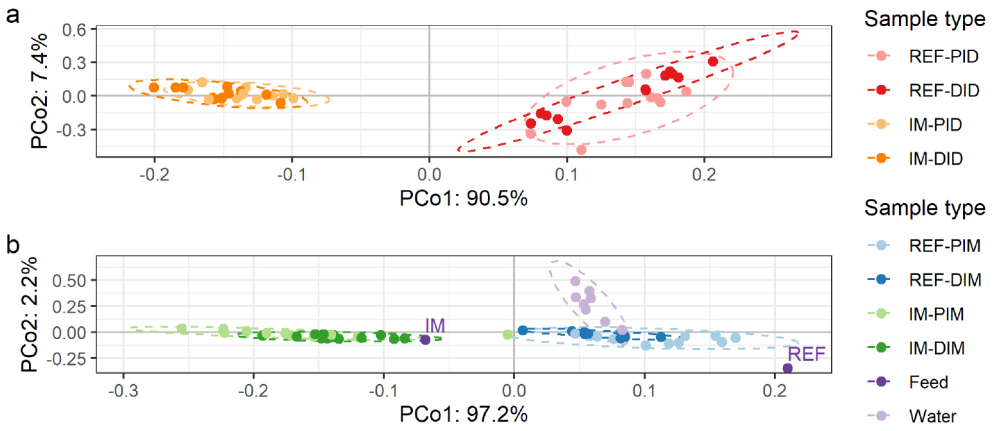


Fig.1. Beta-diversity visualized by robust Aitchison PCA. Upper plot (a), beta-diversity of digesta samples; lower plot (b), beta-diversity of mucosa, feed and water samples. Abbreviations: PCo, principal coordinate; REF, reference diet; IM, insect meal diet; PID, proximal intestine digesta; DID, distal intestine digesta; PIM, proximal intestine mucosa; DIM, distal intestine mucosa.

(Continued on next page)

Results

Overall, the microbial diversity was lower in the digesta of fish fed the insect meal diet but higher in the mucosa. Regardless of the intestinal compartment, the insect meal diet markedly modulated the intestinal microbiota composition in both digesta and mucosa (Fig.1). The diet effect on the mucosa microbiota, however, was stronger in the proximal intestine than the distal intestine. We also found that the feed-borne microbiota showed close resemblance to those observed in the intestine, whereas the water microbiota was distinct from the others. Irrespective of the intestinal compartment, bacterial taxa classified as *Actinomyces*, *Bacillaceae*, *Bacillus*, *Beutenbergiaceae*, *Brevibacterium*, *Corynebacterium 1*, *Enterococcus*, *Lactobacillales*, *Microbacterium*, *Oceanobacillus,* and *RsaHF231* were enriched in both intestinal digesta and mucosa of salmon fed the insect meal diet.

Discussion and conclusion

Our current study largely reproduced what we found in a previous experiment wherein post-smolt Atlantic salmon were fed an insect meal diet containing 15% black soldier fly larvae meal for 16 weeks (Li et al., 2021). Despite the huge difference in the inclusion level of black soldier fly larvae meal, marked changes in the digesta- and mucosa-associated intestinal microbiota were observed in both studies. In particular, insect meal diets seem to enrich specific microbial clades in the salmon intestine as outlined in the Results section. Notably, most of these taxa were also found in the feed pellets. As sequencing-based methods do not differentiate between living and dead cells, the viability of these bacterial taxa in the salmon intestine warrants further investigations. Potential implications of the changes in the intestinal microbiota for salmon physiology and health are being analyzed and will be discussed in the context of their associations with host response data that we reported elsewhere (Li et al., 2020).

Reference

Barroso, F. G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. Aquaculture, 422-423, 193-201. doi:10.1016/j.aquaculture.2013.12.024

Li, Y., Bruni, L., Jaramillo-Torres, A., Gajardo, K., Kortner, T. M., & Krogdahl, Å. (2021). Differential response of digesta- and mucosa-associated intestinal microbiota to dietary insect meal during the seawater phase of Atlantic salmon. Animal Microbiome, 3(1), 8. doi:10.1186/s42523-020-00071-3

Li, Y., Kortner, T. M., Chikwati, E. M., Belghit, I., Lock, E.-J., & Krogdahl, Å. (2020). Total replacement of fish meal with black soldier fly (*Hermetia illucens*) larvae meal does not compromise the gut health of Atlantic salmon (*Salmo salar*). Aquaculture, 520, 734967.

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. Annu Rev Entomol, 58, 563-583. doi:10.1146/annurev-ento-120811-153704

ONE GENERATION OF GENOMIC SELECTION IMPROVES WHITE SPOT SYNDROME VIRUS (WSSV) RESISTANCE IN *Litopenaeus vannamei* SHRIMP

M. Lillehammer[1*], R. Bangera[2], M. Salazar[3], S. Vela-Avitua[2], E. C. Erazo[3], A. Suarez, J. Cock[3], M. Rye[2], and N. Robinson[1]

[1] Nofima AS, Postboks 210, NO-1431 Ås, Norway
[2] Benchmark Genetics Norway AS, 6600 Sunndalsøra, Norway
[3] Benchmark Genetics Colombia, Bogota, Colombia
E-mail: marie.lillehammer@nofima.no

Introduction

White spot syndrome virus (WSSV) is among the most damaging diseases in global shrimp aquaculture regarding production and economic losses (Sánchez-Paz, 2010). Resistance to WSSV in *L. vannamei* has previously been reported to have low but significant heritability in adult shrimp (Gitterle et al., 2006; Caballero-Zamora et al., 2015) and moderate heritability in juveniles (Trang et al, 2019). Selective breeding is therefore one method that is being used to increase WSSV resistance (Cock et al., 2015). Genomic selection has been predicted to be a powerful and accurate way of selecting among non-infected candidates based on challenge test results from relatives and estimated genomic relationships.

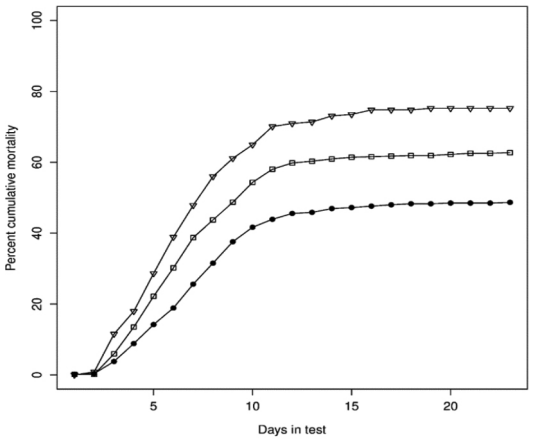
Materials and Methods

Two domesticated shrimp lines were used in this study, known to be resistant (R-line) or susceptible (S-line) for WSSV. Purebred S and R animals as well as crosses between them were produced. From this population 1151 individuals (597 males and 554 females) were used as selection candidates, while 1447 individuals were used as test animals. Selection candidates and test animals were genotyped for 18,643 SNPs to estimate a genomic relationship matrix. The juvenile stage test animals were exposed to a per os infection with WSSV infected tissue in artificial seawater at 30 ppt salinity and 26°C temperature. Genetic parameters and genomic breeding values (Meuwissen et al, 2001) were estimated for survival, and these breeding values were used to select the top 60 high ranking breeding value male and female and the bottom ranking 20 breeding value male and female candidates. These were used to produce 32 resistant and 8 susceptible full sib families. For comparison, 20 random families were produced from candidates not selected for any of these lists. The offspring (1885 individuals) were tagged by family and challenged with WSSV in a controlled test. Mortality of each family was recorded, and genetic parameters were re-estimated in the offspring generation from a sire-dam model, with and without the effect of selection group fitted. For full details on the experiment, see Lillehammer et al., (2020).

Results

Cumulative mortality at the end of the test was 75% in the susceptible, 63% in the random and 49% in the resistant group (Figure 1). The G1 random gEBV group final cumulative mortality during the test was the same as in the G0 training, and the difference between the selection groups was statistically significant. Estimated heritability of WSSV resistance in the offspring was 0.47±0.09, but reduced to 0.41±0.09 if selection groups was accounted for.

Figure 1: Cumulative mortality of high (closed circles), random (open squares) and low (open triangles) selection groups



(Continued on next page)

Discussion and Conclusion

The large, significant, difference in cumulative mortality between the selection groups demonstrate that genomic selection can be used to increase WSSV resistance. Significant and moderately high (>0.4) heritability of WSSV resistance in the offspring population suggest that repeated selection in future generations will lead to further genetic improvement.

To avoid exposing selection candidates for pathogens, family selection without individual selection is commonly used for disease traits. With genomic selection, individual selection is possible without exposing the breeding candidates to the virus. Thus, with genomic resources selection intensity and genetic gain increases. The SNP genotyping we used was sufficient to obtain genomic breeding values suitable for individual selection. This opens the way to genetic improvement of traits not measured on the breeding candidates.

References

Caballero-Zamora, A., Montaldo, H. H., Campos-Montes, G. R., Cienfuegos-Rivas, E. G., Martínez-Ortega, A., & Castillo-Juárez, H. (2015). Genetic parameters for body weight and survival in the Pacific White Shrimp Penaeus (Litopenaeus) vannamei affected by a White Spot Syndrome Virus (WSSV) natural outbreak. Aquaculture, 447, 102-107. doi:https://doi.org/10.1016/j.aquaculture.2014.08.028

Cock, J., Salazar, M., & Rye, M. (2015). Strategies for managing diseases in non-native shrimp populations. Reviews in Aquaculture, 9(3), 211-226. doi:10.1111/raq.12132

Gitterle, T., Ødegård, J., Gjerde, B., Rye, M., & Salte, R. (2006). Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in Penaeus (Litopenaeus) vannamei using different statistical models. Aquaculture, 251(2), 210-218. doi:https://doi.org/10.1016/j.aquaculture.2005.06.008

Lillehammer, M., Bangera, R., Salazar, M., Vela, S., Erazo, E.C., Suarez, A., Cock, J., Rye, M., & Robinson, N.A. Genomic selection for white spot syndrome virus resistance in whiteleg shrimp boosts survival under an experimental challenge test. Sci Rep. 2020 Nov 25;10(1):20571. doi: 10.1038/s41598-020-77580-3

Meuwissen, T., Hayes, B. & Goddard, M. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* **157**, 1819 - 1829 (2001).

Sánchez-Paz, A. (2010). White spot syndrome virus: an overview on an emergent concern. Vet. Res., 41(6), 43.

Trang, T. T., Hung, N. H., Ninh, N. H., Knibb, W., & Nguyen, N. H. (2019). Genetic Variation in Disease Resistance Against White Spot Syndrome Virus (WSSV) in Liptopenaeus vannamei. Frontiers in genetics, 10, 264-264. doi:10.3389/fgene.2019.00264

DIGITAL TWIN PROTOTYPES IN FLOW-THROUGH SYSTEMS FOR FINFISH

Adriano C. Lima[1*], Edouard Royer[1], Matteo Bolzonella[1] and Roberto Pastres[1,2]

[1] Università Ca' Foscari Venezia, Campus Scientifico, via Torino 155, 30172 Mestre, Venice (Italy)
[2] Bluefarm s.r.l., Centro Vega ed. Pegaso, via delle Industrie 15, 30175 Marghera, Venice (Italy)
Email: adriano.lima@unive.it

Introduction
The virtual, digital counterpart of a physical object referred as digital twin derives from the Internet of Things (IoT) and involves real time acquisition and processing of large data sets. A fully implemented system ultimately enables real-time and remote management, as well as the reproduction of real or forecasted scenarios. Despite such potential, the adoption of digital twin features by smaller enterprises, including by aquaculture SMEs, has been comparatively slow (Uhlemann et al., 2017).

Under the emerging framework of Precision Fish Farming, we set up digital twin prototypes for land-based farms of Rainbow trout (*Oncorhynchus mykiss*), European seabass (*Dicentrarchus labrax*) and Gilthead seabream (*Sparus aurata*), with the aim of supporting producers in optimizing feeding practices and oxygen supply with respect to 1) growth performances; 2) fish welfare, and 3) environmental loads. The digital twins were conceptualized targeting rearing cycles at Preore Farm (Trentino-Alto Adige, Italy), for trout, and Vigneto Farm (Tuscany, Italy), for seabass and seabream. The twins rely on integrated mathematical models which are fed with farm data sets and simulate several dynamic processes, allowing the estimation of key parameters such as feed digestibility, fish appetite, ammonia excretion rate, fish size distribution and dissolved oxygen consumption.

Methodology
For the trout cultivation system, the envisaged digital twin block is to be implemented to the liquid oxygen storage and supply system, whereas for the seabass and seabream plant, the focus is on optimizing feeding. These twin blocks will be interconnected to bioenergetic, population management and dissolved oxygen dynamic models, schematized in Figure 1.

The bioenergetic model (Brigolin et al., 2014) simulates the fish growth and metabolism. The forcing variables input to this model are water temperature, feeding rate, feed composition, initial average fish weight and population dynamics variables, which are associated with the population management model. In addition, husbandry parameters are input, including species-specific optimal and lethal extreme temperatures and energy consumed by respiration. The resulted calculated fish biomass is then input into a dissolved oxygen (DO) transport model (Royer et al., 2021; Lima et al., 2021), with the aim to assess the rapidly changing DO concentration in the cultivation ponds.

Results and discussion
The results demonstrated the potential of the models to provide the digital twins with short-term response capabilities, specifically intra-hour adjusted oxygen supply and daily adjusted feeding. The bioenergetic model provided robust estimates for the evolution of individual fish and total biomass weights, based on a single initial value for fish weight. With these values as inputs, the feeding table in the envisaged digital twin is automatically corrected on daily basis. At present, in contrast, these corrections tend to occur at longer time windows, and weight estimates are provided based on sampling. It is noticeable that the uncertainties in sampled fish weights may be significant, as indicated by the fact that farmers may back calculate fish weights once the biomass has been fully harvested.

The assessment of oxygen dynamics in the trout cultivation system considered scenarios based on current practices at the farm, fish welfare, the quality of the water discharged from the cultivation tanks and temperature changes. The results for DO concentration in a spatial-temporal frame obtained from the model indicate that the consumption of liquid oxygen can be reduced significantly with a real-time control.

(Continued on next page)

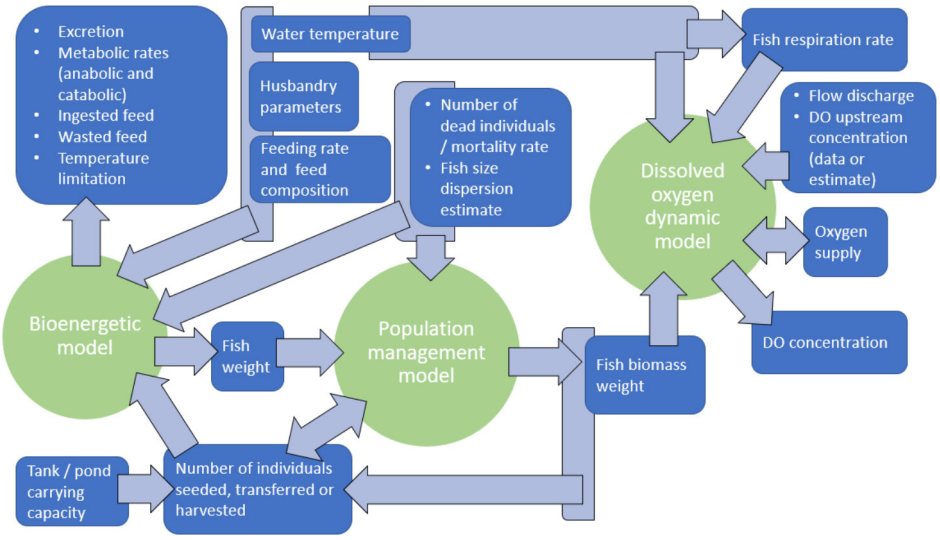


Figure 1. Scheme of the models incorporated into the digital twins.

Acknowledgements

The research leading to these results has received funding from the European Union's HORIZON 2020 Framework Programme under GRANT AGREEMENT NO. 773330 and 862658.

References

Brigolin, D., Meccia, V.L., Venier, C., Tomassetti, P., Porrello, S., Pastres, R., 2014. Modelling biogeochemical fluxes across a Mediterranean fish cage farm. Aquaculture Environment Interactions 5, 71-88.

Lima, A. C., Royer, E., Pastres, R., 2021. Dissolved oxygen consumption and intra-tank distribution of trout biomass. AE2020, European Aquaculture Society, 137-138.

Royer, E., Faccenda, F., Pastres, R., 2021. Estimating oxygen consumption of rainbow trout (Oncorhynchus mykiss) in a raceway: A Precision Fish Farming approach. Aquacultural Engineering, 92, 102141.

Uhlemann, T. H. J., Schock, C., Lehmann, C., Freiberger, S., Steinhilper, R., 2017. The digital twin: Demonstrating the potential of real time data acquisition in production systems. Procedia Manufacturing 9, 113–120.

APPLIED HOLOGENOMICS: LEVERAGING MICROBIOTA SERVICES THROUGH HOLO-OMIC ANALYSES IN FARMED FISH

Morten Limborg*

Center for Evolutionary Hologenomics, GLOBE institute, Faculty of Health and Medical Sciences
Email: morten.limborg@sund.ku.dk

Introduction

Emerging evidence across all areas of life has revealed the evolutionary importance of the intimate biological interactions between animals and their associated microbiota. Both the host genotype and the host microbiota have been shown to influence host phenotypes, such as growth and disease states. The hologenome concept maintains that the host genome and the host microbial metagenome are subject to essential biological interactions; thus, both should be considered simultaneously as a single interconnected 'holobiont system' when investigating how animals respond to e.g., diet and disease.

Methods

Based on challenges in aquaculture, we leverage current knowledge in molecular biology and host microbiota interactions to propose an applied holo-omic framework [1] that integrates molecular data including (meta)genomes, (meta)transcriptomes, epigenomes, and (meta)metabolomes for analysing fish and their associated gut microbiota as interconnected and coregulated holobiont systems (figure 1).

Results

I will present data and results from a suite of ongoing projects - including HoloFish [2] and HoloFood [3] - that all apply our holo-omic framework to understand the essential molecular interactions by which the gut microbiota shapes phenotypic traits in both Atlantic salmon and rainbow trout. In particular, we look at traits related to growth, novel feed additives, and response to a pathogenic bacterium.

Conclusions

I will discuss the feasibility and potential of using our holo-omic framework to combine large -omics data sets for more coherent analyses of host – microbiota systems to help steer a more sustainable growth of aquaculture.

References

1. Limborg MT, Alberdi, A, Kodama, M, Roggenbuck, M, Kristiansen, K, & Gilbert, MTP (2018) Applied hologenomics: feasibility and potential in aquaculture. *Trends in Biotechnology* **36**: 252-264
2. https://www.fhf.no/prosjekter/prosjektbasen/901436/
3. https://www.holofood.eu/

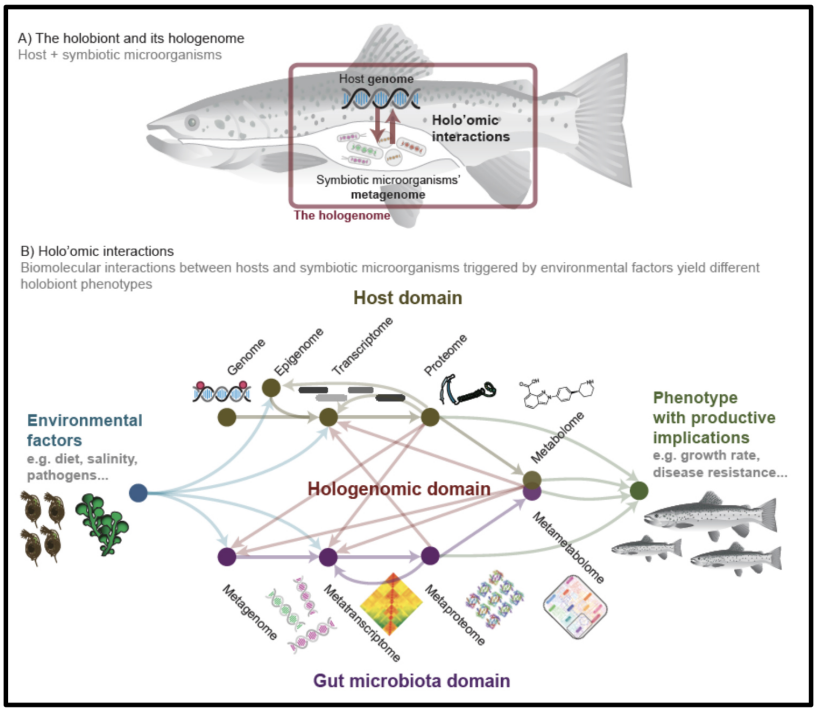


Fig. 1. Conceptual Framework of the Holobiont and the Hologenome (A), and the Holo-Omic Interactions Between the Host and Its Gut Microbiota (B).

EVALUATION OF DIETS USING INSECT FLOUR ON THE GROWTH OF GREEN FROG (*Phelophylax perezi*) TADPOLES

J. Lopes[1,2,*], C. Batista[1], V. Morais[1]

[1] Instituto Politécnico de Viana do Castelo, Escola Superior Agrária
[2] Centre for Research and Development in Agrifood Systems and Sustainability – CISAS
Email: juliocesar@esa.ipvc.pt

Introduction

With the growing need to reduce the use of fishmeal in aquaculture diets, it is important to evaluate the possibility of alternative sources that allow, in a sustainable way, to ensure the increase in aquaculture production (Barroso et al., 2014; FAO, 2020; Van Huis, 2020).
The production of amphibians, particularly in the case of the green frog - *Phelophylax perezi*, has been increasing in interest within Europe. However, there is no diet developed specifically for their food on the market, with producers using feed for carnivorous fish and, as such, highly dependent on fish meal (Finke, 2002; Makkar et al, 2014).

The larval stage is extremely important in the development of amphibians because the tadpoles, when in good nutritional status, produce more resistant imagoes, increasing the probability of a decrease in mortality in the following stages.

Although frog culture is well developed in several countries, little is known about the nutritional needs of amphibians, even more so in the case of green frogs.
The present work intended to start the process of understanding these needs.

Material and methods

From different spawns of *P. perezi*, 360 tadpoles were selected in phase 25 (Gosner, 1960) and divided into 12 groups of 30 tadpoles in 40 liter tanks under a water recirculation regime. Four diets were formulated corresponding to two levels of energy (5000 and 5500 kcal/kg) and two of protein (38 and 44% CP) using raw materials of vegetable origin and insect flour (*Tenebrio molitor*).

Diets were randomly assigned by three repetitions, being fed ad libitum and the animals weighed weekly for 9 weeks. The first two weeks were considered as weeks of adaptation to the controlled environment and experimental diets. Each group was monitored daily, with residues and food not consumed by siphoning and dead animals being removed.

The animals that complete the metamorphosis were transferred to the growth sector.

The comparison of the average daily gain between the experimental diets was carried out, as well as the mortality and metamorphosis rates.

Comparisons were performed using ANOVA and Tukey LSD tests from the SPSS 22 statistical package.

Results

Mortality and metamorphosis rates did not show significant differences between diets ($p>0.05$), varying between 26.05 ± 0.01 and 30.04 ± 0.17% for mortality and between 32.78 ± 0. 08 and 49.11 ± 0.09% for metamorphosis during the test weeks.
The average daily gain varied between 3.79 ± 0.77 and 8.92 ± 1.05 mg/day, with significant differences ($p<0.05$) between the diets being observed.

Conclusions

The observed results indicate a better performance with diets with lower energy value, however, it will be necessary to extend the comparisons to different protein:energy ratios in order to understand the real effect of this relationship.

(Continued on next page)

Table 1. Composition of experimental diets

Diets	Crude Protein (%)	Crude Energy (kcal/kg)
A	44	5500
B	44	5000
C	38	5000
D	38	5500

Table 2. Average daily gain, Mortality rate and Metamorphosis rate.

Diet	ADG (mg/dia)	Mortality (%)	Metamorphosis (%)
A	$6,32^{b} \pm 0,66$	30,04 ± 17,29	39,62 ± 1,89
B	$7,13^{bc} \pm 1,12$	28,65 ± 14,10	49,11 ± 8,83
C	$8,92^{c} \pm 1,05$	26,06 ± 15,00	42,00 ± 12,78
D	$3,79^{a} \pm 0,77$	28,89 ± 7,62	32,78 ± 8,37

Funding

Project: Technical model of intensive frog production - *Rana perezi* (*Phelophylax perezi*) - Funded by the Operational Program MAR2020 - European Fund for Maritime Affairs and Fisheries.

References

Barroso, F.G., Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sáncez, A., Pérez-Bañón, C.,2014. The potential of various insect species for use as food for fish. Aquaculture, 422–423, 193–201.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Feedipedia - Animal Feed Resources Information System, 2012-2020. INRAE CIRAD AFZ and FAO.

Finke, M. D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21, 269-285.

Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol., 197,1-33.

Van Huis, A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. Journal of Insects as Food and Feed; 6(1): 27-44.

LET CONSUMERS DESIGN THE PACKAGING FOR FISH PRODUCTS

L. López-Mas*, A. Claret, A. Bermúdez, M. Llauger, & L. Guerrero

Institute of Agrifood Research and Technology (IRTA), Monells (Spain)
*E-mail: laura.lopezm@irta.es

Introduction

Food packaging is no longer a mere structural element but also a powerful marketing tool able to affect product perception, purchase decision, and consumers' food choices (Ares and Deliza, 2010). Packaging can be the most direct and influential communication element at the point of purchase (van Rompay and Veltkamp, 2014), where most purchasing decisions are made (76%) (POPAI, 2014). Thus, incorporating consumers' opinions during food packaging design (co-creation) could enhance its potential success in the market (Moon et al., 2018).

Packaging attributes could be divided into two groups, (A) visual, which draw attention and transmit non-verbal information (e.g., colour); and (B) textual, which transmit verbal or numerical information (e.g., claims).

This study aimed to involve consumers in the design of a fish product packaging in order to know the combination of visual and textual attributes that best fit into their preferences and expectations.

Materials and methods

An online survey with 200 Spanish participants was conducted to let consumers choose which packaging attributes (visual and textual characteristics) preferred for a 'meagre burger with mushrooms (black trumpet)', an aquaculture fish product developed within the MedAID project (European Commission, Horizon 2020, No. 727315).

Visual attributes of the packaging included the type of container (bowl, bag, tray, box), colour (61 options), window presence and type (16 options), image presence and type (six options), typeface (15 options), package presentation (individual, per serving, without divisions), and product quantity (one, two, four, or more than four servings). Textual attributes were divided into three groups according to the three dimensions of the quality (searched, experienced, and credential). Three conjoint analysis were performed, one per each quality dimension, and four factors were included in each one: (1) convenience, price, products' presentation (e.g., individually wrapped), and recyclability (searched quality), (2) freshness, texture, flavour, and novelty (experienced quality), and (3) health, natural, animal welfare, and sustainability (credential quality).

Table 1. Most frequently selected options of visual attributes (%).

Attribute	%	Selection	Attribute	%	Selection
Container	66.5	Tray	**Typeface**	17.0	Arial Rounded MT Bold
	20.5	Box		11.5	Rage Italic
Colour	12.5	White	**Presentation**	43.0	Packed per serving
	10.0	Light blue		36.5	Individually packed
Window	29.5	Full	**Quantity**	45.0	2 servings
	12.0	Large left side		38.0	4 servings
Image	48.0	Dish ready-to-eat			
	35.5	Ingredients			

Table 2. Mean importance of the textual attributes grouped by quality dimension (%).

Searched		Experienced		Credential	
Factor	**Importance (%)**	**Factor**	**Importance (%)**	**Factor**	**Importance (%)**
Convenience	18.9^{b}	**Freshness**	29.8^{a}	**Health**	25.1^{ab}
Price	29.3^{a}	**Texture**	19.3^{c}	**Natural**	20.7^{b}
Presentation	22.5^{b}	**Flavour**	23.5^{bc}	**Animal welfare**	31.1^{a}
Recyclability	29.4^{a}	**Novelty**	27.4^{ab}	**Sustainability**	23.1^{b}

Superscript a–c: different letters in the same column differ significantly ($p < 0.05$).

(Continued on next page)

Results

The visual attributes of the packaging preferred by consumers for a meagre burger are presented in Table 1. The tray was the most widely selected option, maybe because it is the most common container for burgers (both meat and fish) in Spanish supermarkets. Colours white and light blue were alike in preference, as they may evoke the sea. Participants preferred to see the raw product, through a wide window on the package. Besides, they chose to have a picture of the burgers ready-to-eat or the raw ingredients (e.g., fish, black trumpet) printed in the package. Typefaces most selected were 'Arial Rounded' and 'Rage Italic'. Regarding the presentation, packaged per serving (2 burgers per serving) was the most chosen option, followed by individually packaged burgers. Lastly, two and four servings were picked out a higher number of times, probably due to that 64% of participants had between three and four people living in their household.

The textual attributes preferred by participants are presented in Table 2. 'Recyclability' and 'Price' played the most relevant role when it came to the searched quality. Therefore, it could be hypothesised that claims related to the packaging' disposal and material (i.e., 80% recyclable packaging) and price (i.e., now 5% cheaper) would be positively valued by consumers. On the other hand, 'Freshness' and 'Novelty' were the most important factors for experienced quality. Freshness is regarded as a critical aspect of fish quality, as it is a highly perishable product. Claims like 'New product', also attracted consumers' attention. Finally, 'Animal welfare' and 'Health' stood out for credence quality. Animal welfare and ethical issues have been gaining special attention over the last decades, which explains why consumers are demanding welfare standards for the fish they consume. Health, instead, has been for a long time a consumption driver of fish.

Conclusions

Consumers have clear preferences for specific package designs, both visual and textual. Thus, involving consumers in the final design of a product might improve its potential success in the market.

References

Ares, G., Deliza, R., 2010. Studying the influence of package shape and colour on consumer expectations of milk desserts using word association and conjoint analysis. Food Qual. Prefer. 21, 930–937.

Moon, H., Johnson, J.L., Mariadoss, B.J., Cullen, J.B., 2018. Supplier and customer involvement in New Product Development stages: implications for new product innovation outcomes. Int. J. Innov. Technol. Manag. 15, 1850004.

POPAI, 2014. Mass merchant shopper engagement study. Chicago (U.S.).

van Rompay, T.J.L., Veltkamp, M., 2014. Product packaging metaphors: effects of ambiguity and explanatory information on consumer appreciation and brand perception. Psychol. Mark. 31, 404–415.

CONSUMERS' PERCEPTION OF NEW AQUACULTURE TECHNOLOGIES

L. López-Mas[1,2,*], A. Claret[1], G. Arvisenet[3], R. Romero del Castillo[2], Z. Kallas[2], M. Zuccaro[4], X. Durany[1], and L. Guerrero[1]

[1]Institute of Agrifood Research and Technology (IRTA), Monells (Spain)
[2]Department of Agri-Food Engineering and Biotechnology (DEAB), Universitat Politècnica de Catalunya (UPC), Castelldefels (Spain)
[3]Centre des Sciences du Goût et de l'Alimentation (CSGA), Université Bourgogne Franche-Comté (UBFC), Dijon (France)
[4]International Center for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Bari (CIHEAM Bari), Valenzano (Italy)
*E-mail: laura.lopezm@irta.es

Introduction
In recent years, several aquaculture technologies have been implemented by some producers. Considering that consumers tend to be concerned about the use of new technologies in food production (Yeung and Morris, 2001), it is of great interest to know how they perceive them. Understanding consumers' perceptions, feelings, and opinions is a key element in designing communication and marketing campaigns and in providing guidelines for producers and policymakers, with the ultimate goal of improving the overall perception of aquaculture and aligning it with the green deal strategy.

This study aimed to understand European consumers' perception of new production systems and new managing technologies applied in the aquaculture industry.

Materials and methods
One focus group was carried out in each of the three EU countries under study (France, Italy, Spain). Between seven and eight participants took part in each focus group. Participants met the criteria of being 50% women, older than 18 years old, responsible for food purchase and preparation within their household, and fish consumers (≥ once a week).

During focus group discussion, consumers were asked about their perceptions of different aquaculture production systems (Aquaponics, Recirculating Aquaculture Systems (RAS), Biofloc Technology (BFT)) and new aquaculture managing technologies (Artificial Intelligence (AI), Electrochemical biosensors, Prediction models), all of them explored within the NewTechAqua project (European Commission, Horizon 2020, No. 862658). Sessions were audio/video recorded for further analysis. A subsequent content analysis procedure was carried out to synthesize the main insight that came out during sessions.

Results
A summary table of consumers' perceptions about different aquaculture production systems and managing technologies is shown in Table 1. As a general trend and, as expected, there is a widespread lack of knowledge of all the technologies presented.

Aquaponics was perceived rather positively. Some participants related it with the concept of 'hydroponics' and other plant growing techniques. Natural, sustainable, and environmentally friendly also came out, in addition, avoiding wastes and clean were mentioned. RAS perception was twofold. Participants rejected if it means dirty and not renewed water and appreciated if it means water filtration, recycle water, sustainability, ecological, *etc*. Biofloc technology was perceived unanimously as positive. The main reason is its name, as the prefix 'bio' generates good feelings among participants.

AI was probably the most known technology, and its perception was generally positive. Future, automation, and control were the most cited terms. AI suggested better management, keep standards, mass production, and clean the sea. However, in France, some level of distrust came out due to the relative novelty of this technology. Electrochemical biosensors were mainly associated with control (i.e., fish, water, production). Most participants showed a lack of knowledge about this technology. Finally, Prediction models were perceived rather positively. Control was frequently mentioned (i.e., production, quality, economic), as well as better management and yields. Overconsumption and sustainability aspects also came out.

Conclusions
Understanding consumers' perception, feeling, and opinions of new aquaculture production systems and new aquaculture managing technologies should be the basis for future communication and marketing campaigns in order to promote their general acceptance, especially in those aspects identified as more controversial. Consumers' knowledge and understanding of the new aquaculture production systems and managing technologies seems to be the key element in guaranteeing their acceptance.

(Continued on next page)

Table 1. Comparative table between countries of the main consumers' perceptions about different aquaculture production systems and managing technologies.

	France	Italy	Spain
Technology	**Associated with…**	**Associated with…**	**Associated with…**
New aquaculture production systems			
Aquaponics	• Natural • Sustainable • Use of natural resources • Closed-circuit • Eco-responsible • Avoid waste	• Hydroponics • Natural • Clean • Environmentally friendly	• Hydroponics • Plantation technique • Algae • Greenhouses • Irrigation • Growth control
Recirculating Aquaculture Systems	• Ecological • Recycle water	-	• Dirty water • Not renewed water • Water filtration • Water reuse • Flowing water • Sustainability
Biofloc technology	• Positive connotation (name, prefix 'bio-')	• Positive connotation (name)	-
New aquaculture managing technologies			
Artificial Intelligence	• Automation • Fear • New and unknown • Control	• Future • Smart • Progress • Safer • Keep standards • Better management	• Future • Automation • Mass production • Computer control • Robotics • Industrial • Cleaning the sea
Electrochemical biosensors	• Fish control • Water control (certain compounds)	-	• Fish control (quality of life, diseases) • Production control (yield, exploitation) • Growing sensors • Higher breeding quality
Prediction models	• Production control • Better management • Higher yields (when demand is high) • Sustainable • Overconsumption	• Useful • Fundamental • Necessary • Development of an innovative method	• Production control • Better management • Higher yields • Fish feeding and health • Quality control • Economics • Sales prediction

References

Yeung, R.M.W., Morris, J., 2001. Food safety risk: consumer perception and purchase behaviour. Br. Food J. 103, 170–186.

TEMPORAL CHANGES IN SKIN AND GILL MICROBIOMES ARE IMPACTED BY DEVIATIONS IN THE MICROBIAL COMMUNITY OF REARING WATER IN A RECIRCULATING AQUACULTURE SYSTEM

M. Lorgen-Ritchie[1]*, L. Chalmers[2], M. Clarkson[2], J. Taylor[2], H. Migaud[2] and S.A.M. Martin[1]

[1]School of Biological Sciences, University of Aberdeen, AB242TZ, Aberdeen, UK
[2]Institute of Aquaculture, University of Stirling, Stirling, UK
Email: marlene.lorgen@abdn.ac.uk

Introduction
Atlantic salmon (*Salmo salar*) aquaculture continues to grow and intensify in line with a continually expanding world population. Such demand for sustainable protein sources has driven the expansion of land-based production in recirculating aquaculture systems (RAS). Microbes associated with fish mucosal surfaces are an important component of health and immunity and have become of increasing interest in recent year, particularly in RAS where microbes are also a key component in maintaining high water quality. External mucosal surfaces, skin and gill, are the first lines of defence against opportunistic pathogens as they are in constant contact with surrounding water, but the stability of mucosal microbiomes over time in RAS is unknown.

Materials and methods
We analysed the temporal dynamics of the microbial communities associated with skin and gill mucus in Atlantic salmon during smoltification in a commercial RAS facility and following transfer to a commercial sea site. Mucus swabs were taken from 6 fish from triplicate tanks at 4 timepoints in freshwater (FW1-4) and at 1- and 4-weeks post-seawater transfer (SW1/SW2). Sequencing of the V3/V4 variable region of the bacterial 16S gene was carried out on the Illumina MiSeq platform. DADA2 (Callahan, McMurdie et al., 2016) was used to determine microbial composition in skin and gill mucus, water and diet samples at the level of amplicon sequence variants (ASVs). Functionality was inferred using Piphillin (Iwai, Weinmaier et al., 2016).

Results
Microbial diversity and richness were temporally dynamic in both skin and gill mucus ($p<0.001$) with a distinct and significant drop at FW2 followed by a rising trend to FW4 and stability post-SWT. The drop in diversity in FW was the result of a surge in relative abundance of two taxa belonging to the genus *Hydrogenophaga*. These dynamics were mirrored in tank water samples. Beta diversity revealed more separation between sampling points than between tissues at a single sampling point.

The numbers of identified core microbial taxa (present in >90% of samples) generally increased over time in both skin and gill mucus, and overlap was observed between the two. However, coincident with the surge in *Hydrogenophaga* at FW2, few core taxa were identified and all of these cores were also identified in tank water.

Functional inference identified metabolic pathways associated with microbial communities. Coincident with the surge in *Hydrogenophaga* at FW2, 'Xenobiotics biodegradation and metabolism' increased in contribution at the detriment of 'Carbohydrate metabolism' in both skin and gill mucus.

Discussion and conclusions
Microbial diversity in skin and gill mucus were temporally dynamic in fish reared in a FW RAS during the parr – smolt transformation. A distinct drop in diversity was identified at FW2, coincident with a spike in the relative abundance of two taxa belonging to the genus *Hydrogenophaga* and suggested the occurrence of a dysbiotic event.

Hydrogenophaga is an autotrophic de-nitrifier associated with RAS biofilters (Rurangwa, Verdegem 2015). Previous work in RAS has shown that biofilter-associated taxa are not restricted to the biofilter and have the potential to colonise fish mucus (Schmidt et al., 2016, Minich et al., 2020). The *Hydrogenophaga* spike was associated with 'Xenobiotics biodegradation and metabolism', suggesting a role for this genus in metabolism and bioremediation of potentially harmful inorganic compounds in RAS tank water and mucosal surfaces.

(Continued on next page)

Despite disruption of the mucosal microbiota, no adverse impacts on health or growth were observed, indicating functional redundancy and potentially protective functions of individual genera. This study highlights the importance of considering temporal dynamics when interpreting results of microbiome studies and inferring wider significance.

This work was funded by BBSRC grant RobustSmolt (BB/S004270/1). The authors would like to thank John Richmond, MOWI and ARCH-UK for their contributions to this work.

References

CALLAHAN, B.J., MCMURDIE, P.J., ROSEN, M.J., HAN, A.W., JOHNSON, A.J.A. and HOLMES, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods,* **13**(7), pp. 581-583.

IWAI, S., WEINMAIER, T., SCHMIDT, B.L., ALBERTSON, D.G., POLOSO, N.J., DABBAGH, K. and DESANTIS, T.Z., 2016. Piphillin: Improved Prediction of Metagenomic Content by Direct Inference from Human Microbiomes. *PLOS ONE,* **11**(11), pp. e0166104.

MINICH, J.J., POORE, G.D., JANTAWONGSRI, K., JOHNSTON, C., BOWIE, K., BOWMAN, J., KNIGHT, R., NOWAK, B. and ALLEN, E.E., 2020. Microbial Ecology of Atlantic Salmon (*Salmo salar)* Hatcheries: Impacts of the Built Environment on Fish Mucosal Microbiota. *Applied and Environmental Microbiology,* **86**(12), pp. e00411-20.

RURANGWA, E. and VERDEGEM, M.C.J., 2015. Microorganisms in recirculating aquaculture systems and their management. *Reviews in Aquaculture,* **7**(2), pp. 117-130.

SCHMIDT, V., AMARAL-ZETTLER, L., DAVIDSON, J., SUMMERFELT, S. and GOOD, C., 2016. Influence of Fishmeal-Free Diets on Microbial Communities in Atlantic Salmon (Salmo salar) Recirculation Aquaculture Systems. *Applied and Environmental Microbiology,* **82**(15), pp. 4470.

IMPACT OF MICROALGAE NUTRITIONAL VALUE ON THE DEVELOPMENT, CONDITION, AND FATTY ACIDS PROFILE OF *Paracentrotus lividus* (LAMARCK, 1816) ECHINOPLUTEUS

A. S. Gomes[1], S. Lourenço[1,2*], P. M. Santos[1], M. Neves[1,2], P. Adão[1], C. Tecelão[1,2], A. Pombo[1, 2]

[1]MARE-Marine and Environmental Sciences Centre, Polytechnic of Leiria.
[2]MARE-Marine and Environmental Sciences Centre, ESTM, Polytechnic of Leiria
*Email: slourenco2@gmail.com

Introduction

The high mortality rates occurring during larval development, together with the high running costs of larval rearing systems and juvenile production, remain the major bottlenecks to the full cycle production of sea urchin (Carboni et al., 2012). For this reason, it is necessary to improve experimental protocols for larval production. The provision of optimal feeds is a key factor for successful larval culture. The nutritional value of a diet influences directly the larval growth, condition, and survival. Some nutritional characteristics, such as high carotenoids and lipids levels, are already known to improve larval development. The present study aimed to evaluate the effects of diet nutritional value (lipid and carotenoid content) on larvae of *Paracentrotus lividus* (Lamarck, 1816) by comparing several morphometric indicators of larval growth and condition between dietary treatments and by analysing the reciprocal correlation between the microalgae and larvae Fatty Acids (FA).

Material and Methods

The larval rearing experiment was carried out in triplicate tanks, at a density of 6 ind.mL^{-1}, using nine 50 L cylindroconical tanks in a closed system and organized in a Latin square design. During the experiment, larvae were maintained in aerated static seawater (35 ppm), with an average temperature of 19 °C and continuous fluorescent light (11.14 µE.m^{-2}.s^{-1}). The larvae were fed with three microalgae species, *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna) as monospecific diets. The feeding doses were standardized to cell volume supplying equal bio-volume of microalgae and adapted to larval stages as follows: for Rho diet, the larvae with 4, 6, and 8-arms were fed with 3600, 7200 and 14400 cells.mL^{-1}.day^{-1} respectively; for Chae and Duna diet, the 4, 6, and 8-arms larvae were fed with 7200, 14400 and 28800 cells.mL^{-1}.day^{-}1 respectively. Larval body length (BL), body width (BW), post-oral arm length (POAL) and stomach length (SL) were measured to characterize growth and morphology of larvae fed with the different diets. Larval survival rate (%) was assessed volumetrically every two days and age-at-competence was defined as the number of days post-fertilization (DPF) required for at least 75% of the larvae attain competence for settlement. The larval condition was assessed empirically by analysing the deviation of the larvae size from the expected tendency obtained by fitting linear models BW~BL (model A), SL~BL (model B) and POAL~SL (model C). The FA composition was determined for microalgal diets and larvae by gas chromatography. Total lipids and carotenoid pigments were quantified only for microalgae diets, by spectrophotometry and by High-Performance Liquid Chromatography, respectively.

Results

The larvae attained the competence for settlement at 18 DPF in all dietary treatments. At this stage, the larval survival rate was 2.03 ± 3.29 % for Rho, 4.88 ± 6.29 % for Chae and 1.67 ± 0.60 % for Duna treatments, without significant differences between diets (H_{KW} = 7.44, df=2, P = 0.189). Larvae fed with Chae showed the shortest BL and BW at 12 DPF (BL: 242.76 ± 15.76 µm; BW:182.09 ± 13.21 µm) and 16 DPF (BL: 286.51 ± 57.78 µm; BW:253.05 ± 38.47 µm). And at 18 DPF, these larvae showed the largest stomach (SL: 147.54 ± 20.59 µm) and the shortest POAL (319.69 ± 39.80 µm). The analysis of the residuals of condition model A showed that the larvae fed with the Chae presented the smallest BW, while model B showed that the larvae fed with Duna presented in average the smallest stomachs and model C showed that the larvae fed with Duna presented the longest post oral arms. The microalgae Rho and Duna presented the highest lipidic content. Particularly, Rho showed the highest content in PUFAs (65.34 %), the highest content in docosahexaenoic acid (DHA) and the highest content in erucic acid (C22:1n-9). Reciprocally, larvae fed with this diet also showed a high content of C22:1n-9 and DHA. On the other hand, Chae presented the highest content in arachidonic (ARA) and eicosapentaenoic (EPA), but low content in linoleic acid (LA) and α-linolenic acid (ALA). The larvae fed with this microalga were characterized by a relatively high content of total PUFA (46.06 %), total MUFAs (25.62 %) and EPA/ARA ratio (5.37), but low DHA/EPA ratio (0.11). The microalgae Duna presented the highest content of ALA, LA and total n - 6 PUFA (14.03 %) and the lowest content of n-3/n-6 (2.72). The larvae fed with Duna showed high content of ARA, ALA and LA. Although Duna

(Continued on next page)

did not show any content in EPA, a relatively content of EPA was found when larvae was fed with this diet. Regarding the carotenoid content, Chae presented the highest ß-carotene content (122.51 ± 2.93 $\mu g.mg^{-1}$) and is the only one that presents fucoxanthin (36.55 ± 4.08 $\mu g.mg^{-1}$) of the three microalgae cultures.

Discussion

Age of competence of *P. lividus* larvae was independent of the microalgae nutritional value, and their relatively low survival rate of advanced stages (2-5 %) was apparently related with the high stocking density. Several studies indicate that larvae raised in lower densities show higher survival rates at competence (Suckling et al. 2018)"ISSN":"0044848 6","abstract":"In this study, we present the results of two experiments; in the first one we evaluated the effects of four larval dietary treatments on the survival and growth of the sea urchin Paracentrotus lividus, larvae and post-larvae. In the second experiment we have measured the effects of two different settlement substrates, combined with the presence of conspecifics, on metamorphosis, survival and growth of post-larvae. The microalgae dietary treatments consisted in: Dunaliella tertiolecta (Duna. The larvae FA profile reflected the assimilation of the microalgal diets provided. The increase of EPA and ARA levels in larvae fed with Duna diet, suggests active biosynthesis of EPA and ARA from LA and ALA through the "Δ8 pathway" (Kabeya et al., 2017). The data suggested that high carotenoids content, specific fatty acids, low DHA/EPA ratio and high EPA/ARA ratio and n-3/n-6 ratio present in *C. calcitrans* enhanced larval growth (largest stomach and shortest POAL) and condition. Further research should look to a larger number of phytoplankton species to determine what nutritional characteristics should be supplied to enhance larval development and condition.

Acknowledgments

This study had the financial support of Operational Programme MAR2020 through the project Ouriceira AQUA (16-02-01-FMP-0004) and FCT, through the strategic project UIDB/ 04292/2020 granted to MARE. AP was supported through the Scientific Employment Stimulus Programmes (CEECIND/00095/2017 and CEECINST/00051/2018).

References

Carboni, S., Vignier, J., Chiantore, M., Tocher, D.R., Migaud, H., 2012. Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. Aquaculture 324–325, 250–258.

Kabeya N., Sanz-Jorquera A., Carboni S., Davie A., Oboh A., Monroig, O., 2017. Biosynthesis of polyunsaturated fatty acids in sea urchins: Molecular and functional characterisation of three fatty acyl desaturases from *Paracentrotus lividus* (Lamark 1816). PLoS ONE 12: e0169374.

Suckling, C.C., Terrey, D., Davies, A.J., 2018. Optimising stocking density for the commercial cultivation of sea urchin larvae. Aquaculture 488, 96–104.

EFFECT OF THE INTERACTION BETWEEN DIET AND HEAT CHALLENGE ON THE IMMUNE RESPONSE OF THE SEA URCHIN *Paracentrotus lividus*

C. Lapa[1], S. Lourenço[1,2*], R. Passos[2], A. Pombo[1,2] and T. Baptista[1,2]

[1]MARE –Marine and Environmental Sciences Centre, ESTM, Polytechnic of Leiria
[2]MARE-Marine and Environmental Sciences Centre, Polytechnic of Leiria
*E-mail: silvia.lourenco@ipleiria.pt

Introduction

The sea urchins' welfare is highly affected by changes in water quality. For this reason, it is crucial to understand their tolerance limits in closed and semi-closed aquaculture systems, where the levels of harmful or toxic substances can be higher that normally occur in natural environments (Albrizio *et al.,* 2019). The harmful substances can affect the immune system of sea urchins, particularly the activity of their immune cells, the coelomocytes. The coelomocytes are responsible for cellular responses to external agents and produce a wide variety of humoral factors that are important defences against pathogens (Fernandez-Boo *et al.,* 2018). The main objective of this study was to evaluate the effect of two diets (a macroalgae diet and a dry diet) in the immune response of sea urchins subject to acute exposure to high temperatures.

Materials and Methods

Adult sea urchins (N = 228) with 40 mm test diameter were maintained in two RAS with a density of 0.38 ind./L^{-1} and fed three times per week for 3 months with two diets: a macroalgae (*Ulva rigida*) diet and dry diet formulated with vegetable-based ingredients. During the nutritional trial, the sea urchins were subject to three acute exposure experiments (AEE), at the beginning, mid and at the end of trial. In each AEE, a randomly selected group of urchins of each diet were exposed to high temperature (24ºC) for 24 hours and compared to a control group under ambient temperature. After exposure, the righting response (behavioural test measuring the time the urchin takes to recover its aboral position) of all urchins (exposed and control group) was evaluated. Then, the urchins were measured (test diameter), individually and their gonads weighed, and maturity level assessed. For all urchins (exposed and control group), it was collected a sample of coelomic fluid to determined cell immunity parameters and measure the humoral response parameters. For cell immunity analysis, the different immune cells were identified: phagocytes (pha); colorless granulocytes (cg); red granulocytes (rg) and vibratile cells (vib) and counted using a Neubauer chamber under 400x magnification microscope. For humoral response analysis: lysozyme concentration, protease activity and nitric oxide concentration was quantified by turbidimetric test, azocasein hydrolysis assay and Griess reaction, respectively, following Fernandez-Boo *et al.,* (2018).

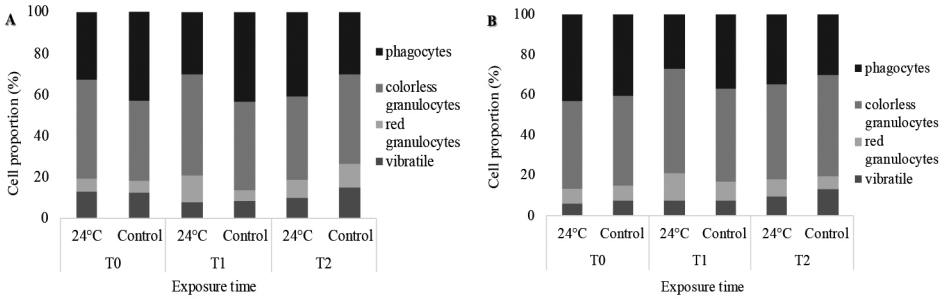


Figure 1- Percentage of different immune cells in the coelomic fluid of *Paracentrotus lividus*, after the three acute exposure experiments: T0- initial experiment, T1– mid trial experiment and T2– final experiment, A- macroalgae diet (*Ulva rigida*) and B- dry diet.

(Continued on next page)

Results

Both diets promoted identical level of gonad maturity, while in the end of the study the dry diet promoted higher GI (12.50 %) than the macroalgae (3.53 %). After the AEE to high temperature, the righting response varied between 01:01 to 03:13 minutes. The sea urchins fed with the macroalgae and exposed to 24°C showed longer righting behaviour time, when compared to the control group. In all the AEE, the most abundant immune cells were the cg (52%) and pha (31%), followed by rg (12%) and vib cells (2%). The macroalgae promoted higher percentage cg and rg and lower percentage of pha and vib whereas the dry diet promoted higher percentage of pha and rg and lower cg and vib when compared to the control group. For the humoral response parameters, lysozyme concentration (0.53 to 2.18 μg/mL) was higher in the sea urchins fed with the dry diet, when compared to the control group. The protease concentration (12 to 18%) was lower in both diets when compared to the control group and nitric oxide production (0.14 to 2.59 μM/mL) only occurred in the dry diet.

Discussion and Conclusion

The coelomic fluid of the sea urchins fed with dry diet and exposed to 24°C presented higher proportion of phagocytes and granulocytes (cg and rg) than the control groups. These results suggest that dry diet can promote a quicker activation of cellular immunity. On the other hand, the rg were the only cells that showed an increased production in both diets. Branco *et al*., (2013) suggests that this cell type plays an important role in the innate immune response, on different types of stress, including lesions on the skeleton and dermis and environmental contamination. Additionally, the increasing lysozyme concentration and the production of nitric oxide in the urchins fed with dry diet also support the conclusion that dry diet can promote sea urchins' innate immunity. Additionally, higher protease activity was observed in sea urchins fed with the dry diet. This result is supported by the study conducted by Johnston & Freeman (2005), which observed an increase of protease activity in the crab *Leptograpsus variegatus* fed with a mixed diet (plant and animal material). The information about sea urchins' immune response to acute changes in the environmental conditions is still scarce and the future research should be carried out to assess the immune response and the physical behavior of *P. lividus* exposed to different extreme environmental conditions and fed with different diets.

Acknowledgements:

This study had the financial support of Operational Programme MAR2020 through the projects Ouriceira AQUA (16-02-01-FMP-0004) and Be4Aquahealth (MAR2020-02.05.01-FEAMP-0013); and Fundação para a Ciência e Tecnologia (FCT), through the strategic project UIDB/ 04292/2020 granted to MARE-Marine and Environmental Sciences Centre. AP was supported through the Scientific Employment Stimulus Programmes (CEECIND/00095/2017 and CEECINST/00051/2018).

References

Albrizio *et al*. (2019) Male reproductive physiology of *Paracentrotus lividus*: monthly and seasonal evaluations to study the effects of the environment on gonadal development and sperm cells kinetic. Aquaculture 503: 620–627.

Branco *et al*. (2013) The impact of rising sea temperature on innate immune parameters in the tropical subtidal sea urchin *Lytechinus variegatus* and the intertidal sea urchin *Echinometra lucunter*. Marine Environmental Research 92: 95–101.

Fernández-Boo *et al*. (2018) Annual assessment of the sea urchin (*Paracentrotus lividus*) humoral innate immune status: Tales from the north Portuguese coast, Marine Environmental Research, 141:128-137.

Johnston, D. & Freeman, J (2005). Dietary preference and digestive enzyme activities as indicators of trophic resource utilization by six species of crab. Biological Bulletin 208:36–46.

DETERMINING KEY FISH WELFARE AREAS AND CORRESPONDING WELFARE INDICATORS IN AQUACULTURE ACROSS DIFFERENT STAKEHOLDER GROUPS

Vincent Lugert*[1], Karina Retter[2], Felix Teitge[2], Dieter Steinhagen[2], Stefan Reiser[1]

[1]Johann Heinrich von Thünen Institute, Institute of Fisheries Ecology, Herwigstraße 31, D-27572 Bremerhaven, Germany
*Email: vincent.lugert@thuenen.de

[2] University of Veterinary Medicine, Fish Disease Research Unit, Bünteweg 17, D-30559 Hannover, Germany

Introduction

The recent decade has seen increasing attention on the subject of the welfare of fishes in aquaculture operations. This trend is due to the constant global growth of the aquaculture sector since the late 1980s. The sector is expected to continue its growth at a rate of approximately 2.3% per annum until 2029 and is projected to overtake capture fisheries in total fish production by 2024 (OECD 2021). This growth is mainly due to three progressions:

1.) expansion, 2.) intensification, and 3.) diversification,

with all three of these potentially leading to numerous new welfare implications and challenges on the farmed specimens. Today, the fulfillment of good husbandry and welfare practice in aquaculture operations is often impaired by the lack of fundamental knowledge addressing the species- and life-stage specific welfare needs of the vast variety of different aquatic organisms. In contrast to terrestrial livestock farming, aquaculture, of finfish in particular, has to cope with an X-fold higher species diversity than all terrestrial animals combined. Fish account for around 60 % of all global vertebrate species (Nelson et al. 2016), and the FAO (2018) reports more than 350 finfish species to be currently cultivated in aquaculture. According to Mood & Brooke (2012), an estimated 79,3 billion individual farmed fish were slaughtered each year by 2010, compared to 63 billion terrestrial livestock, of which 86% accounted for chicken alone. These sheer numbers reveal the need to safeguard fish welfare during all stages of the production cycle. However, a monitoring system consolidated across different stakeholders of the sector has yet to be developed.

Figure 01: Word-cloud on main topics and terms generated from multiple discussion panels with different stakeholder groups. Major topics are feed, staff-competence, health, stocking-density, behaviour and stunning.

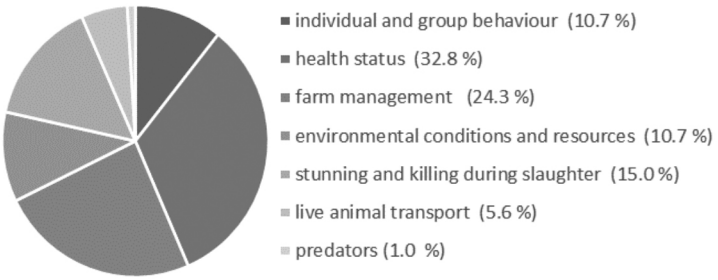


Figure 02: A model of the ideal quantitative distribution of all welfare indicators across the seven key areas (clockwise) desired for a monitoring system as identified by different stakeholder groups and the literature.

(Continued on next page)

Materials and methods

To address the respective welfare needs of the fish, and select a widely accepted, yet broad baseline set of welfare indicators (WIs), we collected and reviewed 109 articles, scientific publications, book chapters, databases, and legal frameworks, as well as guidelines and recommendations from the European Union, expert panels as well as national and international associations. We organized five national meetings with representatives of the scientific community, fish health services, aquaculture associations, national authorities, as well as fish farmers, and the interested public. The meetings were organized in two stages. In the first stage, we collected and discussed main topics and terms regarding potential welfare constrains in aquaculture production (Fig. 01). We then sorted and assigned these terms to the corresponding WIs found in the literature. In the second stage, we asked stakeholders to rank each identified WI for its suitability on a score from 0 (lowest) to 3 (highest) by three different criteria each: validity, reliability, practicability. We merged the results based on multiple statistical selection criteria, to identify those topics and WIs that have the most common denominators across all stakeholder groups.

Results

During the meetings, we collected 234 different terms related to fish welfare in aquaculture. Out of these, we identified 160 individual WIs which can be referred to by the international literature. Indicators fall into two distinct categories: single indicators and combined indicators. All indicators can be classified into seven key areas: individual and group behaviour; health status; farm management; environmental conditions and resources; stunning and killing during slaughter; live animal transport; predators. WIs related to health status dominated (32.8%), followed by management measures (24.3 %), and stunning and killing during slaughter (15.0%) (Fig. 02). Overall, we identified 36 WIs from all key areas to be the most common denominators in fish welfare across all stakeholder groups.

Discussion and Conclusion

Stakeholders have a very diverse view regarding fish welfare indicators and monitoring systems. Some group representatives stated, that only animal-based WIs with regard to the health status were eligible to adequately reflect the state of fish welfare, while others endorsed growth performance to be solitarily sufficient. However, the majority of stakeholders supported a holistic welfare approach, with a broad view across all seven thematic areas. All groups agreed, that water quality parameters are among the most important, yet most complex WIs, due to their strong interconnection among each other, seasonality, and daily rhythms. It was suguessted to closely monitor water quality parameters, and interlink these to the farm management, and the overall staff competence.

Reference

FAO 2018. The State of World Fisheries and Aquaculture 2018 -Meeting the sustainable development goals. Rome.Licence: CC BY-NC-SA 3.0 IGO.

OECD 2021. OECD-FAO Agricultural Outlook 2020-2029. https://www.oecd-ilibrary.org/sites/4dd9b3d0-en/index.html?itemId=/content/component/4dd9b3d0-en

Nelson JS, Grande TC, Wilson MVH (2016). Fishes of the world. 4th Edition. John Wiley & Sons, Hoboken. 624pp

Mood A, Brooke, P, (2012). Estimating the Number of Farmed Fish Killed in Global Aquaculture Each Year. http://fishcount.org.uk/published/std/fishcountstudy2.pdf

THE SEA-URCHIN *Paracentrotus lividus* PRODUCTION: OPTIMIZING LAND-BASED CULTURE

Ricardo Luís[1,2]*, Ricardo José[2,3], Carlos Andrade[2,3,4]

1 ARDITI – Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, 9020-105 Funchal, Madeira, Portugal
2 CMC – Centro de Maricultura da Calheta, 9370-135 Calheta, Madeira, Portugal
3 OOM – Oceanic Observatory of Madeira, 9020-105 Funchal, Madeira, Portugal
4 CIIMAR – Interdisciplinary Centre of Marine and Environmental Research- University of Porto, 4450 – 208 Matosinhos, Portugal
Email: ricardoluis_09@hotmail.com

Introduction

As sea-urchin gonads (roe) became more popular after the 1950s, many *Paracentrotus lividus* populations were exploited in a boom-and-bust model, resulting in a sharp decline of their wild stocks. In this context, aquaculture culture emerges as one of the solutions for the ineffective resource management policies, as a sustainable exploitation of this product.

Sea ranching, and land-based culture have both been utilized for grow-out of sea-urchins. Land-based industrial culture requires large capital costs, and intensive management, while sea ranching is less costly but often results in low recapture rates. Thus, a significant increase in efforts to develop cost-effective, and sustainable methods to culture sea urchins has been observed including research aiming to optimize the reproduction, and juvenile production. A better knowledge in the control, and understanding of the factors controlling sea-urchin's growth, gametogenesis, spawning, and survival is essential for a sustainable production, and supply of the increasing demand for eggs and larvae. This communication presents the broodstock, and gonadossomatic conditioning pilot systems as well as protocol optimization for broodstock rearing, and spwan induction methods at Centro de Maricultura da Calheta.

Material and Methods

For this work, *P. lividus* with test size superior of 35 mm were collected from intertidal rock pools in the southern coast of Madeira island, Portugal. The broodstock rearing system consisted of two 750 liters (L), and one 500 L (quarantine) cylindrical tanks. Temperature was 22.82 ±0.80 ºC, and X L/h of water renovation. Feeding was established at 5% weight. week^{-1} of maize (*Zea maes*). The population collected (n=20) was analyzed *a priori* to determine the gonadal index (GI), and compared with conditioned sea-urchins (n=20) after three months. Four spawning methods were evaluated: KCl 0.5M injection; agitation; microalgae addition, and cospecific gametes addition (spermatozoa), by assessing the spawning response within 30 minutes, and survival up to five days, in wild-caught, and conditioned individuals.

Results

Results indicate that three months conditioned sea-urchins GI average was 8.46 ±2.90 in which 25% of the individuals presented a GI $\geq$ 10, as opposed to wild caught sea-urchins that presented a GI average of 2.84 ±1.93. In the spawn induction techniques evaluation, all methods were able to trigger spawning, and mortality was only observed with KCl 0.5M injection method. The agitation was chosen to induce spawning without impairing broodstock survival, with gamet ejection within 30 min. Further experiments are undergoing to allow improvements in the larvae rearing methods for *P. lividus*.

References

Azad, A. K.; McKinley, S. & Pearce, C. M. (2010). Factors influencing the growth and survival of larval and juvenile echinoids. Reviews in Aquaculture, 2(3), 121-137. doi: 10.1111/j.1753-5131.2010.01030.x

Liu, H.; Chang, Y, n.d. in: Brown, N., & Eddy, S. (2015). Echinoderm Aquaculture. John Wiley & Sons. ISBN: 978-0-470-96038-7

Gago, J. & Luís, O. J. (2011). Comparison of spawning induction techniques on *Paracentrotus lividus* (Echinodermata: Echinoidea) broodstock. Aquaculture international, 19(1), 181-191. doi: 10.1007/s10499-010-9351-6

Acknowledgements

This study and Ricardo Luís research grant had the support of project ISLANDAP ADVANCED (Interreg MAC/1.1a/207). Ricardo José was were financially supported by the Oceanic Observatory of Madeira Project (M1420-01-0145-FEDER-000001-Observatório Oceanico da Madeira-OOM).

A COMPLETE RISK MODEL FOR AQUACULTURE COMPANIES

M. Luna*, L. Luna, J.L. Fernández-Sánchez and I. Llorente

Business Management Department, University of Cantabria, Santander, Spain
E-mail: manuel.luna@unican.es

Introduction
In recent decades, aquaculture has established itself as a flagship industry in the agri-food sector. It is at the top of the food production industries in terms of growth due to its increasing worldwide popularity and its expanding diversity of products. However, their companies inherently face more variability in their results than those from other food production industries (Geurin and Geurin, 1994; Flaten et al., 2011).

Despite this fact, the field of risk in aquaculture is still understudied and subject to discourse. On the one hand, the scientific literature on the risk field in aquaculture is limited and very unbalanced as most contributions are focused on some specific risks. On the other hand, the industry lags behind comparable industries in implementing systematic risk management and only a small fraction of the industry is insured for losses (Holmen and Thorvaldsen, 2015). In this context, the scientific community and the industry have to work together to address some basic research questions on the fundamentals of risk in its broadest sense, before focusing again on the operative "how-to".

For these reasons, the main objective of this study is to provide a comprehensive risk framework for the aquaculture industry with regard to all those risks that could adversely affect the companies' economic or financial situation.

Materials and methods
The development of a comprehensive risk model for the aquaculture industry requires to identify and classify the risks faced by companies in practice, and to define them in detail.

To do this, the literature providing empirical evidence on aquaculture producers' risk perceptions has been reviewed and consolidated, , which is formed by eight empirical studies (Bergfjord, 2009; Le and Cheong, 2010; Ahsan and Roth, 2010; Ahsan, 2011; Le Bihan et al., 2013; Lebel et al., 2016; Alam and Guttormsen, 2019; Rahman et al., 2021). Furthermore, other studies on risk assessment and management have also been examined in order to properly define each type of risk.

After that, a panel of independent experts - consisting of researchers, distributors and policymakers – have been asked to revise the model, seeking to go beyond the producers' perceptions.

Results and conclusions
The process carried out allowed us to cover a wide range of periods, species, and countries, which is crucial for the development of a consolidated and comprehensive risk model as risk perceptions are usually influenced by different socio-economic aspects. In this way, we aggregated the perceptions of more than 1500 producers from 8 publications, resulting in a model with 7 risk categories comprised of 40 risk sources (Table 1). Moreover, the producers' valuations on the importance of each risk source were also collected and processed to make them comparable, which allowed us to estimate an importance ranking.

This showed that Production, Market, and Operational risks gather the most important sources of risk, while those belonging to the Strategic, Regulatory, Financial, and Reputational risks are slightly behind. With regard to the main specific sources of risk, diseases, price volatility, and quality control have been ranked as the most worrying aspects for producers. On the contrary, they see the uncertainty around technological and HR as not very significant. Lastly, the independent evaluation of the model, based on the opinion of a panel of experts from industry and academia, allowed us to validate the model.

This work responds to the current need for a standardized approach to risk analysis that takes into account the different dimensions of risk, which has been stressed several times. This will help both the scientific community and the industry to standardize and improve risk assessment and management practices. Furthermore, as this model is based on those applied in the financial sector, it could also be useful for other stakeholders, such as financial agents and policymakers, improving the access of the industry to external funds.

(Continued on next page)

Exogenous	Market/Price Risks	Selling Prices
		Market Related
		Interest Rates
		Input Prices/supply
	Production Risks	Climatic Shocks
		Bio-sanitary
		Technological
	Regulatory Risks	Legal Requirements
		Governmental Aid
Endogenous	Financial Risks	Credit
		Liquidity
		Structural
	Strategic Risks	Location
		Infrastructures
		Integration and Scale
	Operational Risks	Production System
		Control Processes
		Human Resources
	Reputational Risks	Company Reputation

Table 1. Risk model for the aquaculture industry

References

1. Ahsan, D. (2011). Farmers' motivations, risk perceptions and risk management strategies in a developing economy: Bangladesh experience. Journal Of Risk Research, 14(3), 325-349. doi: 10.1080/13669877.2010.541558
2. Ahsan, D., & Roth, E. (2010). Farmers' Perceived Risks and Risk Management Strategies in an Emerging Mussel Aquaculture Industry in Denmark. Marine Resource Economics, 25(3), 309-323. doi: 10.5950/0738-1360-25.3.309
3. Alam, M., & Guttormsen, A. (2019). Risk in aquaculture: farmers' perceptions and management strategies in Bangladesh. Aquaculture Economics & Management, 23(4), 359-381. doi: 10.1080/13657305.2019.1641568
4. Bergfjord, O. (2009). Risk perception and risk management in Norwegian aquaculture. Journal Of Risk Research, 12(1), 91-104. doi: 10.1080/13669870802488941
5. Flaten, O., Lien, G., & Tveterås, R., (2011) A comparative study of risk exposure in agriculture and aquaculture. Acta Agriculturae Scandinavica, Section C — Food Economics 8(1), 20-34. Doi: 10.1080/16507541.2011.566418
6. Geurin, L.J., & Geurin, T.F. (1994). Constraints to the Adoption of Innovations in Agricultural Research and Environmental Management: A Review. Australian Journal of Experimental Agriculture 34, 549–571.
7. Holmen, I.M., & Thorvaldsen, T. (2015) Good Safety Work - Examples from Several Industries. (In Norwegian). Trondheim SINTEF Fisheries and Aquaculture, Norway
8. Le Bigan, V., Pardo, S. & Guillotreau, P. (2013). Risk Perception and Risk Management Strategies of Oyster Farmers. Marine Resource Economics 28(3), 285-304.
9. Le, T., & Cheong, F. (2010). Perceptions of risk and risk management in vietnamese catfish farming: an empirical study. Aquaculture Economics & Management, 14(4), 282-314. doi: 10.1080/13657305.2010.526019
10. Lebel, L., Lebel, P., Lebel, B., (2016). Impacts, Perceptions and Management of Climate-Related Risks to Cage Aquaculture in the Reservoirs of Northern Thailand. Environmental Management 58, 931–945. doi:10.1007/s00267-016-0764-5
11. Rahman, M., Nielsen, R., Khan, M., & Ahsan, D. (2021). Perceived Risk and Risk Management Strategies in Pond Aquaculture. Marine Resource Economics, 36(1), 43-69. doi: 10.1086/711066

TEMPERATURE DEVELOPMENT IN PACIFIC OYSTER (*Crassostrea gigas*) UNDER EVERYDAY FOOD PREPARATION HEAT TREATMENTS – IMPLICATIONS FOR NOROVIRUS INACTIVATION

S.M. Stoppel, A. Duinker, S. Mortensen and B.T. Lunestad*

Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway
E-mail: bjorn-tore.lunestad@hi.no

Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg, 1793) originates in South-East Asia, but has been extensively translocated for aquaculture purposes and is now one of the main cultured bivalve species. Norovirus (NoV) is one of the most common causes of gastroenteritis globally and outbreaks are often linked to bivalves, including the Pacific oyster (Bellou et al., 2013; Hardstaff et al., 2018). Two main challenge in assuring NoV absence in food is the lack of cultivation based methods for detection and high number of false positive results from RNA in non-infective NoV with damaged capsid. Thus, to improve food safety in raw oysters, temperature treatment prior to consumption has been suggested to inactivate pathogens, including NoV. This study describes temperature development in oyster tissue during several commonly applied thermal treatments (steaming, broiling, baking, barbecuing, boiling water, hot broth). In addition, changes in the sensory quality during heating were evaluated.

Materials and methods

Sixty Pacific oysters of an average weight of 100 g and harvested from a wild population on the South-Eastern Norwegian coast, were included. Oyster heat treatment regimens (time-temperature) were chosen from CDC guidelines (Centre for Disease Control and Prevention, 2019a; 2019b) as well as in accordance with the most common recipe suggestions in both printed and online sources. The following heat treatments were examined:

Steaming: The steaming was done in a steel pot with tap water. Live closed oysters were positioned in a steamer basket and placed into the pot containing boiling water without touching the water surface. Temperature probes were placed in the oyster digestive tissue through a drilled and sealed hole in the flat shell half. The pot was covered with a lid and oysters were steamed for 8 min.

Broiling and baking: During broiling and baking oysters were opened, the upper shell removed, and placed on a metal baking sheet. Thermometer probes were positioned inside the digestive tissue and a tablespoon of butter placed on top. For broiling, the sheet was put into a pre-heated oven set to the grill heater at 250°C, ~8 cm below the heat source at the top of the oven. For baking, the sheet was put into a pre-heated oven at 225°C (top and bottom heat). Oysters were left in the oven for 8 min (broiling) or 10 min (baking).

Barbecue: During the barbecue treatment, the temperature sensors were placed in the digestive tissue of opened oysters. The oysters were put on a small 30x25 cm disposable charcoal barbecue lit 20 min before the trial and temperature was measured for 8 min. Ambient temperature was 20°C.

Broth trial: Oysters were opened, and the soft parts removed from the shell. Thermometer probes were placed in the digestive tissue and the oyster soft parts put into porcelain cups of 250 mL capacity, at room temperature. Broth was brought to a boil and 100 mL were poured into the cups holding the oyster soft tissue. Temperature was monitored for 5 min.

Boiling water: Oysters were opened, and the soft parts removed from the shell. Thermometer probes were placed as above, and the soft parts placed in a steamer basket. Salted water (1 L) was brought to a boil in a metal pot and the heat was turned down so that water was slightly simmering throughout the experiment. The steamer basket holding the oysters was lowered into the simmering broth and the temperature was measured for 5 min.

Temperature development was measured by two digital thermometers each with two K temperature sensors (Thermocouple ET-959). The thermometer displays were filmed throughout each experiment. At least eight replicates prepared in separate batches were analysed per heat treatment, except for baked and boiled oysters with only six replicates.

(Continued on next page)

Results

The highest temperatures were recorded during steaming, broiling and submersion in boiling water. Steamed and boiled oysters were at >90°C after 6 and 4.5 min, respectively. Broiling at 250°C, lead to 78°C when oysters were visually considered ready. Lowest temperatures were observed in oysters on a small disposable barbecue or covered with broth. Temperatures in broth peaked at 56°C and barbecued oysters reached 37°C-68°C, depending on placement on the barbecue. Consequently, both barbecuing and broth-heating would not lead to norovirus inactivation. Virus may be inactivated only in steamed oysters or in boiling water, while baking and broiling shucked oysters in the oven for ≥10 min should increase food safety as well. Steaming for >8 min or broiling and baking at 250°C for ≥10 min is suggested to reduce the number of infectious norovirus, even though this might lead to a less sensory acceptable product with a decline in taste and texture after prolonged heating.

Conclusion

As the highest temperatures were recorded during steaming, broiling and submersion in boiling water, these heating methods would give the best reduction of NoV infectivity, if present. Of all the heating methods boiling water lead to the most rapid temperature increase. Lowest maximal temperatures were observed in oysters on a small disposable barbecue or covered with broth, and both heating methods would not lead to norovirus inactivation. Future analysis of contaminated oysters for assessing infectivity of norovirus would be preferable for more accurate temperature recommendations.

References

Bellou, M., Kokkinos, P., & Vantarakis, A., 2013. Shellfish-Borne Viral Outbreaks: A Systematic Review. Food and Environmental Virology, 5(1), 13-23.

Centers for Disease Control and Prevention, 2019a. Preventing Norovirus Outbreaks. Retrieved November 18, 2020, from https://www.cdc.gov/vitalsigns/norovirus/infographic.html#graphic

Centers for Disease Control and Prevention, 2019b. *Vibrio* Species Causing Vibriosis - *Vibrio* & Oysters: Prevention Tips. Retrieved November 18, 2020, from https://www.cdc.gov/vibrio/prevention.html#cooking

Hardstaff, J. L., Clough, H. E., Lutje, V., McIntyre, K. M., Harris, J. P., Garner, P., & O'Brien, S. J. (2018). Foodborne and Food-Handler Norovirus Outbreaks: A Systematic Review. Foodborne Pathogens and Disease, 15(10), 589-597.

GASTRIC EVACUATION IN THREE SIZES OF GROW-OUT ATLANTIC HALIBUT (*Hippoglossus hippoglossus*) FED DIFFERENT PELLETS SIZES

E. Lygre[a,b,*], A.S. Gomes[a], O-K Hess-Erga[b], B. Norberg[c], J. Nilsson[d], P. Perrichon[c], I. Rønnestad[a]

[a] Department of Biological Sciences, University of Bergen, Bergen, Norway
[b] Sogn Aqua AS, Bjordal, Norway
[c] Institute of Marine Research, Austevoll, Norway
[d] Institute of Marine Research, Bergen, Norway
*Corresponding author. email: Endre.Lygre@uib.no

Introduction

The production of farmed Atlantic halibut has stagnated with an average production of 1686 tons/year since 2007 (Statistics Norway, 2020). One of the major bottlenecks hindering the expansion of the industry is the slow growth rates of halibut above 1kg (grow-out phase), linked to the low feed intake rates observed. Ingestion of feed requires appetite stimulating signals such as visual, olfactory, and physiological sensation of hunger. The amount of feed ingested is eventually limited by the filling capacity of the stomach and generally appetite decreases during feeding as satiation (fullness of the stomach) increases (Brett, 1971; Grove et al., 1978). After ingestion of a meal, the food in the stomach is mechanically and chemically digested before it is gradually evacuated from the stomach and enter the intestine for further digestion, followed by absorption, and assimilation of nutrients. The effectiveness of digestive processes is affected by several parameters, including fish size (Flowerdew and Grove, 1979), water temperature (Brett and Higgs, 1970), meal size and type of food. Halibut appetite varies on a day-to-day basis, and it has been observed that it prefers to eat a small meal the day after a large meal (Tuene and Nortvedt, 1995). This suggests that halibut may have a relatively slow gastric evacuation time, negatively affecting the time to next meal. In this study we investigated the gastric evacuation time for 3 different Atlantic halibut sizes using different pellet sizes.

Material and methods

Three size groups (1, 2 and 3 kg) of Atlantic halibut in duplicates were randomly allocated to 12 tanks with a bottom surface of 4.9 m^2 supplied with fresh seawater with a mean temperature of 8.9 °C and 91 % O_2. After the acclimatization period of 14 days, where the fish were hand fed every day using a commercial diet (Hippo Express, Skretting, Norway), the halibut were deprived of food for 120 hours to ensure no feed residuals were present in the gastrointestinal tract (Davenport et al., 1990)Hippoglossus hippoglossus L., eat larger satiation meals (mean 11.7% body weight. The fish groups were then fed a single meal *ad libitum* with respective pellets sizes (7, 9, 13 and 17 mm), and 3 fish were sampled from each tank at 1, 3, 6, 10, 23, 32, 48, 71, 96 and 120 hours after feeding. The content of stomach, pyloric caeca, midgut and hindgut were collected as well as information regarding the round weight, fork length, sex and weight of gonads, liver and heart. Content of the gastrointestinal tract compartments were dried for 48 h at 105 °C and the dry weight normalised to the fish weight.

Results and discussion

The maximal meal ingested by any fish was 1.1 % of body weight. However, there was a large variability, and some fish did not ingest any feed despite 120 hours of fasting. Preliminary analysis of the data indicates a negative relationship between pellet size and feed intake (measured at 1h). To determine the optimal feeding frequency for Atlantic halibut, gastric evacuation time should be accounted for, since satiation is mainly affected by the fullness of the stomach (Brett, 1971; Grove et al., 1978). In our study, the dry weight of the stomach content was reduced to 50 % at 14 hours after feeding and 25 % after 23 hours (Figure 1). This result might explain the variable day-to-day feed ingestion levels observed in commercial halibut farms and the results obtained by Tuene and Nortvedt (1995). The content of the pyloric caeca and midgut reached the highest dry weight at 23 hours, and hindgut at 71 hours. No feed residuals were observed in the gut at 120 hours which corresponds with previous observations (Davenport et al., 1990). This study provides valuable information to design optimal timed feeding regimes for grow-out Atlantic halibut.

(Continued on next page)

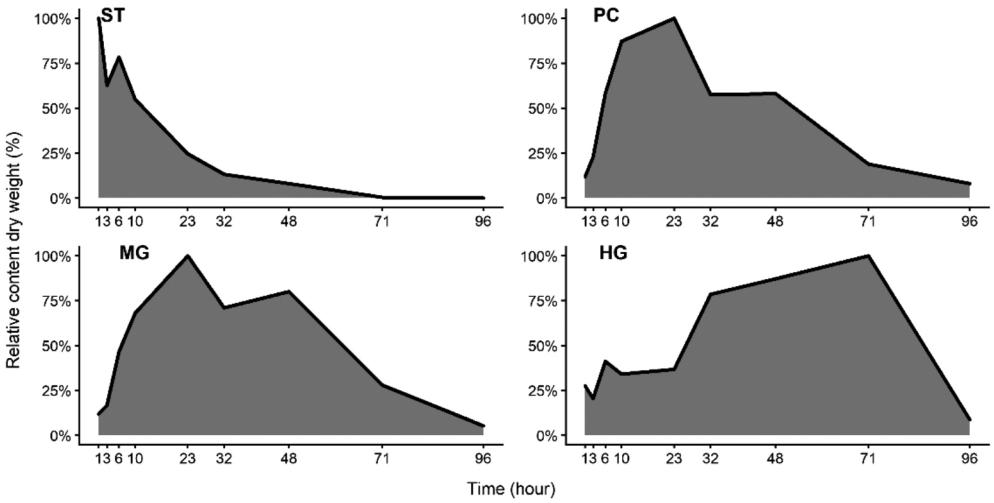


Figure 1. Relative dry weight content from stomach (ST), pyloric caeca (PC), midgut (MG) and hindgut (HG) at 1, 3, 6, 10, 23, 32, 48, 71 and 96 hours after feeding. Dry content weight was divided by the fish weight and subsequently normalized by the maximum content weight within each tissue.

Sources

Brett, J. R. (1971). Satiation Time, Appetite, and Maximum Food Intake of Sockeye Salmon (*Oncorhynchus nerka*). *Fish. Res. Board Canar.* 28, 409–415.

Brett, J. R. and Higgs, D. A. (1970). Effect of Temperature on the Rate of Gastric Digestion in Fingerling Sockeye Salmon, *Oncorhynchus nerka . J. Fish. Res. Board Canada* 27, 1767–1779.

Davenport, J., Kjørsvik, E. and Haug, T. (1990). Appetite, gut transit, oxygen uptake and nitrogen excretion in captive Atlantic halibut, *Hippoglossus hippoglossus* L., and lemon sole, *Microstomus kitt* (Walbaum). *Aquaculture* 90, 267–277.

Flowerdew, M. W. and Grove, D. J. (1979). Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. *J. Fish Biol.* 14, 229–238.

Grove, D. J., Loizides, L. G. and Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *J. Fish Biol.* 12, 507–516.

Statistics Norway (2020). Akvakultur. Salg av slaktet matfisk, etter fiskeslag, statistikkvariabel og år. *SSB*.

Tuene, S. and Nortvedt, R. (1995). Feed intake , growth and feed conversion efficiency of Atlantic halibut , *Hippoglossus hippoglossus* (L.). *Aquac. Nutr.* 1, 27–35.

MODELLING THE RESILIENCE OF THE UK SEAFOOD SYSTEM

Alan MacDonald* and Sofia C. Franco

Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, Scotland, United Kingdom
Email: alan.macdonald@sams.ac.uk

COVID-19 and the corresponding human response have tested the resilience of seafood supply chains (SC) (Ivanov, 2020) and the wider supply network (SN) where they sit in. The changes to global markets and trade triggered by the pandemic, coupled with factors such as national public health measures (e.g., lockdowns, COVID-secure restriction to operations) or behaviour changes (e.g., to consumer purchase patterns, workforce mobility) have forced business to quickly adapt how they operate and have placed strain on many UK seafood businesses. At the same time, businesses have been responding to systemic changes associated with the withdrawal of the UK from the EU (BREXIT). Changes following COVID-19, meant that UK shellfish and finfish aquaculture producers, for example, have had to cope with risks such as fluctuating demand and supplies due to collapsing export markets, while trying to maintain ongoing production and ensure animal welfare standards. Our ongoing work, as part of the RiseUp project, is brought to light that, to limit disruption, aquaculture businesses have employed a range of in-farm and out-of-farm strategies, from re-routing products, changing export markets, to delaying harvest time to prolong grow-out periods, amongst others. These knock-off effects highlight the relevance of considering impacts and resilience of specific industries, such as aquaculture, in the context of the wider complex SN in which it operates, and which consists of interdependencies to multiple suppliers and customers and whose long-term viability is dependent on how individual and food system resilience interact.

This study, part of the RiseUp project (www.sams.ac.uk/science/projects/riseup/), collected evidence of context of COVID-19 impacts on businesses across the UK seafood industry and the responses that were put in place to limit disruption. The aim of the work herein presented is to develop approaches that can serve to inform how to increase the resilience of the UK seafood businesses and seafood supply network to current and future disruption, by combining expert knowledge from seafood industry stakeholders, collected through interviews and a survey, with resilience modelling using Bayesian networks.

Bayesian networks (BN), directed acyclic graphs encoding conditional probabilistic relationships among variables through feedback, are powerful tools for risk analysis and decision support in real-world problems. Probabilities are set based on expert knowledge and can be updated by Bayesian inference. An advantage of using BNs are that variables can be discrete, continuous or Boolean making it easy to incorporate different data from survey and questionnaires. A new area of research has been the application of BNs to model SC resilience (Hosseini et al., 2019; Yodo and Wang, 2016), however, to our knowledge this has not been done for food SCs and SN in general. Further, we extend the resilience modelling framework by linking risks to SC businesses with mitigation/prevention strategies and concepts of resilience. Defined in Zavala-Alcívar et al. (2020), several resilience concepts (e.g., business innovation, visibility, trust, leadership etc.) help negate the impact of disruptive events. That is, improving resilience should minimise negative impacts of risks such as changes in non-tariffs barriers to trade (i.e., a BREXIT related risk) or market demand (i.e., a COVID-19 related risk). Resilience concepts are dependent on pro-active or reactive strategies. For example, visibility (resilience concept), defined as the ability to respond rapidly to disruptive events by efficiently distributing your critical resources, is likely dependent on having competent staff (mitigation/prevention strategy).

A challenge in the use of BNs to model SN is the proper identification of risks that can impact the different supply chains and the possible mitigation/prevention strategies to resist potentially disruptive events (e.g., COVID-19, BREXIT, climate change, future trade deals) (Lockamy and McCormack, 2012). To overcome this challenge, we combine findings from semi-structured interviews with results from a follow-up online survey, which asked respondents on business characteristics, business risks during times of disruption and 'business-as-usual' operating conditions, impacts of different ongoing disruptive events (e.g., COVID-19, BREXIT), their business responses and attributes and actions that they perceive to have contributed to a successful (or not) response to these shocks. First, data contextualising COVID-19 impacts and business responses, collected from interviews conducted with stakeholders across the UK seafood industry (between October 2020 and February 2021; see Franco et al. AE21 abstract for details), was used to identify business risks, corresponding attributes and actions that may buffer their impacts. Next, a different pool of industry stakeholders was asked to assess the importance of these risks and actions/attributes in an online survey. Following the methodology of Nepal and Yadav (2015) interviewees were asked to rank the detectability, occurrence and severity of risks (e.g., changes in market demand) on a 6-point Likert scale. Interviewees were also asked to rank the importance of several mitigation and prevention strategies to their business response to COVID-19 (e.g., planning and preparedness, situational awareness, capacity to innovate), as well as change in demand, costs, capacity, workforce and turnover before and after the pandemic, further to BREXIT impacts.

(Continued on next page)

We will present our ongoing work on BN development, through a schematic of the conceptual framework for modelling the resilience of the UK seafood industry at the conference and preliminary analysis of connections between risks and resilience of seafood businesses. To facilitate the analysis, risks are divided into environmental, social, economic, external and internal categories. Mitigation and presentation strategies tied to concepts of resilience are then linked to broader resilience categories. A unique property of BNs is the ability to carry out forward and backward propagation analysis, that is, model the overall resilience of a particular business sector e.g., aquaculture business or seafood importer (forward propagation), or determine the best mitigation/prevention strategies given a completely resilient SC (backward propagation). With these different views of the network, such models can provide a useful risk management tool for industries such as aquaculture to build their resilience, as well as a practical approach to inform government policy, for instance to identify unintended consequences of new policy and to support food system resilience in context of change.

Acknowledgments

The RiseUp project 'Resilience of the UK Seafood industry to COVID-19' is supported by the Economic and Social Research Council grant ref.ES/V009907/1 to Franco. The project website is accessible at www.sams.ac.uk/science/projects/riseup/ . The authors would like to extend their recognition to Seafish (www.seafish.org) and are grateful to the businesses that took part in the interviews and online surveys.

References

Hosseini, S., Ivanov, D. and Dolgui, A. "Review of quantitative methods for supply chain resilience analysis." Transportation Research Part E: Logistics and Transportation Review 125 (2019): 285-307.

Ivanov, D. "Viable supply chain model: integrating agility, resilience and sustainability perspectives—lessons from and thinking beyond the COVID-19 pandemic." Annals of Operations Research (2020): 1-21.

Lockamy, A., & McCormack, K. (2012). Modeling supplier risks using Bayesian networks. Industrial Management & Data Systems.

Nepal, B., & Yadav, O. P. (2015). Bayesian belief network-based framework for sourcing risk analysis during supplier selection. International Journal of Production Research, 53(20), 6114-6135.

Yodo, N., & Wang, P. (2016). Resilience modeling and quantification for engineered systems using Bayesian networks. Journal of Mechanical Design, 138(3).

Zavala-Alcívar, A., Verdecho, M.J. and Alfaro-Saíz, J.J., 2020. A conceptual framework to manage resilience and increase sustainability in the supply chain. Sustainability, 12(16), p.6300.

EFFLUENT-GROWN ULVA AS A FUNCTIONAL INGREDIENT FOR FARMED ABALONE: IMPACTS ON GROWTH, PHYSIOLOGY AND MICROBIOME

B.M. Macey[1,2]*, M.J. Brand[2], M. Brink-Hull[2], M.D. Cyrus[1,2] and J.J. Bolton[2]

[1]Department of Forestry, Fisheries and the Environment, Cape Town, 8001 South Africa
[2]Department of Biological Sciences, University of Cape Town, 7701 South Africa
Email: BMacey@environment.gov.za

Introduction

Integrated Multi-Trophic Aquaculture (IMTA) represents a sustainable production method that can reduce the environmental impacts of aquaculture, facilitate diversification and increase production. Several large-scale commercial abalone farms in South Africa practice IMTA, growing *Haliotis midae* in land-based raceway tanks, interconnected with *Ulva* **in** adjacent paddle raceways using abalone effluent. *Ulva* **serves as a biofilter**, allowing water from the *Ulva* **systems to be re-circulated back to abalone tanks**, and *Ulva* is used as supplementary feed for abalone. Dietary *Ulva* supplementation has conveyed benefits to a variety of cultured animals, enhancing feed consumption, growth, product quality and health, with some of the improvements to abalone culture believed to be linked to the carbohydrate fraction of *Ulva* (Naidoo et al., 2006; Mulvaney et al., 2013; Kemp et al. 2015; Bansemer et al., 2016). The aim was to investigate the effects of (1) *Ulva* as a partial or complete replacement of formulated feed, and (2) the inclusion of *Ulva,* or specific components of *Ulva*, on feed consumption, growth, physiology and the gut microbiome of cultured *H. midae* to gain a better understanding of the functional potential of *Ulva* as an aquafeed ingredient.

Materials and Methods

To test the effects of *Ulva* as a partial or complete replacement of a local formulated feed Abfeed®S34 (diet AB) on total feed consumption, abalone (67.30±5.49g; n=10 per basket) were fed for 28 days with AB at a rate of 1.27% BW.day^{-1} or with graded levels of AB (75, 50, 25 & 0% of AB); with the balance of the feed constituting of fresh IMTA grown *Ulva* (FU). A separate one-year on farm growth trial was conducted under farm conditions to assess the extent to which AB can be replaced by FU. Abalone (51.10±3.09g; n=200 per basket) were fed AB at a rate of 0.27% BW.day^{-1} or graded levels of AB (100, 80, 70, 60 & 40% of AB) supplemented with *Ulva* (0.21% BW.day^{-1}, dry weight approximation). All treatments were offered in triplicate and growth and condition were assessed on days 112, 223 and 366.

A controlled laboratory trial was conducted to test the effects of specific components of *Ulva* on abalone (20-30g) growth, physiology and gut microbiome. Isonitrogenous diets consisting of dried *Ulva* (10% w/w; AB10U), Ulvan (1% w/w; AB1U), and glucuronic acid (0.1% w/w; AB0.1U) were formulated. Feed conversion ratio (FCR), specific growth rate (SGR), tissue glycogen, and gut microbiome was assessed around day 0, 105 and 215 and compared to abalone fed diets AB, FU, or a combination of the latter (ABFU). 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.

Results and Discussion

Incorporation of small amounts of fresh IMTA grown *Ulva* (25%; diet 75A25U) were shown to significantly improve total feed consumption by ca. 90%, compared with abalone fed diets AB and FU (**Error! Reference source not found.** 1). No significant differences in SGR, CF and tissue glycogen content were recorded between treatments in the 1 year growth trial, suggesting as much as 60% of a formulated feed can be substituted with FU without negatively affecting growth and condition of abalone.

The controlled laboratory trial, showed that abalone maintained on ABFU and AB0.1U had the highest SGR, significantly higher than abalone fed AB, AB10U, and FU (Fig. 2). Abalone maintained on FU alone grew at a rate not statistically different to abalone maintained on AB, suggesting farmed abalone can be maintained on a diet consisting only of FU during the grow-out phase of production. Abalone fed diets supplemented with 0.1% glucuronic acid not only had improved SGR, but significantly lower FCR than abalone fed AB; suggesting glucuronic acid may be one of the components within *Ulva* contributing towards growth. 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 OTUs 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. This study has demonstrated that dietary supplementation with IMTA-grown *Ulva* can reduce an abalone producer's reliance on fishmeal-based dry formulated feeds and have several other health benefits.

(Continued on next page)

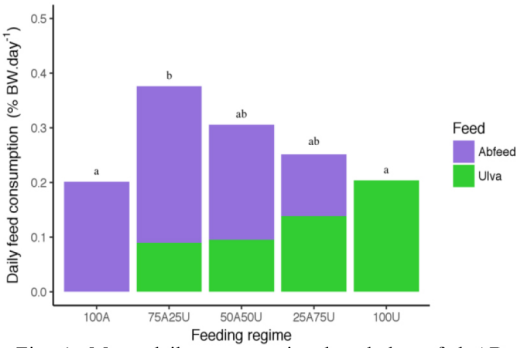


Fig. 1. Mean daily consumption by abalone fed AB or graded levels of AB (100, 80, 70, 60 & 40% of AB) supplemented with *Ulva*.

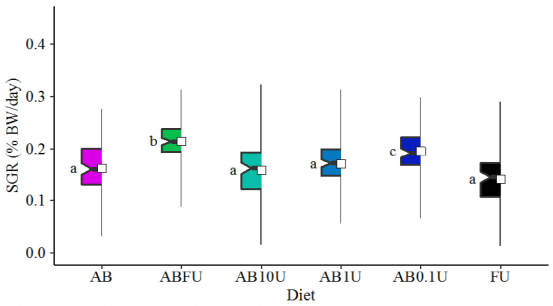


Fig. 2. SGR of abalone fed 6 different dietary treatments. Date represents the mean (white squares), median (notch) and the 1st (lower), 3rd (upper) and interquartile range. Different letters indicate significant differences ($p<0.05$).

References

Bansemer, M.S., Qin, J.G., Harris, J.O., Duong, D.N., Hai, T., Howarth, G.S., Stone, D.A.J. 2016. Growth and feed utilisation of greenlip abalone (*Haliotis laevigata*) fed nutrient enriched macroalgae. Aquaculture. 452, 62–68.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857.

Kemp, J.O.G., Britz, P.J., Agüero, P.H.T. 2015. The effect of macroalgal, formulated and combination diets on growth, survival and feed utilisation in the red abalone *Haliotis rufescens*. *Aquaculture*. 448(C), 306–314.

Mulvaney, W.J., Winberg, P.C., Adams, L. 2013. Comparison of macroalgal (*Ulva* and *Grateloupia* spp.) and formulated terrestrial feed on the growth and condition of juvenile abalone. Journal of Applied Phycology. 25(3), 815–824.

Naidoo, K., Maneveldt, G., Ruck, K., Bolton, J.J. 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology*. 18(3–5), 437–443.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41(D1), D590–D596.

CHARACTERIZATION OF THE MICROBIOME OF A PARTIALLY RECIRCULATED ABALONE-ULVA IMTA SYSTEM

N.B. Makhahlela[a]*, B.M. Macey[b], M. Greeff-Laubscher[c], M. Brink-Hull[a], V.E. Coyne[a]

[a]Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa
[b]Department of Agriculture, Forestry and Fisheries, Aquaculture Research, Private Bag X2, Roggebaai, Western Cape 8012, South Africa
[c]Department for Environmental Sciences and Management, Potchefstroom Campus, North West University, Private Bag X6001, Potchefstroom 2520, South Africa
E-mail: bridgetmakhahlela@gmail.com

Introduction

Several commercial abalone farms in South Africa use *Ulva* to bioremediate farm effluent water, allowing for water to be recirculated back to abalone raceways and the *Ulva* is often used as a supplementary feed source. Despite *Ulva*'s versatility in integrated multi-trophic aquaculture (IMTA) systems, there are biosecurity concerns with using effluent grown *Ulva* as abalone feed, preventing wider adoption of this technology. To better understand the potential disease risks, this project aims to characterize the microbial and fungal communities associated with the seawater and *Ulva* obtained from an integrated abalone-*Ulva* systems (with 50% water recirculation). The findings from this study will provide critical information on biosecurity of IMTA systems, species and system health that may promote broader uptake of more sustainable aquaculture production technologies.

Materials and methods

This study was conducted on a commercial abalone farm along the south-western Cape coast of South Africa. *Ulva* and seawater samples were collected from two separate *Ulva* raceway systems. One system consisted of tanks that received seawater directly from the adjacent coastline, hereafter referred to as the seawater (SW) raceway. The other system comprised raceways receiving abalone effluent water, with 50% recirculation between the abalone and *Ulva* raceways, referred to as abalone effluent water (AEW) raceways. *Ulva* samples were collected from within each raceway, whereas the water samples were collected at the inlet and outlet of each raceway. One SW raceway (only one exists on the farm) and 4 AEW raceways were sampled in winter, summer, and spring. Culture-dependent techniques were used to assess changes in the abundance of specific bacteria on *Ulva* and in seawater using three selective media, namely Tryptic Soy Agar (TSA; a general media routinely used for isolation of marine bacteria), Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar (vibrio selective), and Ulvan agar plates, where the primary carbohydrate of *Ulva* was utilized as the main carbohydrate source. The non-culture-based approach used next generation sequencing (NGS) and downstream bioinformatics analysis to describe the microbiome. The bacterial microbiome was characterised by sequencing the V4 hyper-variable region of the 16S rRNA gene, while fungi and oomycetes were identified by sequencing the ITS2 region of rRNA genes. 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).

Results and discussion

Culturable bacterial numbers on all media types were significantly higher ($P < 0.05$) in the *Ulva* raceway systems receiving AEW when compared with *Ulva* raceways receiving seawater. Bacterial abundance on all three selective media types was also higher on *Ulva* blades sampled from AEW systems. *Ulva* appeared to have an inhibitory effect on the number of culturable bacteria in the water column, as indicated by the general reduction in bacteria recovered from seawater from the inlets to the outlets of each raceway. A total of 15 917 078 individual bacterial amplicon sequence variants (ASVs) were identified in MicrobiomeAnalyst, belonging to 203 family-, 305 genus-, and 154 species level ASV groups. The most abundant phyla detected was Proteobacteria (58%), followed by Bacteroidata (23%) and Campilobacterota (4%). The most abundant genus detected was *Vibrio* (11%), followed by *Pseudoalteromonas* (8%) and *Leucothrix* (5%). The three most prevalent ASVs at genus level in each group consisted of bacteria such as *Rhodobacteriaceae, Saprospiraceae,* and *Vibrio* (Fig.1). Various *Vibrio* sp. and *Psuedoalteromonas* were more prevalent in the water systems (inlet and outlet), whereas *Granulosicoccus*, and *Glaciecola* were more prevalent on *Ulva.* General taxa, such as *Leucothrix*, *Psychrilyobacter*, and Psychromonas were present in both the seawater as well as on the *Ulva* itself. Bacteria inhabiting the *Ulva*, or the *Ulva* itself, can release antimicrobial compounds capable of inhibiting *Vibrio* spp. and other bacteria (Long et al., 2005). This was supported in the current study (Fig. 1) as *Vibrio* spp. were prevalent in the water systems (Inlet and Outlet), but less prevalent on *Ulva*, indicating that *Ulva* is effective at wastewater treatment.

(Continued on next page)

Conclusion

This study describes the diversity and functional roles of microbial communities in an aquaculture system using next-generation sequencing. The use of advanced molecular techniques will promote a better understanding of the complex microbial communities that exist within IMTA systems, and their contribution to the health of the systems and the animals cultivated in these systems..

References

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857.

Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research 45, 180–188.

Long, R.A., Rowley, D.C., Zamora, E., Lui, J., Bartlett, D.H., Azam, F. 2005. Antagonistic interactions among marine bacteria impede the proliferation of *Vibrio cholerae*. Applied and Environmental Microbiology 71(12), 8531-8536.

HEALTH PROMOTING EFFECTS OF *Salicornia ramosissima* BIOMASS IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax*)

M. Machado[1*], F. Cruz[1,4], S. Fernández-Boo[1], L. Ramos-Pinto[1], A. Laranjeira[2], R. Serradeiro[2], R. Rocha[2], J. Dias[5] and B. Costas[1,3]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR),Portugal
[2] Riaserach, Lda, Portugal
[3] Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal
[4] Escola Superior de Turismo e Tecnologia do Mar de Peniche, Instituto Politécnico de Leiria (IPL), Portugal
[5] Sparos Lda, Portugal
*mcasimiro@ciimar.up.pt

Introduction:
Halophyte plants such as *Salicornia ramosissima* have the ability to grown in saline soils (marginal land) and/or be irrigated with seawater. Despite the green tips of Salicornia are sold as food, the woody part is still considered a residue. However, this residue biomass is rich in valuable bio-active molecules that can be extracted using simple and affordable processing[1,2]. These compounds include hydroxycinnamic acids that may present strong antinflammatory and antioxidant effects. The present study aimed to assess the effects of *Salicornia ramosissima* biomass inclusion in diets for European seabass *Dicentrarchus labrax*. Fish immune condition during the feeding trial and the inflammatory response to inactivated *Photobacterium damselae* subsp. *piscicida* (*Phdp*) were evaluated.

Material and methods:
European seabass juveniles (mean initial weight: 7.26 ± 0.06 g) were reared at Riasearch Lda. facilities (Murtosa, Portugal). Fish were randomly distributed by 12 tanks of 350 L with 80 individuals allocated to each tank. Four diets were tested in triplicates. A commercial like diet was used as control (CTRL) whereas three experimental diets including *S. ramosissima* biomasses were formulated to have similar proximal compositions to CTRL. In the experimental diets, whole plant biomasses of *S. ramosissima* were included at three different levels: 2.5, 5 and 10% of feed (ST2.5, ST5 and ST10, respectively) and fish were given 3 meals per day, by hand until visual satiety for 62 days. To evaluate seabass immune condition, blood, plasma and head-kidney were collected from 5 fish per tank on day 34 and 62 for haematological and immune condition assessment and gene expression analysis. At the end of the feeding trial, fish were subjected to an inflammatory challenge by intraperitoneal injection with an inactivated *Phdp* (strain PP3). Blood, plasma, peritoneal cells and head-kidney samples were collected from 3 fish per tank at 4, 24, 48 and 72 hours after injection. The sampling point 62 days was used as time 0 h during the time-course study, as they represent unstimulated animals prior to inflammation.

Results and discussion:
No changes were observed among dietary treatments after both 34 and 62 days of feeding on the haematological profile. Similarly, no changes were observed among dietary treatments after both 34 and 62 days on the plasma humoral immune parameters analysed (peroxidase, anti-protease and bactericidal activities and lysozyme). However, in response to inflammation, fish fed ST5 showed a decrease of hematocrit and mean corpuscular values compared to those fed CTRL and ST10, respectively. Also, 4 hours after the inflammatory stimulus, seabass fed ST10 presented a higher concentration of leucocytes found in the peritoneal cavity in response to local inflammation (Figure 1) compared to fish fed CTRL. Head-kidney gene expression is being performed in fish sampled for all treatments and sampled times.

In conclusion, the results obtained in this trial demonstrate that the inclusion of *Salicornia ramosissima* biomasses of up to 10% can be performed successfully in diets for juvenile European seabass with no compromises to fish hematological and innate immune status. In fact, the highest inclusion level showed to improve leucocyte recruitment to the inflammatory focus (peritoneal cavity), what could be key in response to infection.

Acknowledgements: This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 86283 (project AQUACOMBINE). This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein. BC was supported by FCT - Foundation for Science and Technology (IF/00197/2015).

[1]Parvaiz A. and Satyawati S. (2008) Plant, Soil and Environment 54: 89–99.
[2]Stuchlık M. and Zak S. (2002) Biomedical Papers 146: 3–10.

DIFFERENCES IN IMMUNE RESPONSE TO KOI HERPESVIRUS INFECTION BETWEEN KOI CARP AND AMUR WILD CARP

R. Machat, L. Pojezdal, N. Hodkovicova, H. Minarova, and M. Faldyna

Veterinary Research Institute, Hudcova 296/70, 621 00 Brno (Czech Republic)
Email: machat@vri.cz

Introduction

Koi herpesvirus (KHV) is infective agent causing emerging KHV disease (KHVD), which affecting exclusively breeds of common carp (*Cyprinus carpio*) and carp hybrids with other cyprinids (Bergmann *et al*., 2010; Hedrick *et al*., 2006). KHVD is seasonal disease, which manifests itself in temperatures of 18-28°C (Pokorova *et al*., 2005)several cases have been confirmed all over the world. At present, this viral disease is considered to be one of the most risky factors affecting populations of common carp and koi carp. Affected fish become disoriented and swim erratically with high breathing frequency, swollen gills and partially local skin lesions. The virus was isolated from the tissues of fish showing signs of the disease and subsequently cultured on koi fin (KF-1 and it is typical with high morbidity and mortality. As no treatment or vaccine has been approved in EU so far, all that remains is complete eradication of affected fish farm. KHVD causes significant economic losses, which increase need for understanding nature of the host immune reaction against it. Significant difference in susceptibility among carp breeds was observed (Piačková *et al*., 2013). In this research, we focused on differences in antiviral immune response of two common carp breeds with significantly different susceptibility to KHVD.

Material and methods

Two breeds of common carp were chosen as experimental organisms, highly KHV resistant breed- Amur wild carp (AS), and KHV susceptible breed- koi. 26 fish from both carp breeds were divided into two groups: control (10 fish) and infected (16 fish). Carps from control groups were injected intraperitoneally with virus free media. Carps from infected groups were injected intraperitoneally with dosage 10^4 TCID50/ml of KHV suspension. Both groups were kept in separated water tanks with the same temperature 23°C for 7 days. Samples of gills, spleen, and head kidney were taken at 3 and at 7 dpi (days post infection). Relative expression levels of selected genes were measured using qRT-PCR. RNA for analysis of gene expression were isolated from gills, spleen and head kidney using commercial kit (Qiagen, Germany) according to manufacturer's instructions. Further, cDNA was obtained using LunaScript RT SuperMix Kit (New England Biolabs, USA). Target cDNA was labelled by intercalating dye SYBR Green (Qiagen, Germany) and gene expression was measured at LightCycler 480 (Roche, Switzerland).

Results and discussion

Relative gene expression of several genes participating in immune response to KHV disease infection was measured in this research (Tab. 1). According our not yet published data following genes were chosen: *c9* and *b/c2* genes participating in complement cascade, genes *mx* and *vig1,* as class I interferon signalling pathway (IFN-I) products and gene encoding Fas ligand (*faslg*) activating cytotoxic reaction of cytotoxic cells like NK cells. Following data were obtained by comparison of corresponding groups of infected koi and AS and results were related to the controls.

Infected group	Gills	Spleen	Head kidney
Koi carp 3 dpi	*faslg*, c9***	-	*b/c2**
Koi carp 7 dpi	-	*faslg****	*c9*, b/c2***
Amur wild carp 3 dpi	*vig1**	*mx****	*mx**
Amur wild carp 7 dpi	-	*mx**	*mx**

Tab.1: Significant differences between relative mRNA expressions of targeted genes in gills, spleen and head kidney of fish from infected groups. This data was obtained by comparing 3 dpi koi with 3 dpi AS and 7 dpi koi with 7 dpi AS. *p<0,05, **p<0,01, ***p<0,001

(Continued on next page)

Significantly increased levels of expressions of *faslg* and *c9* genes were detected in koi gills at 3 dpi as well as overexpression of *b/c2* gene was observed in head kidney of koi at 3 dpi. Further, higher expression of *faslg* was revealed in koi spleen and upper expression of *c9* and *b/c2* was observed in head kidney of koi carp. Superior expression of products of IFN-I pathway was detected in AS. Concretely higher expression of *vig1* was observed in AS gills at 3 dpi and *mx* at 3 dpi and 7 dpi time points in spleen and head kidney of AS.

According to obtained data it seems that AS immune response to KHV infection was stronger at 3 dpi than at 7 dpi, which at least partially support data obtained by Adamek *et al*., 2019. On the contrary immune reaction in koi is, at least in spleen and head kidney stronger later during infection in the monitored factors. Moreover it seems that immune response to KHV infection of koi is based on complement cascade and cytotoxic cells in contrary of AS immune response is based at overexpression of IFN-I effectors.

Conclusion

Data acquired in this study showed interesting clue in case of the origin of Amur wild carp resistance. Nevertheless, further research is recommended to understand actual influence of possible different immune response strategy after KHV infection.

Acknowledgements

This study was supported by European Regional Development Fund in the Operational Programme Research, Development and Education and The Czech Ministry of Education, Youth and Sports, project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869.

References

Adamek, M., Matras, M., Dawson, A., Piackova, V., Gela, D., Kocour, M., Adamek, J., Kaminski, R., Rakus, K., Bergmann, S. M., Stachnik, M., Reichert, M., & Steinhagen, D. (2019). Type I interferon responses of common carp strains with different levels of resistance to koi herpesvirus disease during infection with CyHV-3 or SVCV. *Fish & Shellfish Immunology*, *87*, 809–819. https://doi.org/10.1016/j.fsi.2019.02.022

Bergmann, S. M., Sadowski, J., Kiełpiński, M., Bartłomiejczyk, M., Fichtner, D., Riebe, R., Lenk, M., & Kempter, J. (2010). Susceptibility of koi × crucian carp and koi × goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD). *Journal of Fish Diseases*, *33*(3), 267–272. https://doi.org/10.1111/j.1365-2761.2009.01127.x

Hedrick, R. P., Waltzek, T. B., & McDowell, T. S. (2006). Susceptibility of Koi Carp, Common Carp, Goldfish, and Goldfish × Common Carp Hybrids to Cyprinid Herpesvirus-2 and Herpesvirus-3. *Journal of Aquatic Animal Health*, *18*(1), 26–34. https://doi.org/10.1577/H05-028.1

Piačková, V., Flajšhans, M., Pokorová, D., Reschová, S., Gela, D., Čížek, A., & Veselý, T. (2013). Sensitivity of common carp, Cyprinus carpio L., strains and crossbreeds reared in the Czech Republic to infection by cyprinid herpesvirus 3 (CyHV-3; KHV). *Journal of Fish Diseases*, *36*(1), 75–80. https://doi.org/10.1111/jfd.12007

Pokorova, D., Vesely, T., Piackova, V., Reschova, S., & Hulova, J. (2005). Current knowledge on koi herpesvirus (KHV)- a review. *Veterinární Medicína*, *50*(No. 4), 139–148. https://doi.org/10.17221/5607-VETMED

DIETARY ARA/DHA RATIOS AND CARBOHYDRATE LEVEL EFFECTS ON GILTHEAD SEA BREAM LIVER AND INTESTINE WELFARE AND GUT MICROBIOTA

Magalhães, R.*[1,2], Martins, N[1,2]., Fontinha, F.[1,2], Couto, A.[1,2], Serra, C.R.[2], Olsen R. E.[3], Peres, H. [1,2], Oliva-Teles, A.[1,2]

*rmagalhaes@ciimar.up.pt
[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal.
[2]Departamento de Biologia, Faculdade de Ciências, University of Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal.
[3]Department of Biology, Norwegian University of Science and Technology, Trondheim, N-7491, Norway.

Introduction
Presently, the utilization of plant feedstuffs and oils in commercial aquafeeds arise the necessity to evaluate the effects of dietary essential fatty acids (EFA), such as arachidonic acid (ARA) and docosahexaenoic acid (DHA), and carbohydrate levels, and possible interactions between them in marine fish welfare. For that purpose, the effects of dietary ARA/DHA ratio and carbohydrate level on liver and intestine oxidative stress status, liver histology, and intestinal microbiota were evaluated in gilthead sea bream juveniles.

Material and methods
Four isoproteic (47% crude protein) and isolipidic (18% crude lipids) diets were formulated to include 18% or 0% gelatinized starch (HS and LS diets) and dietary ARA/DHA ratios of 2.3/0.3 or 0/2.6 (ARA and DHA diets). Triplicate groups of fish (initial body weight = 47.5 g) were fed each diet to satiety for 84 days. At the end of the trial, the liver and intestine of 3 fish from each tank were collected for the determination of oxidative stress parameters and liver histology. Two other fish per tank were sampled under aseptic conditions for autochthonous (mucosa) microbiota characterization.

Results
No differences in growth performance were observed between dietary treatments, but HS diets increased feed efficiency and protein efficiency ratio. The ARA diets reduced liver and intestine lipidic peroxidation (LPO), and improved liver glutathione redox status (total, reduced (GSH) and oxidized glutathione (GSSG)) when combined with HS (Table 1). In the intestine, ARA diets reduced GSSG content and oxidative stress index (OSI). DHA diets increased hepatic SOD and GR activities but in the intestine, antioxidant enzymatic activity was not affected by the dietary EFA ratios (Table 2). HS diets increased liver OSI and reduced intestinal GSSG. HS also decreased LPO values, but only in DHA diets (Table 1). HS increased liver G6PDH, GR, and GPX (only in the DHA diets) activities but in the intestine, it decreased GR and SOD activities (Table 2). Neither dietary EFA nor carbohydrates induced major histomorphology alterations in the distal intestine but HS, when combined with DHA, promoted hepatocyte hypertrophy and alterations in nuclei position (Fig. 1). Dietary ARA modified mucosa bacterial profile by reducing the number of operational taxonomic units, richness, and diversity, and promoted similarity between bacterial communities. In conclusion, a high dietary ARA/DHA ratio reduced oxidative stress (LPO) and hepatocyte histomorphological alterations.

Acknowledgments
This work was supported by the Fundação para a Ciência e a Tecnologia, Portugal and Fundo Europeu de Desenvolvimento Regional (FEDER), from COMPETE 2020-Programa Operacional Competitividade e Internacionalização (POCI) (Project Eicobream: PTDC/MAR-BIO/1949/2014). This research was partially supported by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through national funds provided by FCT. Magalhães, R. was supported by an FCT grant (SFRH/BD/115870/2016) and European social fund (ESF). Martins, N., Fontinha, F., and Moutinho, S., were supported by an FCT grant (SFRH/BD/137919/2018, 2020.07212.BD, SFRH/BD/138224/2018, respectively).

(Continued on next page)

Table 1. Liver and intestine total glutathione (tGSH), oxidized glutathione (GSSG), reduced glutathione (GSH), oxidative stress index (OSI), and lipid peroxidation (LPO) levels of gilthead sea bream fed the experimental diets.

	Liver					Intestine				
Diets	**ARA/HS**	**ARA/LS**	**DHA/HS**	**DHA/LS**	**SEM**	**ARA/HS**	**ARA/LS**	**DHA/HS**	**DHA/LS**	**SEM**
tGSH	4892^{b}	4857	^{A}3665^{a}	^{B}4749	154.1	1847	1995	2170	1606	102.1
GSSG	37^{b}	52	^{A}27^{a}	^{B}59	3.3	80.0	63.0	128	77	6.5
GSH	4855^{b}	4805	^{A}3637^{a}	^{B}4690	153.7	1767	1932	1878	1529	92.2
OSI[1]	1.5	1.9	1.5	2.0	0.1	8.7	7.0	11.8	10.3	0.6
LPO	18.6	18.2	18.9	22.9	0.6	55.2^{a}	65.0^{a}	^{A}104.0^{b}	^{B}244.9^{b}	17.0
Two-way ANOVA	**Variance source**					**Variance source**				
	EFA	**CHO**	**Interaction**			**EFA**	**CHO**	**Interaction**		
tGSH	0.018	NS	0.044			NS	NS	NS		
GSSG	NS	0.000	0.038			0.006	0.003	NS		
GSH	0.019	NS	0.048			NS	NS	NS		
OSI[1]	NS	0.021	NS			0.006	NS	NS		
LPO	0.027	NS	NS			0.000	0.000	0.009		

Values presented as means (n = 9) and pooled standard error of the mean (SEM). LPO values expressed as nmols MDA g^{-1} tissue and GSH, tGSH, and GSSG as nmol g^{-1} tissue. Two-way ANOVA: ns: non-significant (P > 0.05). The capital letter means differences between the CHO level in each EFA and subscript letters between EFA in each CHO Level.

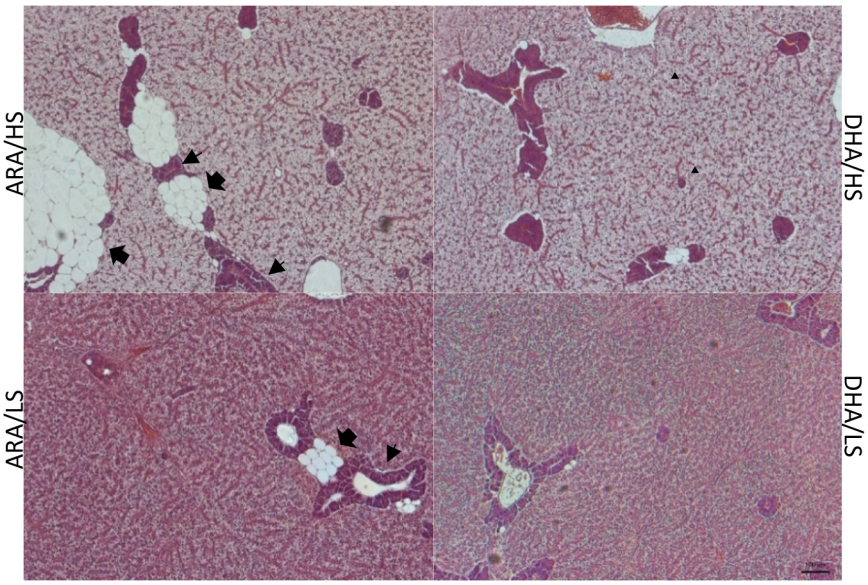
[1]OSI = 100 x (2 x GSSG/tGSH).

Table 2. Liver and intestine antioxidant enzymes activity of gilthead sea bream fed the experimental diets.

	Liver					Intestine				
Diets	**ARA/HS**	**ARA/LS**	**DHA/HS**	**DHA/LS**	**SEM**	**ARA/HS**	**ARA/LS**	**DHA/HS**	**DHA/LS**	**SEM**
CAT	857.6	787.6	1055.7	766.6	55.6	233.2	235.4	179.9	396.8	33.4
G6PDH	171.4	81.5	166.0	94.0	9.3	34.1	44.9	38.5	31.5	2.7
GR	2.0	1.6	3.7	1.9	0.2	27.2	35.2	27.6	31.7	1.3
SOD	444.3	370.2	749.1	489.1	53.6	2089.7	2748.4	2158.8	2555.3	114.3
GPX	45.2	49.8	^{B}63.8	^{A}41.9	3.1	15.0	17.9	15.6	15.6	1.0
Two-way ANOVA		**Variance source**				**Variance source**				
		EFA	**CHO**	**Interaction**		**EFA**	**CHO**	**Interaction**		
CAT		NS	NS	NS		NS	NS	NS		
G6PDH		NS	0.000	NS		NS	NS	NS		
GR		0.006	0.001	NS		NS	0.023	NS		
SOD		0.042	NS	NS		NS	0.022	NS		
GPX		NS	NS	0.030		NS	NS	NS		

Values presented as means (n = 9) and pooled standard error of the mean (SEM). Enzyme activities are expressed as mU mg $protein^{-1}$ for G6PDH, GR, and GPX and as U mg $protein^{-1}$ for CAT and SOD. Two-way ANOVA: ns: non-significant (P ≥ 0.05). The capital letter means differences between the CHO level in each EFA and subscript letters between EFA in each CHO Level.

Figure 1. Histomorphology features of gilthead sea bream juvenile's liver fed the experimental diets.



ARA/HS and DHA/HS diets show increased hypertrophy compared to diets ARA/LS and DHA/LS diets. DHA/HS shows decentralized nuclei (▲). Lipidic patches: ⬆ Pancreatic area: ▶ H-E staining.

IMPROVED GROWTH PERFORMANCE OF WHITELEG SHRIMP (*Penaeus vannamei*) USING A PHYTOGENIC FEED ADDITIVE

Alex Makol*, Tobias Aumiller and Karola R. Wendler

Delacon Biotechnik GmbH, Langwiesen 24, 4209 Engerwitzdorf, Austria
Email: alex.makol@delacon.com

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.

In summary, these studies indicate the benefits of supplementing Syrena® Boost, a specific formulation of selected phytogenics, being efficient to promote whiteleg shrimp growth performance and to optimize feed conversion ratio. All this supporting Syrena® Boost as an ideal phytogenic product to enhance the profitability production in a cost-effective way.

OXYGENATION OF SEA CAGES AND MONITORING OF OXYGEN FLUCTUATIONS IN TWO FISH FARMS IN GREECE

P. Makridis*, A. Grimpampi, E. Kakaridi, I.E. Papadakis, and A. Bergheim

University of Patras, Department of Biology, Rio Achaias, 26504, Greece
Email: makridis@upatras.gr

Introduction

Renewal of the water in sea cages is based on sea currents. During summer and early autumn, there is a critical phase for cultured populations as temperature may rise close to the tolerance level of the fish species cultured, and there is a consequent increase of fish metabolism and the need for an increased amount of oxygen. At the same time, the available amount of dissolved oxygen in seawater is lower (Makridis et al., 2018). An additional problem is that during this period of the year, fish pens exhibit increased fouling due to epiphytic organisms, which in its turn may decrease the renewal of the water inside a fish cage.

The aim of this study was to: i) monitor the effect of aeration of the water inside two cages (with and without aeration) by measuring the oxygen concentration every 30 min inside the cages throughout the experiment, ii) take samples before, in the middle, and at the end of the experimental period and measure the expression of important digestive enzymes in liver, intestine and pyloric ceca, iii) evaluate the state of the digestive system at the same sampling points through histological examination in liver, intestine and pyloric ceca, and iv) monitor the oxygen fluctuations in four cages at Galaxidi Marine Farms A/S during one year in four cages, two with seabass and two with gilthead seabream.

Materials and Methods

A population of seabass of approximately 14 tons was split into two cages in a fish farm in Greece (Zervas Kyriazis A/S) which was situated in the Vorios Evoikos Gulf (38°39′1.26″N, 23°6′13.34″E). The cages had a circumference of 40 m and a depth of 8 m. One optical oxygen and temperature sensor was used in each cage and a third one was used as a reference outside the cages. The probes were placed at a depth of 3.5 m and took a measurement every 30 min. The air was injected to the cage through a ring of AirX (Oxyvision A/S Norway) hose. The injection of air took place in one cage in two periods per day from 13th of August to 15th of September 2014, 9-12 a.m., and 9-12 p.m. Tissue samples were taken twice from liver, gut, and pyloric ceca of fish from both cages for histology analysis fixed in 4% formaldehyde and for enzyme gene expression stored in RNA later at -80ºC. Tissues were analyzed using histological methods (embedded in Technovit 7100, cut in sections of 5 μm, stained with methylene blue/azure II and basic fuchsin). The sections were then observed through light microscopy and photographed. Lipid deposition in liver was quantified by use of ImageJ software. The expression of two genes related to lipid metabolism was studied, phospholipase A2 (PLA2) and Hormone Sensitive Lipase (HSL). Phospholipase A2 catalyzes the hydrolysis of phospholipids and serves for the initial digestion of phospholipid compounds in dietary fat, while lipase hydrolyzes stored triglycerides to free fatty acids. Real-time qPCR was performed with housekeeping genes ACTb, L17 and EF1a.

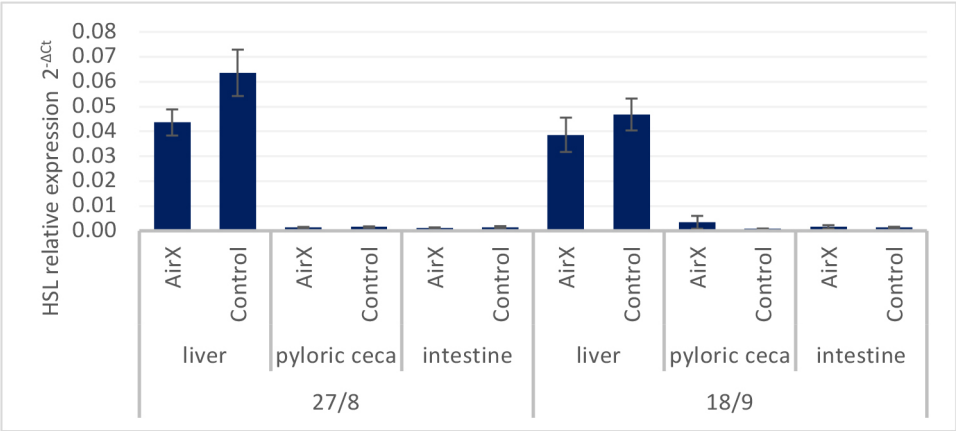


Fig. 1. Relative expression of hormone sensitive lipase in different tissues of seabass sampled during the first trial.

(Continued on next page)

In a second trial performed at Galaxidi Marine Farms A/S, four Guardian xO2 sensors (Meox A/S) for monitoring of oxygen and temperature were placed in two cages with seabass (*Dicentrarchus labrax*) and two cages with gilthead seabream (*Sparus aurata*). Data were recorded and transmitted in real-time through MEOX cloud for an 11-month period from April 2020 to February 2021.

Results and Discussion

In the first trial, the aerated cage showed a higher average oxygen saturation level and a lower FCR compared with the control cage. A higher percentage of lipid deposition was observed in liver of seabass in aerated cage compared with the control cage ($P<0.05$).

The expression of PLA2 increased in pyloric caeca samples from the aerated cage, suggesting that aeration improved the absorption rate of dietary phospholipids. Expression of HSL increased significantly in liver samples from the control cage, in comparison with the aerated cage (Fig. 1). Reduced oxygen levels induced lipolysis and mobilization of the stored triglycerides, to produce energy. The increased energy demand had negative impact on the growth rate of sea bass. A previous histological examination of the same sea bass samples revealed an increased fat accumulation in hepatocytes of fish in the aerated cage. The results of the present study confirm the lipolytic effect induced by low DO levels in farmed sea bass and induced by HSL, as has been shown in other fish species (Li et al., 2018).

In the second trial, oxygen saturation level reached 15% at different periods of the summer and early autumn indicating that there is a important problem with hypoxia in cages in the sea during the most productive period of the year.

References

Li H., Xu, W., Jin, J., Yang, Y., Zhu, X., Han, D., ... & Xie, S. (2018). Effects of starvation on glucose and lipid metabolism in gibel carp (*Carassius auratus gibelio* var. CAS III). Aquaculture, 496, 166-175.

Makridis P., Mente E., Grunvig H., Koutsikopoulos C., and Bergheim A., 2018. Monitoring of oxygen fluctuations in seabass cages (*Dicentrachus labrax* L.) in a commercial fish farm in Greece. Aquaculture Research 49(2), 684-691.

DETERMINING SKIN PIGMENTATION OF GILTHEAD SEABREAM *Sparus aurata:* A COMPARATIVE APPROACH OF CHROMATOMETRY AND DIGITAL PHOTOGRAPHY

E.E. Malandrakis and C. Zantioti

Laboratory of Applied Hydrobiology, Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.
E-mail: emalandrak@aua.gr

Introduction

In aquaculture industry, among other production challenges, is the inconsistency of fish skin coloration between different batches produced. This pitfall, although in a less extend compared with more colorful fish species, can be considered for gilthead seabream (*Sparus aurata*). Pigmentation biology, species characteristics and, defects have been extensively reviewed, for gilthead seabream and Sparidae in general, by Pavlidis and Mylonas (2011). Many factors may affect seabream skin pigmentation in aquaculture, such as feed ingredients (Gouveia et al., 2002, Pulcini et al., 2020) and genetics (Bertolini et al., 2020). Moreover, in intensively reared fish species, stressors or other environmental factors substantially affect skin coloration (Pavlidis et al., 2008). Therefore, the aim of the present study was to find a proper way to analyze skin pigmentation patterns of gilthead seabream, with a view to standardize optimal coloration patterns.

Materials and methods

Gilthead seabream individuals (>350g) produced from different production sources, were purchased from the fish market. Fish were weighted and both total and standard length were measured. Twenty-five (25) spots were measured on each side of the fish (50 in total for each fish) as presented in Figure 1, with a Konica Minolta CM-600d Spectrophotometer at an 8mm target spot. CIELAB coordinates were determined for each spot. Each fish was photographed in the left lateral and frontal sides with a high-resolution digital camera mounted on a tripod in a photo box with two LED lamps (2x1890LM - 5600K). The distance between the camera and the fish was 50cm and color calibration was carried out with a Colour Checker. RGB color values were determined by randomly selecting six pixels for three images per spot and per fish, in characteristic areas of gilthead seabream (both frontal and lateral).

In order to check the integrity of testing correlation analysis was applied between both sides of each fish and the differences among different batches were tested with ANOVA at a 0.05 significance level.

Figure 1 Skin spots tested with the chromatometer

(Continued on next page)

Results

Significant positive correlation patterns between both sides were reported only for the lightness value (L) with high correlation coefficients (R^2>0.85). On the other hand, the parameters a and b (red-green, blue-yellow), correlations between both sides were weak. In general, the application of the spectrophotometer exhibited high inconsistency. RGB values, exported from the digital photographs, exhibited higher consistency and, in some cases, they could discriminate fish between batches.

Discussion

Skin pigmentation in fish is usually measured in specific body areas with a colorimeter. Digital image analysis has the advantages of examining the pigmentation of the whole body or selecting specific areas and applying uniform photography conditions. Digital photography was considered as a more accurate technique to discriminate among fish batches. The heterogeneity of the skin renders the use of the chromatometer difficult to apply, since the specific circular area of 8mm diameter includes many different colours and tones. Therefore, digital photography of the skin is an appropriate tool to study pigmentation patterns, create prototype colours and apply them in nutritional trials and selective breeding.

Acknowledgements

This study has been funded by the Operational Programme Maritime and Fisheries 2014-2020 and co-funded by the European Maritime and Fisheries Fund (MIS 5074567).

References

Bertolini, F., Ribani, A., Capoccioni, F., Buttazzoni, L., Utzeri, V.J., Bovo, S., Schiavo, G., Caggiano, M., Fontanesi, L. and Rothschild, M.F. (2020), Identification of a major locus determining a pigmentation defect in cultivated gilthead seabream (*Sparus aurata*). Animal Genetics, 51: 319-323.

Gouveia, L., Choubert, G., Pereira, N., Santinha, J., Empis, J. and Gomes, E. (2002), Pigmentation of gilthead seabream, *Sparus aurata* (L. 1875), using *Chlorella vulgaris* (Chlorophyta, Volvocales) microalga. Aquaculture Research, 33: 987-993.

Pavlidis, M.A. and Mylonas, C.C. (2011) Sparidae: Biology and aquaculture of gilthead sea bream and other species. Oxford, UK: Blackwell Publishing Ltd.

Pavlidis, M., Karkana, M., Fanouraki, E. and Papandroulakis, N. (2008) Environmental control of skin colour in the red porgy, *Pagrus pagrus*. Aquaculture Research, 39: 837-849.

Pulcini D., F. Capoccioni F., Franceschini S., Martinoli M. and Tibaldi E. (2020) Skin Pigmentation in gilthead seabream (*Sparus aurata* L.) fed conventional and novel protein sources in diets deprived of fish meal. Animals 10, 02138

Ribeiro, A.R., Gonçalves, A., Barbeiro, M., Bandarra N., Nunes M. L., Carvalho M. L., Silva J., Navalho J., Dinis M. T., Silva T. and J. Dias (2017) *Phaeodactylum tricornutum* in finishing diets for gilthead seabream: effects on skin pigmentation, sensory properties and nutritional value. Journal of Applied Phycology 29, 1945–1956

EFFECTS OF TEMPERATURE ON HATCHING RATE AND EARLY LARVAL DEVELOPMENT OF LONGFIN YELLOWTAIL *Seriola rivoliana*

Minerva Maldonado-García *[1], Miriam Viader Guerrero [1], Laura T. Guzmán-Villanueva [2], Milton Spanopoulos-Zarco [3], José Antonio Estrada Godínez [4], Deneb Maldonado García [2], Vicente Gracia López [1], Alexia Omont [1]

[1] Centro de Investigaciones Biológicas del Noroeste S.C. Av. Instituto Politécnico Nacional 195. Col. Playa Palo de Santa Rita Sur, 23096. La Paz, Baja California Sur, México
[2] CONACYT-CIBNOR Av. Instituto Politécnico Nacional 195. Col. Playa Palo de Santa Rita Sur, 23096. La Paz, Baja California Sur, México
[3] Universidad Autónoma de Baja California Sur, Carretera Sur KM 5.5, 23080, Baja California Sur, México
[4] Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen s/n, Col. Los Pinos, Mazatlán, Sinaloa, 82000, México
Email: Minerva Maldonado-García (minervam04@cibnor.mx)

Introduction

The longfin yellowtail *Seriola rivoliana* has been recognized as a potential species for aquaculture diversification and as relevant for commercial farming (Sicuro and Luzzana, 2016). However, optimal culture conditions and reproduction protocols for this species are scare. Survival past the larval stage has been up until now a bottleneck in the larvae early development process and is extremely variable between reports depending on the rearing conditions (Pacheco-Carlón *et al*., 2021). The temperature has been determined as the main physicochemical factor that performs a fundamental role in producing high-quality fish larvae (Burt, Hinch and Patterson, 2011). Water temperature exerted a direct influence on the accumulation of yolk in the eggs and consequently affected the larval development events by stimulating the growth of various organs, mouth opening, initial inflation of the swim bladder and eye pigmentation of *L. peru* (Moreno Figueroa, 2011). Therefore, the accurate timing of each step of physiological development becomes critical for the fish larvae to receive their first feeding, and finally govern optimal hatchability, the highest survival rates and successive growth (Neuheimer, MacKenzie and Payne, 2018).

Consequently, the purpose of this research was to find out the optimal temperature for hatching time, survival rate, growth performance, biochemical quality and early development (eye pigmentation and mouth opening) of the Longfin yellowtail *Seriola rivoliana* larvae.

Materials and Methods

Breeders are wild-sourced fish that have been maintained in captivity for 4 years by the Mexican company King Kampachi, inside the facilities of CIBNOR, La Paz, Mexico. Fertilized eggs were obtained by natural spawning from a batch of 20 *S. rivoliana* breeders (1:1 female:male). The selected spawning (1.1 L) was obtained in August 2018 (concentration: 1821 ± 74 eggs/ml; diameter: 1.1 ± 0.002 mm).

The effect of fourteen temperatures was evaluated on the larval development of *S. rivoliana*: 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 32, and 34°C, all with a ± 0.3°C variation. The experiment was carried out in 100-L containers, used as temperature incubators, filled with filtered seawater. Inside each 100 L tank, 100 fertilized eggs were deposited inside six 1-L containers (to determine hatching rate and survival time) and 1 ml of eggs in six 4-L containers (to determine notochord length, growth rate, yolk sac and oil droplet volumes, mouth opening, eye pigmentation and larvae proximal composition), all filled with filtered seawater. Larvae were unfed during the experiment.

Results

The 23°C comfort temperature that showed the significantly highest hatching rate (75.5%) of *S. rivoliana* fertilized eggs. The longer survival times were observed for the larvae hatched in the 22, 24, 26, and 28°C containers, which varied from 90 hours after hatching (hah), up to 120 hah. (Figure 1).

Larvae hatched at 23°C revealed the significantly highest initial notochordal length (2.97 ± 0.02 mm). At 96 hah, only 24°C (72 CTU) larvae presented notochord sizes (2.72 ± 0.09 mm) statistically indifferent compared to the initial length (2.65 ± 0.06 mm).

(Continued on next page)

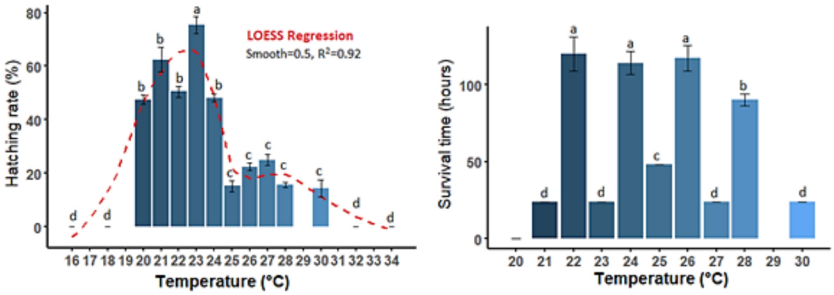


Figure 1. Hatching rate and survival time of *Seriola rivoliana* larvae cultured at different temperatures (°C). Values are given as mean ± standard error (n = 100). Different superscripts indicate a significant difference between treatments. The red dashed line represents the growth rate curve as a function of temperature, generated using LOESS regression.

The higher the incubation temperature, the faster the larvae yolk sac and oil droplet was consumed. Larvae incubated at 22°C and 24°C had a significantly higher amount of protein and lipid than larvae hatched at upper temperatures (26 and 28°C).

Larvae hatched at 24°C started to show pigmentation in the ocular area at 24 hah, while larvae hatched at 22°C presented their first eye pigmentation at 48 hah. By the end of the experiment (96 hah) larvae hatched at 24°C reached an eye pigmentation area of 76.3 ± 2.4 $\times 10^3$ μm^2 and larvae hatched at 22°C, an eye pigmentation area of 54.6 ± 1.2 $\times 10^3$ μm^2. Finally, at 96 hah, larvae hatched at 22°C (88 CTU), had significant wider lateral jaw and a larger mouth opening compared to larvae hatched at 24°C (96 CTU).

Conclusion

Thanks to this study, it can be concluded that modest changes in hatching and larvae rearing temperature can lead to large changes in the survival, growth and larval development for further feeding. According to the results obtained, we recommend incubating the eggs at 23°C to obtain the highest hatching rate. Then, increasing the temperature to 24°C (0.5°C·h^{-1}) to increase the survival rate of larvae during the first 48 hours (48 CTU) and finally reducing incubating temperature to 22°C (0.5°C·h^{-1}) in order to allow larvae to reach an optimal mouth formation for the further endogenous food intake and simultaneously, a sufficient eye pigmentation. The present results could be used for the management of this specie in the aquaculture industry, as well as provide information that would help to acknowledge the effect of temperature on the use of energy reserves at larval development of *S. rivoliana* in captivity.

References

Burt, J. M., Hinch, S. G. and Patterson, D. A. (2011) doi: 10.1007/s11160-010-9179-1.
Moreno Figueroa, L. D. (2011) Available at: http://cibnor.repositorioinstitucional.mx/jspui/handle/1001/229.
Neuheimer, A. B., MacKenzie, B. R. and Payne, M. R. (2018) doi: 10.1126/sciadv.aar4349.
Pacheco-Carlón, N. *et al*. (2021) doi: 10.1111/are.14871.
Sicuro, B. and Luzzana, U. (2016) doi: 10.1080/23308249.2016.1187583.

A POLICY DEFINITION OF INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) AS A MEANS TO STIMULATE ITS UPTAKE IN THE EUROPEAN ATLANTIC AREA

E. Malta[1*], M.M. Agraso-Martínez[1*], M.B. Dunbar[1*], M.E. Cunha[2*], B. Jacquemin[3*], J. Ratcliff[4*] and L. Ribeiro[2*]

1 - CTAQUA- Andalusian Aquaculture Technology Center, Muelle Comercial s/n, 11500, El Puerto de Santa María (Cádiz), Spain
2 - IPMA - Instituto Português do Mar e da Atmosfera, Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
3 - Centre d'Etude et de Valorisation des Algues, 83 Presqu'île de Pen-Lan, 22610, Pleubian, France
4 - Irish Seaweed Consultancy, Ryan Institute Annex, National University of Ireland, Galway, Ireland
E-mail: e.malta@ctaqua.es

Introduction

Integrated Multitrophic Aquaculture (IMTA) is an innovative and sustainable aquaculture practice combining cultivation of species at two or more different and complementary trophic levels that ultimately minimizes waste and optimizes resources. Although application of this concept has a history of centuries in various parts of the world, commercial uptake of the concept in western aquaculture is still limited. As part of the Integrate project, we attempted to identify the bottlenecks hampering the implementation of the IMTA concept in the European Atlantic Area (Spain, Portugal, France, Ireland and the UK). In round table discussions with the main stakeholders, a common problem appeared: although the conceptual definition of IMTA was clear, a more utilitarian definition making some of the details explicit was necessary, for instance for regulatory purposes. In addition, by doing this, it would also help preventing administrations from inventing their own definitions and interpretations, something that is already happening at least at regional levels and that in the long run could easily lead to confusion and "dilution" of the original meaning of the concept. Here we explain the process we used to come to this definition and discuss its main results.

Methods

The infographic (Fig. 1) summarizes both the methods and the main results. In short, expert opinions of stakeholders (aquaculture, administration, environmental NGOs, consumers, academic, etc.) from the European Atlantic Area were recollected in three steps:

- Thematic national workshops were organised in the participating countries on the technical, economic, social/legislative and environmental aspects of IMTA.
- A questionnaire was sent out to experts from academics, industry and administration sectors.
- The organisation of an international expert workshop. Here, the results were discussed both in separate groups (one for land-based IMTA and one for sea-based IMTA) and in a plenary session.

Results

Reports with the results from the thematic workshops, questionnaire and the final workshop are all available on the project's webpage (http://integrate-imta.eu/category/downloads/).

There are widely contrasting opinions on the desirability of defining the IMTA concept, nevertheless, there was clear consensus that it is an essential step in furthering the development of the industry.

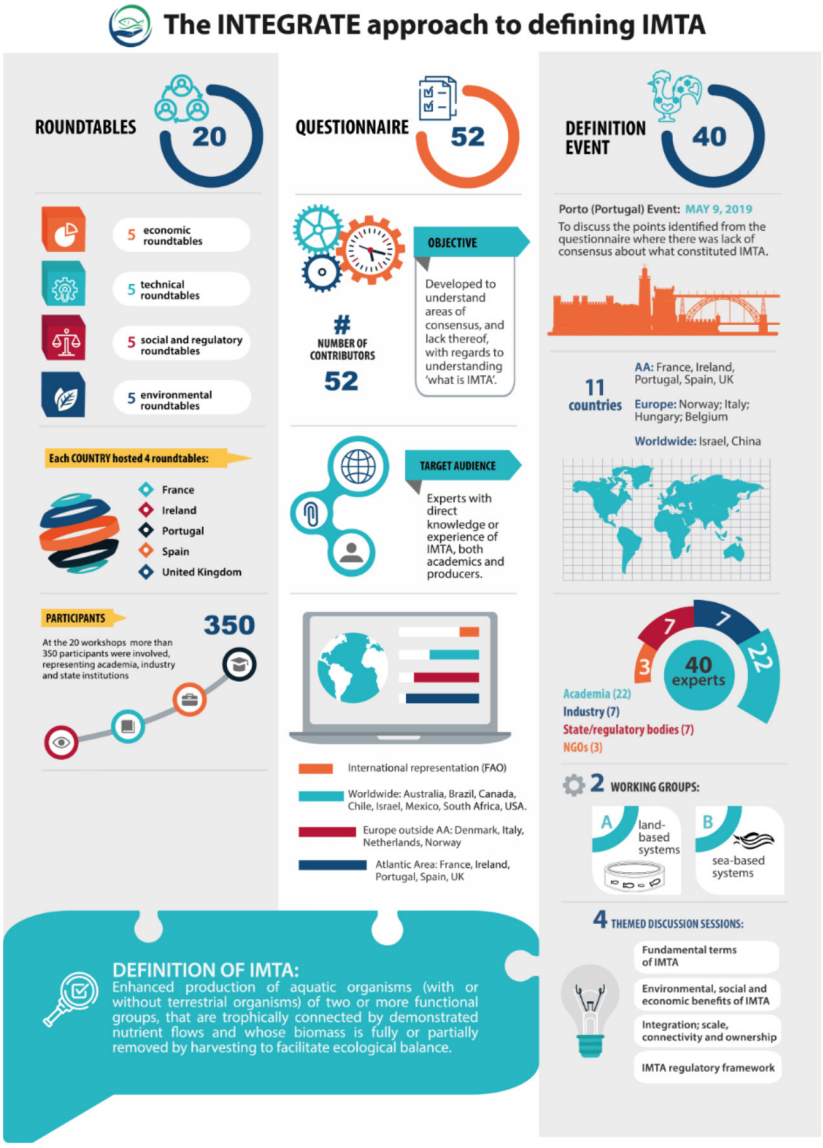
It was concluded that the definition should contain reference to the following components:

- IMTA should be principally aquatic
- Demonstrated flow of nutrients
- Between 2 (or more), managed, functional groups
- The secondary (tertiary etc.) species must be harvested

Furthermore, it was also widely felt that the definition should NOT go into details as degree of connectivity, number of species, etc. or include social or economic performance markers. Taking the above in considerations, the following consensus definition was reached:

IMTA = Enhanced production of aquatic organisms (with or without terrestrial organisms) of two or more functional groups, that are trophically connected by demonstrated nutrient flows and whose biomass is fully or partially removed by harvesting to facilitate ecological balance.

(Continued on next page)



Discussion

We are aware that defining the concept of IMTA is a topic that is not without controversy. It should be stressed that the definition has not been made for the mere sake of defining, nor that it should be interpreted as a fundamental textbook definition. Rather the aim is for it to be useful in policy terms, to facilitate funding and also to enable national governments to be better able to direct licensing among others. We will discuss the further steps that can be taken based on this definition to stimulate the commercial uptake of this concept, for instance in improving consumer acceptance of aquaculture (for instance by means of an ecolabel) and the uses it can have in integrated coastal zone management and spatial planning, topics that are currently being implemented by the Interreg AQUA&AMBI project.

Acknowledgements

We would like to thank all partners of the Integrate consortium for their contribution to this work and all participants of the expert roundtables, respondents of the questionnaire and participants of the IMTA workshop in May 2019. INTEGRATE is funded by the ERDF through the INTERREG Atlantic Area 2014-2020 Programme (project grant number EAPA_232/2016).

GROWING SEAWEEDS FOR POLYSACCHARIDES EXTRACTS AS POTENTIAL IMMUNOSTIMULANTS IN FISH AQUACULTURE

E.-j. Malta*, B. Partida, M. Macías, J. Cabello, I. Folgueira, M.M. Agraso Martínez

Centro Tecnológico de Acuicultura de Andalucía (CTAQUA), Muelle Comercial S/N, 11500, El Puerto de Santa María, Spain
Contact person: e.malta@ctaqua.es

Introduction

Research on the use of seaweeds in aquaculture feeds has been growing exponentially over the last few years. We have been building a literature database over the last few years. First publications date from the 1980s, with a total of only eight references in the past century, 19 in the first decade of this century and no less than 116 in the period 2011-2020. Moreover, 99 of these are from the last five years. Of this total of 143 references, 85 are studies on the use of seaweeds in fish aquaculture, indicating that this a topic of high scientific interest. The majority of the studies focus on the use of whole seaweed meal, mainly as a source of protein and carbohydrates substituting basically wheat, soy and to a lesser extent, fish meal, in artificial diets for aquaculture. Only in the last five years, studies started concentrating on the use of seaweed extracts as potential functional feed components, where the sulphated polysaccharides appear to be of particular interest (e.g. Coste et al., 2015).

At CTAQUA, we started the IMMUNO&ALGAE project in January 2019 with the aim to further explore the use of seaweeds in fish aquaculture as a source of functional feed compounds, in particular that can have immunostimulating effects. Objectives of the project are to optimize and upscale the cultivation of three pre-selected seaweed species from the southwest of Spain, to optimize the extraction of sulphated polysaccharides and to evaluate their use as potential immunostimulating compounds in finfish aquaculture. Seaweeds were selected based on their commonness in the area, prior experience with cultivation and their apparent potential for upscaling cultivation. In addition, the ambition was to test at least one species of each of the major seaweed groups. This led to the selection of the chlorohyte alga *Ulva ohnoi*, the rhodophyte *Gracilaria gracilis* and the phaeophyte *Dictyota dichotoma*.

The first phase of the project was dedicated to the optimization of the cultivation, to experiment with different cultivation conditions and to obtain biomass, either from culture or from harvest of wild populations, to obtain a sufficient amount of polysaccharide extract for inclusion in experimental diets. This phase is nearing completion and we here present the first results of these efforts and the next steps planned in the project.

Material and Methods

Ulva ohnoi was isolated from an earthen pond extensive aquaculture system in the SW of Spain in 2017 and maintained in culture at CTAQUA since then. The majority of the biomass (90%) used for polysaccharide extraction was grown at CTAQUA, 10% is from harvest from wild populations. Its taxonomic status has been confirmed by DNA sequence analysis. *Gracilaria gracilis* was isolated from the same earthen pond and brought to CTAQUA for cultivation trials. Additional biomass was harvested at the end of 2020 and beginning of 2021 (delay due to pandemic) for extraction. *Dictyota dichotoma* biomass was harvested from the La Caleta beach, city of Cádiz. Unfortunately, no sufficient biomass could be harvested for nutritional trials, also due to the lower percentage of polysaccharides in the algae and the potential confusion with the exotic seaweed *Rugulopteryx okumurae* that recently invaded the area.

As a first step towards cultivation, algal fronds were grown in filtered (1 µm) natural seawater under constant temperature, light and nutritional conditions in an attempt to obtain unialgal cultures. To increase the amount of clean biomass, cultivation volumes were increased stepwise from 250 mL to 5 L bottles. In the next step, algae were grown indoor in photobioreactors under LED lighting at controlled temperature, following a specific nutrient addition regime. Finally, for larger scale cultivation, outdoor tanks were deployed and cultivation was followed through an annual cycle with weekly harvests and weekly nutrient addition.

Polysaccharide extraction was carried out on dried algal biomass by the research group "Photobiology and Biotechnology of Aquatic Organisms" of the University of Málaga (Spain) using repetitive cycles of hot water extraction followed by selective precipitation, purification, flocculation and dialysis. Finally, dry extracts were obtained by lyophilization (see Abdala Díaz et al. 2019 for details).

(Continued on next page)

Results and Discussion

Clean cultivation for *U. ohnoi* was obtained at the first attempt and clean, fast-growing fronds were obtained. For *G. gracilis*, despite various attempts, including cultivation of the youngest growth tips only, cleaning with diatom sand, etc., completely unialgal cultures could not be obtained. Nevertheless, epiphyte growth could be maintained at low levels using a specific pulsed nutrient addition regime. For *D. dichotoma*, no successful cultures could be obtained and more studies are required for this species.

For both *U. ohnoi* and *G. gracilis*, steady growth was obtained in photobioreactors with growth rates ranging between 10 – 13 % d^{-1} and stable weekly biomass yields, although occasional collapses occurred in *U. ohnoi*. In a separate project, the potential relation with the algae microbiome is studied. *U. ohnoi* could be successfully cultivated in outdoor tanks all year long, with maximum biomass yields in spring (May – June) and minimum in winter (Jan – Feb). The annual cycle is still not completed for *G. gracilis*, provisional results also indicate that spring is the most favourable period, whereas in late summer growth rates decreased to close to zero, most likely due to high temperatures (≥ 30 ° degrees daily maximum temperature).

As a next step for the optimization of growth in photobioreactors and maximization of useful compounds, experimental trials are being carried out using diffent LED colours.

Polysaccharides could be successfully extracted from all algae, with the highest yields for *G. gracilis*, followed by *U. ohnoi* and *D. dichotoma*. Carbohydrate contents were considerably lower for the latter. Extracts are currently being tested for antibacterial activity. Subsequently, their potential immunostimulating effect will be tested *in vitro*, using cell line cultures and *in vivo* in a nutritional trial with sea bass. The most promising polysaccharide will finally be tested at different addition levels in a second nutritional trial, followed by a challenge study.

References

Abdala Díaz R.T., Casas Arrojo V, Arrojo M.A., Cárdenas C. Dobretsov S and Figueroa F.L. (2019). Immunomodulatory and Antioxidant Activities of Sulfated Polysaccharides from *Laminaria ochroleuca*, *Porphyra umbilicalis*, and *Gelidium corneum*. Marine Biotechnol 21:577–587.

Coste O., Malta E.j., Callejo López J.A. and Fernández-Díaz C. (2015). Production of sulfated oligosaccharides from the seaweed *Ulva* sp. using a new ulvan-degrading enzymatic bacterial crude extract. Algal Res 10:224-231.

Acknowledgments: This project has received funding within the framework of the call for subsidies on a competitive basis corresponding to the 2018 call for subsidies for the implementation of R&D&I projects of the Andalusian Autonomous Government no. PY18-RE-0006.

THE EFFECT OF DIFFERENT LIVE FEED ORGANISMS ON LARVAL BALLAN WRASSE (*Labrus bergylta)*

A.M. Malzahn*[1], B. Kvæstad[1], A. Sarno[1], E. Kjørsvik[2], L. García-Calvo[2], A.S. Norberg Aase[2], H. Hagen[2], B.H. Hansen[1], D. Ribicic[1], R. Netzer[1], A. Hagemann[1]

[1] SINTEF Ocean, 7465 Trondheim, Norway. [2] Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway.
Email: Arne.malzahn@sintef.no

Introduction:
The use of cleaner fish is one important tool in the tool-box to combat the sea lice problem in salmon farming. The major proportion of wrasse used as cleaner fish by the Norwegian salmon industry is coming from capture-based activities, depleting wild stocks. Amongst the four wrasse species used, ballan wrasse (*Labrus bergylta*) is the only species which is also cultivated, though only about 30% of the approx. 3 million fish used per year is produced in aquaculture (Fiskeridirektoratet 2021). The major obstacle in wrasse cultivation preventing large scale production is the difficult first feeding phase and a lack of functional feeding protocols.

Like most marine fish larvae ballan wrasse do not accept inert diets as feed. The current solution to this problem is the use of rotifers as the first feed with a transition to artemia nauplii after about 2 weeks. The initiation of formulated diets usually takes place around 4 weeks into the life of a ballan wrasse larvae. Feeding regimes based on rotifers and artemia usually produce mixed results and are way from being optimal for the larvae (Øie, Galloway et al. 2017), and there is good evidence that effects implanted into any animal during early life history have lasting effects (Winick and Noble 1966).

Material & Methods:
In a 48-day start feeding experiment with ballan wrasse, we studied the feasibility of replacing (Phase 1) rotifers by an experimental cirriped diet or copepod nauplii (*Acartia tonsa*), and (Phase 2) replacing artemia by nauplii of the cirriped *Semibalanus balanoides*. During phase 3 all treatments received the same commercially available formulated diets. Four different treatments were studied: (1) Control: Rotifers-Artemia-Dry diets; (2) Copepods-Artemia-Dry diets; (3) Copepods-Large cirripedia-Dry diets; and (4) Small experimental cirriped diet- Large cirripeds-Dry diets. We sampled at each feed transition for a suite of different response variables such as growth, morphometry, gene expression, lipidomics, histology, and microbiology.

Results:
We found significant differences in mortality rates. Larvae started with copepods died at significantly lower rates than larvae started on either rotifers or small experimental cirripeds (Figure 1 A). The experimental cirripede diet comprised a mix of small and large cirripede nauplii at a ratio of 1:1, whereof the larger nauplii were too large for the larvae to ingest.

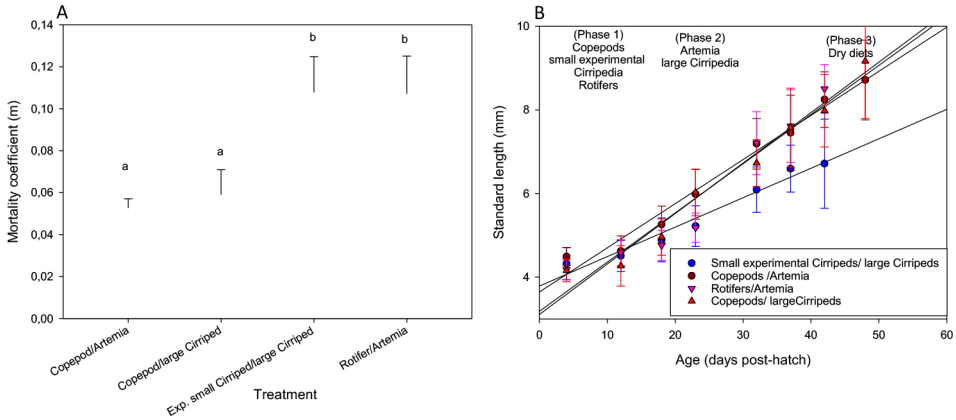


Figure 1 (A) Mortality coefficient of larval ballan wrasse (L. bergylta) fed different diets. (B) Growth in length of ballan wrasse (L. bergylta) larvae fed on different diets.

(Continued on next page)

This pattern was also reflected in early growth (Figure 1 B), copepods fed larvae grew faster than the other two groups. However, once the rotifer-started larvae were fed artemia, they picked up growth rates which were then comparable to larvae receiving copepods in the beginning. There was no pronounced difference between copepod started larvae feeding on artemia or large cirripeds during phase 2 of the experiment.

Gene expression studies at day 42 post-hatch revealed pronounced differences between the rotifer started group and the other three treatments which received natural, unenriched diets. Here, genes involved in fatty acid elongation, terpenoid biosynthesis and purine/pyrimidin metabolism were significantly higher expressed in the rotifer started group. Pronounced differences were also observed in amino acid metabolism. Within the three unenriched groups differences in gene expression were very small. Lipidomics revealed that a group of phosphatidylethanolamines (PE) correlated negative with larval growth rates, while a group of triacylglycerids (TAG) correlated positive with growth. Contrary, a group of monoacylglycerids (MAG) correlated positive with mortality rates.

Discussion & Conclusion:

The first diets in the life of the of the larvae clearly had effects on growth and mortality, but more important, clear effects of the first feed were still visible in both, gene expression pattern and lipidomics data at the end of the experiment. At this time, the larvae received other live feed organisms and the same formulated diets for three weeks. This clearly points towards a nutritional programming in larval fish, as described e.g. in mammals (Hou and Fuiman 2019). This concept opens unique opportunities to not only increase our knowledge on the importance of early life history nutrition in larval fish, but also to use this knowledge to program metabolic pathways early in the life of a fish to increase fish welfare throughout the whole lifespan.

References:

Fiskeridirektoratet. (2021). “Use of cleaner fish in the production of Atlantic salmon and Rainbow trout.” from https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Rensefisk.

Hou, Z. and L. A. Fuiman (2019). “Nutritional programming in fishes: insights from mammalian studies.” Reviews in Fish Biology and Fisheries.

Winick, M. and A. Noble (1966). “Cellular response in rats during malnutrition at various ages.” The Journal of nutrition **89**(3): 300-306.

Øie, G., T. et al (2017). “Effect of cultivated copepods (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*) larvae.” Aquaculture Nutrition **23**(1): 3-17.

GENETIC ESTIMATES TO COLD TOLERANCE IN GILTHEAD SEA BREAM (*Sparus aurata*)

I. Guerrero-Cózar, P. Gayo, C. Carballo, C. Berbel and M. Manchado*

IFAPA Center "El Toruño", Camino Tiro de Pichón, 11500 El Puerto de Santa María, Cádiz (Spain)
E-mail:mailto:Israel.guerrero@juntadeandalucia.es manuel.manchado@juntadeandalucia.es

Introduction

Temperature plays a key role in fish physiology as they cannot regulate their body temperature. As consequence, environmental thermal shifts trigger complex adaptive responses that include a wide range of physiological, metabolic and behavioral changes depending on the magnitude and time of such challenges. Gilthead sea bream, is one of the most important aquaculture species in Mediterranean basin. This species is produced under different systems such as inshore and off-shore cages, open flow land facilities and estuarine ponds. In this latter system, fish is highly exposed to severe thermal fluctuations between seasons with a special sensitivity to low temperatures in winter that triggers a specific pathological process named as "winter syndrome". Animals become lethargic and anorexic when water temperature drops close to 8ºC and lethality appears when these values are below 5ºC (Ibarz et al. 2010). Genetic breeding programs have been proven a useful approach to select animals with a better tolerance to extreme temperatures. This approach is highly sustainable to mitigate mortalities and losses in growth during winter (Charo-Karisa et al. 2005; Ma et al. 2007). The aim of this study was to evaluate the genetic estimates associated with cold tolerance of a F3-growth selected broodstock of gilthead sea bream. Heritabilities for survival at four times after an acute cold challenge were estimated. Data are relevant for genetic selection schemes to support aquaculture industry.

Materials and methods

Broodstock (males n = 32; females n = 38) used to generate families was a third generation (F3) from the PROGENSA® breeding program selected for weight at harvest. To generate families, mass spawning was induced by photoperiod modulation and eggs were pooled for four consecutive days. Hatched larvae were cultivated as previously described (Carballo et al. 2020). Animals cohabited in the same tank from larvae until performing the cold shock challenge at 248 days post-hatch. A total of 1 350 animals were randomly selected and distributed into four replicate tanks (0.640 m^3) connected to a recirculating system (RAS) equipped with a cooling, mechanical filter, skimmer, ultraviolet lights and biofilter. The number of individuals (mean weight 42.0 ± 16.1g) per tank ranged between 316 and 362. Animals were kept at 15.7±0.3 ºC (t_0) for 48 h before the challenge. Thereafter, temperature was decreased by 1.7°C per hour by setting the RAS cooler at 4ºC and adding ice made from seawater to the RAS water reservoir until reaching 8.2°C (threshold) in 4.2 h. To assess cold tolerance, more ice was added to rapidly reduce temperature below the threshold and loss of equilibrium (LOE) was set as sampling endpoint at three times: 0.5h (t_1) at 6.5º±0.3 ºC, 1.5h (t_2) 7.0º±0.2 ºC and 3.0h (t_3) at 6.5º±0.3 ºC. As temperature between t_2 and t_3 increased higher than 8ºC (LOE threshold), ice was newly added to the system between such two times. After t_3 sampling, cooler was set to 15ºC and animals were allowed for recovering and sampled 24 h (t_4) after starting the challenge. All alive fish in t_4 were considered as cold tolerant. Temperature was continuously recorded using a temperature data logger. Oxygen was always higher than 6 ppm and salinity was 37 ppt.

Weight was individually recorded in each sampling and caudal fin samples were taken for genotyping. DNA isolation, microsatellite analysis and parentage assignment were carried out as previously reported (Carballo et al. 2020). Genetic estimates were calculated using animal models fitted by REML in WOMBAT. Tank replicates were used as fixed factors. Cold tolerance was treated as a binary trait for cumulative LOE vs non-affected fish at each sampling point (t_1, t_2, t_3, t_4). Weight was added as covariate. Heritability (h^2) was transformed to the underlying liability scale according to Dempster and Lerner (1950).

Results and discussion

No mortality or disease signs were evident before the challenge. Animals displayed a normal swimming behavior at temperatures higher than 8ºC. The LOE was observed at temperatures lower than 7.2. Cumulative LOE ranged between 13.2 and 18.2% at t_1, 28.2-43.6% at t_2 and 50.6-60.4% at t_3. No additional mortality or behavior alterations were registered between t_3 and t_4 and hence the last sampling time was discarded for genetic estimates. Mean weight of sampled fish for LOE at each time point increased progressively from 33.6±15.2 g at t_1 to 40.6±15.1g at t_3. Mean weight of tolerant fish sampled at t_4 was 48.3±15.4 g. Genotyping assigned 85% individuals (n = 1 156) to a unique parent pair comprising 130 families that contained between 3 and 22 individuals. Familial variability for cold tolerance (t_3) ranged between 31 and 79%.

(Continued on next page)

Heritability estimate for weight was low (0.159±0.049). A similar heritability was observed in a study of our group four young seabream juveniles (Carballo et al. 2020), however, higher values (0.32) were obtained by Navarro et al. (2009) for a similar age. Our broodstock was a F3-growth selected generation for weight at harvest with a lower genetic variability explaining such differences. With respect to cold tolerance after an acute challenge, heritability on the liability scale ranged between 0.204±0.070 for t_3 and 0.225±0.092 for t_1. Previous genetic studies that evaluated tolerance as survival by cooling-degree-hours in long experiments found a wide range of heritabilities from 0.08 to 0.32 (Charo-Karisa et al. 2005; Ma et al. 2007). Interestingly, genetic correlations between weight and cold tolerance were positive and ranged between 0.230±0.244 for t_1 and 0.391±0.205 for t_3. Moderate-high positive correlations between cold tolerance and body weight or length are consistently observed in fish supporting that selective breeding for growth in sea bream could also improve on cold tolerance in the selected broodstocks.

Acknowledgment

This study was funded by the European Maritime and Fisheries Fund (EMFF) project PP.FEM.PPA201700.14 to MM. P.G. is recipient of a PhD fellowship from AEI.

References

Carballo C., Shin H.S., Berbel C., Zamorano M.J., Borrego J.J., Armero E., Afonso J.M., Manchado M. (2020) Heritability estimates and genetic correlation for growth traits and LCDV susceptibility in gilthead sea bream (*Sparus aurata*). Fishes 5, 2.

Charo-Karisa H., Rezk M., Bovenhuis H. & Komen H. (2005) Heritability of cold tolerance in Nile tilapia, *Oreochromis niloticus*, juveniles. Aquaculture 249, 115–23.

Dempster E.R., Lerner I.M. (1950) Heritability of threshold characters. Genetics 35, 212-36.

Ibarz A., Padrós F., Gallardo M.Á., Fernández-Borràs J., Blasco J. & Tort L. (2010) Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'Winter Syndrome'. Rev. Fish Biol. Fish 20, 539-56.

Ma L., Saillant E., Gatlin D.M., Neill W.H., Vega R.R., Gold J.R. (2007) Heritability of cold tolerance in red drum. North American Journal of Aquaculture 69, 381-7.

Navarro A., Zamorano M.J., Hildebrandt S., Ginés R., Aguilera C. & Afonso J.M. (2009) Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus* L.), under industrial conditions. Aquaculture 289, 225-30.

EFFECT OF THERMOCYCLES AND HORMONAL TREATMENTS WITH DOPAMINE INHIBITORS AND GONADOTROPIN-RELEASING HORMONE ON SPERM PRODUCTION AND QUALITY IN SENEGALESE SOLE

P. Gayo*[1], E. Fatsini[2], C. Berbel[1], E. Cabrita[2], R. Zerolo[3], M. Manchado[1]

[1]IFAPA Centro El Toruño, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
[2]Center for Marine Sciences-CCMAR, University of Algarve, 8005-139 Faro, Portugal
[3]CUPIMAR, Ctra. Carraca, s/n, Salina San Juan Bautista, San Fernando, Spain
Email: patricia.gayo@juntadeandalucia.es

Introduction

Senegalese sole is one of the most important aquaculture species in Southern Europe. However, its production is not yet sustainable since F1 broodstocks, unlike wild breeders, has no reproduction success. Artificial fertilization has been identified as a potential solution although currently it is limited by the low volume of sperm produced and the high individual variability in sperm quantity and quality (Beirao et al. 2009). Thus, more knowledge is required about the role of temperature and hormonal therapies. Physical factors such as temperature (García-López et al. 2009) and hormonal therapies such as the dopamine inhibitors (DIs) combined or not with gonadotropin-released hormone (GnRHa) (Guzman et al. 2011) have been reported as modulators of spermatogenesis and milt production . In this study, the effects of two DIs, metoclopramide and sulpiride, and the interaction with GnRHa were assessed in soles cultivated under weekly thermocycles. Sperm production and quality parameters were evaluated using Computer Assisted Sperm Analysis (CASA). Results are highly relevant to standardize artificial fertilization in sole.

Material and methods

A total of 27 male F1 breeders (mean weight = 684.6 ± 243.3 g) were randomly selected from genetic breeding program of CUPIMAR company. These animals were cultivated in open flow at constant temperature (20ºC) and moved to a 12 m^3 tank attached to a recirculation system (RAS) to apply the thermocycles. A total of 18 females (mean weight 1242.5 ± 436.6 g) were also added to simulate a functional broodstock unit. Before handling, fish were anesthetized with 2-phenoxyethanol (300 ppm) and males were evaluated for sperm production. In the RAS, initial temperature was set at 20 °C and then decreased 1ºC d^{-1} until 15 °C. Thereafter, four thermocycles ranging from 15 to 18ºC were applied as follows: 2 d at 15ºC, 2 d increasing 1.5 ºC d^{-1} until 18ºC, 1 d at 18ºC and 2 d decreasing 1.5 ºC d^{-1} until 15ºC. Hormonal treatments with DIs were applied in the thermophase of each cycle (always on wednesday). Three groups were established: 1) control (CTRL) injected with the carrier PBS (n = 9); 2) sulpiride, SUL (n = 9; 20 mg kg^{-1}); and 3) metoclopramide, MET (n = 9; 2 mg kg^{-1}). In the last thermocycle, five males of each group (CTRL, SUL and MET) were also injected GnRHa (25 μg/kg) remaining four animals as negative control. Sperm was collected in the fourth thermocycle at 48 h after hormone injection and immediately diluted 1:3 in Marine freeze®. Sperm evaluation was carried out at 3 h using IVOS II™ CASA system at 15 s after activation. In addition, sperm quality was also evaluated at 24 h for the motility parameters using the ISAS-CASA software. Statistical differences were examined using a GLM with DI groups and GnRHa stimulation as fixed factors in SPSS v25.

Results and Discussion

No mortality or sign of stress were observed during the trial. Before trial, only three out of 27 males (11.1%) were fluent. After four thermocycles and hormonal treatments, 70.4% of fish (nineteen out of 27 males) were spermiating. Statistical analysis did not show significant differences in the number of spermiating fish between DI and GnRH injected fish with respect to the PBS-injected CTRL indicating that thermocycles had a major role in the activation of sperm production in sole.

The volume of sperm collected for each animal ranged between 5 and 50 µl (average 16.2 ± 13.2 μl) without significant differences among DI- or GnRHa-treated groups. With respect to cell concentration and total spermatozoa production, no significant differences associated with DI treatments were found. However, GnRHa injection modified both traits with a significant interaction DI □ GnRHa. Soles from SUL group and injected with GnRHa increased significantly cell concentration (3.3-fold) and total spermatozoa (7.6-fold) with respect to non-GnRHa injected fish. In contrast, soles from the CTRL or MET groups injected with GnRHa remained as unresponsive and they did not modify or even reduced cell concentration (1.9-fold in the MET group) or total spermatozoa (2.8-fold in CTRL group) with respect to those non-GnRHa injected fish.

(Continued on next page)

Effects of hormones on sperm motility were similar to those obtained for sperm quantity parameters. While no significant differences associated with DI treatments were found, GnRHa injection increased values of total motility (TM) and progressive motility (PM) traits with a significant interaction DI GnRHa. GnRHa-injected fish from SUL group increased significantly TM (3.0-fold) and PM (4.0-fold) with respect to non-GnRHa injected fish. Moreover, GnRHa increased PM in fish from the MET group (1.5-fold) with respect to non-GnRHa injected fish. The CTRL group remained as unresponsive to GnRHa injection. With respect sperm velocities, GnRHa injection increased VCL and VSL in fish from MET and SUL groups (between 1.3- and1.6-fold) but not in CTRL with respect to non-GnRHa injected fish. All these data indicate the important role of DIs to modulate the response of GnRHa to enhance sperm production and quality.

When the effects of hormonal treatments on sperm quality were evaluated 24h after sperm collection, all parameters drastically dropped with an average loss of 72.1% and 65.7% for TM and PM, respectively and 27.6% and 16.4% for VCL and VSL respectively. It should be noted that PM and VSL reduction in fish from the SUL group (85 and 33.8 %, respectively) was significantly higher than CTRL group (58.3 % and 0.8 %, respectively). Nevertheless, the differences in such parameters associated with GnRH-injection in SUL and MET for were still observable after 24h.

In conclusion, thermoperiod increased the rates of fluent males. Moreover, SUL treatments improved the responses of GnRH to enhance spermatogenesis and improve sperm quality. The extender or did not preserve the sperm of hormonal treatments quality after 24h.

Acknowledgments
This work is funded by project ERANET-BLUEBIO COFUND “BestBrood” code PCI2020-111994/ AEI/10.13039/501100011033. PGL is granted with a predoctoral scholarship funded by AEI.

References

Beirao J., Soares F., Herraez M.P., Dinis M.T. & Cabrita E. (2009) Sperm quality evaluation in *Solea senegalensis* during the reproductive season at cellular level. Theriogenology 72, 1251-61.

García-López A., Sarasquete C. & Martínez-Rodríguez G. (2009) Temperature manipulation stimulates gonadal maturation and sex steroid production in Senegalese sole S*olea senegalensis* Kaup kept under two different light regimes. Aquac Res. 40:, 103-11.

Guzman J.M., Cal R., García-López Á., Chereguini O., Kight K., Olmedo M., Sarasquete C., Mylonas C.C., Peleteiro J.B., Zohar Y. & Mañanós E.L. (2011) Effects of in vivo treatment with the dopamine antagonist pimozide and gonadotropin-releasing hormone agonist (GnRHa) on the reproductive axis of Senegalese sole (*Solea senegalensis*). Comparative Biochemistry and Physiology - Part A 158, 235-45.

THE INTERNATIONAL AQUACULTURE FEED FORMULATION DATABASE (IAFFD): NUTRIENT FORMULATION TARGETS FOR DIFFERENT PRODUCTION SYSTEMS

Lukas Manomaitis*, Dominique P. Bureau, Neda Nemati, Sirri Kayhan

United States Soybean Export Council (USSEC)
541 Orchard Road , #11-03 Liat Towers, Singapore 238881
LManomaitis@ct.ussec.org

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. One of the primary targets for USSEC are aquaculture feedmills, as this is where soy enters aquaculture production value chain. USSEC identified a critical weakness in the aquaculture industry that generally does not exist in the terrestrial livestock industry, namely the availability of a standardized feed formulation database. Since 2014 USSEC has partnered with others in the aquaculture nutrition space to create the first known standardized aquaculture feed formulation database, now known as the International Aquaculture Feed Formulation Database (IAFFD).

Now entering v7.0, the IAFFD contains almost 700 feed ingredients in the Feed Ingredient Composition (sub) Database (FICD), and the Aquaculture Species Nutritional Specifications (ASNS) (sub) database has information on 31 species at different age groups (typically six for fish and four for crustaceans). This database has been made freely available to the public to help improve aquaculture feed formulation approaches and is available at www.IAFFD.com. It was envisioned that this database would be used for training purposes, and as a reference, but increasingly we are seeing the database being adopted, in full or in part, as an actual database by commercial industry

There are many novel characteristics to the IAFFD, but one of importance is that an attempt is being made to link target nutrient levels in the ASNS to specific production systems. Similar to how different species and different life stages may require different nutrient levels, it is anticipated that different production systems, from extensive to highly intensive (such as Recirculating Aquaculture systems, or RAS) may require different nutrient targets for optimal performance. As this approach is developed it will be important to get input from industry for what important parameters need to be considered to allow more targeted nutrient levels and what additional ingredient information may be needed. For example, in RAS it may be important to target faster growth and a different energy/protein level due to the cost of the production system and desired body conformation, and to add information on ingredients that quantifies their impact on water quality.

AN OPTIMISED NON-ACTIVATING MEDIUM FOR SHORT TERM STORAGE OF BARRAMUNDI *Lates calcarifer* MILT

A.F. Marc[ab*], J.L. Guppy[b], H. Marshall[a], D.R. Jerry[b], D. Rudd[a†], and D.B.B.P Paris[ab†]

[a] College of Public Health, Medical & Veterinary Sciences, James Cook University, Townsville, QLD 4811, Australia
[b] Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia
[†] The work was jointly conducted in the laboratories of these two senior authors
E-mail: adrien.marc@my.jcu.edu.au

Introduction

A reliable milt storage procedure is a critical requirement for the use of advanced reproductive techniques for farmed barramundi (*Lates calcarifer*). Specifically, integrating artificial fertilization within current barramundi selective breeding programs would eliminate challenges to efficient family production seen with the current mass-spawning approach (e.g. skewed parental contribution and uncontrolled pairings; Robinson et al., 2010). The success of artificial fertilization lies in an ability to store and handle gametes effectively, while also ensuring that the functional and structural integrity of gametes is maintained (Beirao et al., 2019). A previous study successfully stored spermatozoa collected from wild barramundi using marine Ringer's solution as a non-activating medium (NAM; Palmer et al., 1993). However, the outcome could not be replicated when using spermatozoa collected from captive-bred individuals; the use of marine Ringer's solution leads to major cell lysis within 30 minutes of incubation. As such, the cause of cell lysis was investigated, and the NAM composition was optimized to suit the cellular requirements of spermatozoa collected from captive-bred barramundi.

Materials and methods

The ionic and metabolite composition of seminal plasma from captive-bred barramundi (n = 10) were characterized to refine the non-activating medium (NAM) composition. The effect of NAM osmolality, pH, and Na^+ and K^+ concentrations on sperm quality were then examined using methods described in Marc et al. (2021). Milt samples were collected through testicular cannulation. Sperm motility parameters were evaluated by a computer-assisted sperm analyzer (CASA; AndroVision®, Germany). Sperm viability was assessed using a dual staining method Hoechst 33342/Propidium Iodine by flow cytometry (CyanADP, Beckman Coulter, USA). To assess the effect of NAM osmolality on spermatozoa, sperm samples (n = 5) were initially diluted 1:10 in NAMs adjusted to 260, 300, 350, 400, and 450 mOsm/kg and incubated at 4 °C for 1 h. Sperm motility and sperm viability were then assessed. After determining the optimal osmolality, sperm samples (n = 10) were used to assess the effect of NAM pH (i.e. 6.5, 7.4, 7.8, 8.1, and 8.5) on sperm motility. This trial was repeated with sperm samples (n = 7) using HEPES as a replacement for $NaHCO_3$ buffering agent and sperm motility was assessed after 1 h and 24 h incubation at 4 °C. Finally, sperm samples (n = 6) were used to investigate the effect of NAM NaCl/KCl ratio (i.e. 0/190, 140/50, 160/30, 185/5, and 190/0 mM) on sperm motility. Sperm motility was assessed after 1, 24, 48, 72, and 96 h incubation at 4 °C.

Results

The viability of barramundi spermatozoa was significantly higher after 1 h incubation in NAM adjusted to 400 mOsm/kg (78.2 ± 2.9%) when compared to the original NAM at 260 mOsm/kg (44.8 ± 3.3%; $P < 0.05$). However, sperm motility did not differ significantly between NAMs and remained low (23.5 ± 2.1%). After determining the optimal NAM osmolality, the effect of NAM pH was investigated. After 1 h incubation, sperm motility was negatively affected by the increase in pH, and was only observed at pH 6.5 after 24 h incubation (11.2 ± 2.4%). Biochemical analysis of the NAMs revealed the presence and an incremental increase of pCO_2 in NAMs with a pH between 7.4 and 8.5. Therefore, to determine the intrinsic effect of pH on sperm motility, the organic buffering agent $NaHCO_3$ was replaced by HEPES. After 24 h incubation in NAM buffered HEPES, sperm motility was at the highest at pH 7.4 (35.1 ± 3.3%), followed by 7.8 (29.7 ± 3.4%). Finally, the effect of NaCl/KCl ratio was tested. After 1 h incubation in NAM buffered HEPES adjusted at 7.4, spermatozoa stored in the 185/5 mM (56.8 ± 5.0%) and 190/0 mM (56.5 ± 4.3%) showed the highest sperm motility, while sperm motility was inhibited in the Na^+ free NAM. After 24 h incubation, 185/5 mM NAM had the highest sperm motility (47.7 ± 7.2%), and was able to maintain motility for up to 72 h.

(Continued on next page)

Discussion and conclusion

The characterization of the ionic composition and osmolality of seminal plasma provided valuable insight into the potential cause of damage to spermatozoa stored in MRS (Palmer et al., 1993). The original MRS had a total osmolality of 260 mOsm/kg, which was lower than the seminal plasma osmolality (mean: 396.1 ± 13.4 mOsm/kg) of captive-bred barramundi. This difference in osmolality caused the cell lysis as sperm viability was restored when the NAM osmolality was adjusted to 400 mOsm/kg. It is therefore possible that the osmolality of seminal plasma might fluctuate according to the environment and that MRS may be isotonically balanced with spermatozoa collected from wild barramundi inhabiting brackish environments. In this study, it was found that the presence of $NaHCO_3$ in the NAM was at the origin of the sperm motility inhibition. While the sperm motility mechanism in barramundi remained to be elucidated, this finding suggest that barramundi spermatozoa might be sensitive (1) to HCO_3^- as $NaHCO_3$ dissociates into HCO_3^- and H^+ at pH 7.4 and above or (2) to Ca^{2+} as HCO_3^- ionized at high pH into CO_3^{2-}, causing Ca^{2+} to precipitate in the form of $CaCO_3$, and rendered Ca^{2+} bio-unavailable for sperm activation. Furthermore, barramundi spermatozoa appeared to require Na^+ but not K^+ in the NAM to maintain activation potential as sperm motility was rapidly inhibited in Na^+ but not in K^+ free medium. Finally, the optimized NAM containing 185 mM NaCl, 5 mM KCl, and 10 mM HEPES at pH 7.4 allowed for the first time to reliably stored barramundi spermatozoa for 2 – 3 days at 4 °C, thereby facilitating the further development of advanced reproductive technologies for the species. These results lay the foundation for further studies looking at the regulatory role played by different ions in barramundi sperm motility to further improve medium composition.

References

Beirao, J., Boulais, M., Gallego, V., Brien, J.K.O., Peixoto, S., Robeck, T.R., Cabrita, E., 2019. Sperm handling in aquatic animals for artificial reproduction. Theriogenology 133, 161–178. https://doi.org/10.1016/j.theriogenology.2019.05.004

Marc, A.F., Guppy, J.L., Bauer, P., Mulvey, P., Jerry, D.R., Paris, D.B.B.P., 2021. Validation of advanced tools to evaluate sperm function in barramundi (*Lates calcarifer*). Aquaculture 531, 735802. https://doi.org/10.1016/j.aquaculture.2020.735802

Palmer, P.J., Blackshaw, A.W., Garrett, R.N., 1993. Successful fertility experiments with cryopreserved spermatozoa of barramundi, *Lates calcarifer* (Bloch), using dimethylsulfoxide and glycerol as cryoprotectants. Reprod. Fertil. Dev. 5, 285–293. https://doi.org/10.1071/RD9930285

Robinson, N.A., Schipp, G., Bosmans, J., Jerry, D.R., 2010. Modelling selective breeding in protandrous, batch-reared Asian sea bass (*Lates calcarifer*, Bloch) using walkback selection. Aquac. Res. 41, 643–655. https://doi.org/10.1111/j.1365-2109.2010.02584.x

TOWARDS A FREE WILD FISH AND SOY DIET FOR EUROPEAN SEABASS USING BY-PRODUCTS FROM FISHERY AND AQUACULTURE

A. Marchi[1,*] L. Parma[1], P. Nicole[1], L. Morsiani[1], L. Mariani[1], F. Dondi[1], E. Brini[1], M.C. Sabetti[1], P.P. Gatta[1], F. Capozzi[2-3], G. Picone[2], C. Di Gregorio[2], A. Di Biase[4], A. Bonaldo[1].

[1]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy
[2]Department of Agri-Food Science and Technologies (DISTAL), University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy
[3]Interdepartmental Centre for Industrial Agri-Food Research (CIRI), University of Bologna, Piazza Goidanich 60, 47521 Cesena, Italy
[4]Veronesi Holding S.p.A., Via Valpantena 18/G, 37142 Quinto di Valpantena, Verona, Italy
Contact: arianna.marchi5@unibo.it

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 (FAO, 2018). Meanwhile, the future development of aquaculture will be severely limited by the lack of proteins intended for animal feed (as a competitor of human nutrition). Fishery and aquaculture by-products can be considered as promising alternative feed ingredients for fish farming in terms of nutritional quality and availability; however these products are still underused resulting in economic and environmental issues (Gasco et al., 2020). At the same time, the need for limit the use of soy in fish diets has become necessary for the sustainability of aquaculture production. The effects of total replacement of wild fish meal, (FM) fishoil (FO) and soy product (SP) by using fishery and aquaculture by-products were tested on the growth, 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 contain increasing levels of fisheries and aquaculture by-product, up to a total replacement of wild FM and FO and SP. Diets were administered to triplicated fish groups of 50 seabass individuals (initial weight: 75.96±19.03g). Animals were reared in RAS for 119 days until triplicate their initial weight. Specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), somatic indexes and blood plasma biochemistry were detected. Also, 30 fish fillets per diet were sampled and then stored at -80 °C. A perchloric acid extraction was performed and a high resolution 600 MHz 1H Nuclear magnetic resonance (NMR) spectrum was recorded. 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 (Picone et al., 2011). Differences among treatments were considered significant at $P < 0.05$

Results
At the end of the trial, final body weight (FBW) was significant higher in fish fed C diet then 0FM100FO, 0FMFO and 0FMFO-50SP diets while in 0FMFO-0SP was the lowest. SGR was significant higher in fish fed C diet than 0FMFO, 0FMFO-50SP and 0FMFO-0SP. No significant differences in FI were detected between C and the other diets, while it was higher in 0FMFO than 0FMFO-0SP. FCR was significant lower in C diet then 0FMFO and 0FMFO-50SP diets. Viscerosomatic index and hepatosomatic index were both significant higher in fish fed 0FMFO-0SP than the other treatments while in C diet were the lowest.

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.

(Continued on next page)

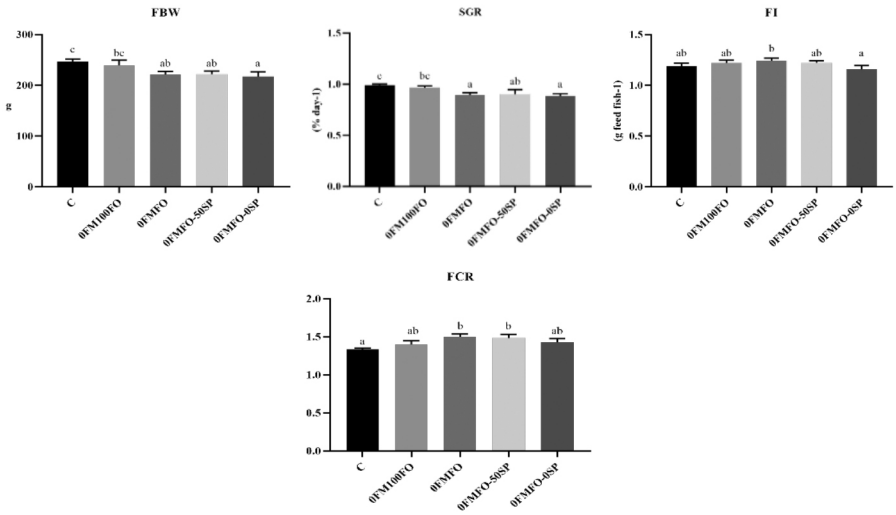


Fig 1. Growth performance and feed intake of E. seabass fed the experimental diets over 119 days.

Reference

FAO, a c. di. 2018. *Meeting the Sustainable Development Goals*. The State of World Fisheries and Aquaculture 2018. Rome.

L. Gasco, G.Acuti, P. Bani, A. Dalle Zotte, P.P. Danieli, A. De Angelis, R. Fortina, et al. 2020. «Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition». *Italian Journal of Animal Science* 19 (1): 360–72.

G. Picone, SB. Engelsen, F. Savorani, S. Testi, A. Badiani, F. Capozzi 2011. «Metabolomics as a Powerful Tool for Molecular Quality Assessment of the Fish Sparus Aurata.» *Nutrients* 3 (2): 212–27.

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/).

TRIPLOID RAINBOW TROUT *Oncorhynchus mykiss* AS A RECIPIENT OF BROWN TROUT GERMLINE STEM CELLS

Z. Marinović[1]*, J. Lujić[2], S. Sušnik Bajec[3], I. Djurdjevič[3], A. Snoj[3], B. Urbányi[1] and Á. Horváth[1]

[1]Hungarian University of Agriculture and Life Sciences, Department of Aquaculture, Páter Károly u. 1., H-2100 Gödöllő, Hungary
[2]Cornell University, Department of Biomedical Sciences, Center for Reproductive Genomics, Ithaca, NY 14850, USA
[3]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, Sl-1230 Domžale, Slovenia
E-mail: zor.marinovic@gmail.com; Marinovic.Zoran@uni-mate.hu

Introduction

Intra- or interspecific transplantation of germline stem cells (GSCs) such as spermatogonial and oogonial stem cells (SSCs and OSCs) has been recognized as an efficient tool for the conservation of genetic resources in fish (Yoshizaki et al., 2011). This methodology allows surrogate production of donor-derived gametes by the recipient individuals where the type of produced gametes (sperm or eggs) depends on the sex of the recipient fish. In our previous study, we tested the plausibility of transplanting brown trout and grayling GSCs into diploid rainbow trout recipients as a proof-of-concept which could be used for the conservation of many endangered Balkan trout species (Lujić et al., 2018)additional ex situ strategies such as surrogate production are needed. Steps required for transplantation such as isolation of high number of viable germ cells and fluorescent labeling of germ cells which are to be transplanted have been optimized. Isolated and labeled brown trout and grayling germ cells were intraperitoneally transplanted into 3 to 5 days post hatch rainbow trout larvae. Survival of the injected larvae was comparable to the controls. Sixty days after transplantation, fluorescently labeled donor cells were detected within the recipient gonads indicating successful incorporation of germ cells (brown trout spermatogonia and oogonia—27%; grayling spermatogonia—28%; grayling oogonia—23%. In the present study, we tested the plausibility of utilizing triploid rainbow trout as a surrogate for obtaining brown trout gametes, as triploid fish are generally sterile and would enable production of only donor-derived gametes after reaching maturity.

Material and methods

Three trials were conducted in order to test the plausibility of surrogate production of brown trout gametes from rainbow trout recipients. In the first trial, isolated GSCs were transplanted into all-female recipients, while in the second and third trial, GSCs were transplanted into mix-sex recipients. Donor fish in all trials were euthanized by an overdose of 2-phenoxyethanol. Gonads were removed, placed in a dissociation solution comprised of L-15, 10% FBS, 2 mg/ml collagenase I and 30 µg/ml DNase I, they were minced, and incubated for 1 h (ovaries) or 1.5 h (testes) at room temperature (RT; 23 □) on a shaking plate. The dissociation was terminated by adding an equal volume of L-15 supplemented with 10% FBS. Samples were filtered through 50 µm filters and centrifuged at 200 ×g for 10 min at 10 □. Viability was assessed by trypan blue exclusion staining.

Transplantation of brown trout GSCs into 3n rainbow trout larvae was conducted as described in Lujić et al. (2018) additional ex situ strategies such as surrogate production are needed. Steps required for transplantation such as isolation of high number of viable germ cells and fluorescent labeling of germ cells which are to be transplanted have been optimized. Isolated and labeled brown trout and grayling germ cells were intraperitoneally transplanted into 3 to 5 days post hatch rainbow trout larvae. Survival of the injected larvae was comparable to the controls. Sixty days after transplantation, fluorescently labeled donor cells were detected within the recipient gonads indicating successful incorporation of germ cells (brown trout spermatogonia and oogonia—27%; grayling spermatogonia—28%; grayling oogonia—23%. In short, three- to five-day post-hatch 3n rainbow trout larvae (approximately 33 - 36 dpf) were used as recipients. Approximately 15000 GSCs were injected into the abdominal cavity of each recipient, and the larvae were transported to the hatchery the following day where they were reared until further work. In the first trial, isolated GSCs were stained by the fluorescent linker dye PKH-26, and after 60 days, some of the recipients were dissected to visualize the incorporation of donor GSCs into recipient gonads. Further, in all trials, recipients were reared until the age of three years when their gonads were excised, and the fate of transplanted cells was determined by histological observation of gonad morphology and species-specific PCR amplification of mtDNA control region.

(Continued on next page)

For histological verification, gonad pieces of recipient fish were fixed in 10% neutral-buffered formalin, processed through standard histological procedure and embedded into paraffin. Subsequently, 5-µm thick sections were cut and stained with hematoxylin and eosin staining. For molecular analyses, gonadal pieces of recipient fish were placed in RNA later and were stored at -20 °C. DNA from each sample was isolated using First-DNA all tissue kit (Genial) according to the manufacturer's instructions and the amplification of mtDNA control region (mtDNA CR) was conducted as described in Lujić et al. (2018) additional ex situ strategies such as surrogate production are needed. Steps required for transplantation such as isolation of high number of viable germ cells and fluorescent labeling of germ cells which are to be transplanted have been optimized. Isolated and labeled brown trout and grayling germ cells were intraperitoneally transplanted into 3 to 5 days post hatch rainbow trout larvae. Survival of the injected larvae was comparable to the controls. Sixty days after transplantation, fluorescently labeled donor cells were detected within the recipient gonads indicating successful incorporation of germ cells (brown trout spermatogonia and oogonia—27%; grayling spermatogonia—28%; grayling oogonia—23%.

Results and discussion

Fluorescently-labelled GSCs transplanted into 3n recipients in the first trial were observed within the recipient gonads at 60 days post-transplantation. Incorporation rate of OSCs was 50% (5/10 individuals), while the incorporation rate of SSCs was 66.7% (4/6 individuals). These results indicate successful incorporation of both brown trout SSCs and OSCs in recipient gonads.

Histological analyses of gonads dissected from mature, three years old transplanted rainbow trout demonstrated that female recipients in all trials did not display signs of gonadal development, and that the ovaries appeared immature with dominant germ cell nests containing OSCs. Primary oocytes were not observed, while single vitellogenic oocytes were scarce. In the first two trials, brown trout-specific mtDNA CR was amplified in only one female recipient, while in the third trial it was amplified in four female recipients. These results indicate that in some cases donor GSCs were present within recipient gonads, however, they did not differentiate into functional gametes. As for testes, all males of the second trial displayed well developed spermiating testes, while in the third trial, all testes were immature, and did not display significant gonadal development. Brown trout-specific mtDNA CR was not amplified in any recipient males in any of the trials indicating that donor GSCs were not present within recipient testes.

Conclusions

In this study, we demonstrated that both SSCs and OSCs are able to incorporate into 3n rainbow trout larvae. However, after rearing the recipients for three years, we did not observe significant gonadal development indicating that the donor cells were not able to differentiate into functional gametes, even though presence of donor-derived cells was observed in ovaries of some female individuals. Therefore, based on the current study, 3n rainbow trout are not suitable recipients for brown trout surrogate production.

References

Lujić J, Marinović Z, Sušnik Bajec S, Djurdjevič I, Urbányi B, Horváth Á. Interspecific germ cell transplantation: a new light in the conservation of valuable Balkan trout genetic resources? Fish Physiol Biochem 2018;44:1487–98. https://doi.org/10.1007/s10695-018-0510-4.

Yoshizaki G, Fujinuma K, Iwasaki Y, Okutsu T, Shikina S, Yazawa R, et al. Spermatogonial transplantation in fish: A novel method for the preservation of genetic resources. Comp Biochem Physiol Part D Genomics Proteomics 2011;6:55–61. https://doi.org/10.1016/j.cbd.2010.05.003.

EUROPEAN CONSUMERS' AWARENESS AND INFORMATIONAL NEEDS FOR FISH FARMING PRACTICES

T. Latvala[1], C. Mariojouls[2]*, R. Ginés[3], L. Muller[4], A. Kause[1]

[1]Natural Resources Institute Finland (Luke), Finland
[2] AgroParisTech, UMR SAD-APT, University Paris-Saclay, France
[3] Universidad de las Palmas de Gran Canaria (ULPGC), Spain
[4] Grenoble Economics Applied Lab (GAEL), Frnace
Email: catherine.mariojouls@agroparistech.fr

Introduction
For many consumers, aquaculture is not a well-known sector of food production (Fernández-Polanco & Luna 2012). In EU, the consumers who consume fish and seafood less frequently are those who have no understanding of the information accompanying the products (Cantillo et al., 2021). Hence, increased awareness of consumers on fish farming practices and their sustainability can be one potential way to increase farmed fish consumption. To study the potential ways to increase the awareness, we assessed consumers' perceptions on the quality and quantity of current information on fish farming, and wishes for information.

Material and methods
A subcontracted market research agency conducted online cross-cultural surveys in three countries (Finland n=412 people, France n=417, Spain n= 413). The master questionnaire, developed in English and translated into national languages, included items about the participants' fish consumption, objective knowledge, information received today and wishes for more information, and socio-demographic characteristics (e.g. country, gender, age, education level). The development of the questionnaire was based on the qualitative results of focus-groups done in the earlier phase of the project (Mariojouls et al., 2021). SPSS Statistics (version 26) was used for the analysis of correlations between the frequency of fish consumption, knowledge on aquaculture and respondent's nationality.

Results and discussion
Results show that there is a relatively weak knowledge of fish farming in the three countries, as only 14% to 24% of total respondents indicated they know aquaculture 'well' or 'very well', and 33% stated that they do not know fish farming at all. Spanish population confirms having better knowledge about fish farming than Finnish or French respondents.

We asked how the consumers qualify the received information, and most respondents stated that information given is 'neutral', but much more frequently in Finland (59%), and less in France (34%), Spain being in an intermediate situation with 46%. The second qualification is "mostly positive" in Spain (23%) and Finland (18%), while it is "mostly negative" in France (25%). Interestingly, the opinion of 'sufficient information' is expressed by a larger share in Finland (23%) and Spain (20%) than in France (10%), and among males (22%) compared with women (16%). The answer 'no opinion' was chosen by 24% of respondents in both Finland and France, and 20% in Spain.

Results show a clear interest of respondents for receiving information about fish farming and most respondents consider it as useful. Spanish respondents are the most interested (84% for the total 'very useful' and 'useful'), followed by Finnish respondents (70%), and French respondents (67%).

While in the three countries the available information about fish farming is considered insufficient by most of the respondents, the starting point for communication is not the same:

France has the strongest share of consumers expressing they have no knowledge about fish farming (40%) and that information about fish farming is insufficient (61%). The overall profile is characterized by low knowledge and an opinion on available information as neutral or negative.

Spain has the largest share of consumers having some knowledge about fish farming (only 24% knowing 'not at all'), but they also mostly consider that available information is insufficient (60%) while 20% say it is sufficient. The overall profile is a relatively high level of knowledge, and an opinion of a rather good quality of available information.

(Continued on next page)

Finland is an intermediate situation: the respondents with no knowledge about fish farming represent 34% of total population, there is the highest share of total respondents saying the available information is sufficient (23%). The overall profile is characterized by a relatively low knowledge about fish farming and an opinion about available information being neutral or positive, while the need for receiving information is less high than in Spain and France.

Conclusions

There is a rather low consumer awareness about aquaculture and the specific production system practices. Understanding of the knowledge gaps and consumer perceptions will benefit to design actions to increase awareness about aquaculture. Informed consumers could be more positive towards the emergence of new and sustainable production approaches, without being misled by the negative information that may follow when new technological advantages in food production are adopted.

Acknowledgments

This study is part of the AquaIMPACT project and was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 818367.

References

Cantillo, J. Martin J.C. & Román, C. 2021. Determinants of fishery and aquaculture products consumption at home in the EU28, Food Quality and Preference, Accessed Dec 31 2020. https://doi.org/10.1016/j.foodqual.2020.104085

Fernández-Polanco, J. & Luna, L. 2012. Factors affecting consumers' beliefs about aquaculture, Aquaculture Economics & Management, 16:1, 22-39, doi: 10.1080/13657305.2012.649047

Mariojouls C., Latvala T., Gines R., Muller L. 2021. Do consumers want information about aquaculture ? Communication, Aquaculture Europe 2020, Online conference April 12-15, 2021. Abstract, 2p.

SUSTAINABLE RECIRCULATING AQUACULTURE SYSTEMS: FROM PREDICTIVE ANALYTICS TO INTELLIGENT PHOTONIC PLATFORM

Carlos Marques, et al

i3N & Physics Department, University of Aveiro, Portugal

In the last decade, aquaculture production in intensive systems have been rising rapidly, often using RAS with limited water exchange. The benefits of RAS are the reduced fresh and saltwater usage, reduced land requirement due to the high stocking density, reduced wastewater effluent volume, increased biosecurity by effectively treating disease outbreaks, and independence from weather and variable environmental conditions. RAS satisfy the objectives of European Union for sustainable aquaculture by producing food while sustaining natural resources with a minimum ecological impact [1]. However, RAS are complex systems where fish biomass and water chemistry/quality interact and small variations may result in sub-optimal conditions, inducing stress, reduced feed intake and may result in reduced growth performance or mortalities [2]. This is a major concern for the industry which needs to be addressed by scientific community.

In order to produce quality fish and promote their wellbeing, the effective and POC monitoring of fishes' stress and water quality is key. Even though there have been remarkable developments in aquaculture, the quality assessment of the water and the fishes' welfare is still based on water and fish sample analysis, and its management is still empiric. The industry is now appealing for technology for POC, real-time monitoring, as well as artificial intelligence and machine learning (ML) tools to prevent catastrophic events (i.e. fish death, diseases prediction).

When analyzing the pros and cons of the available technologies applied to real-time aquatic media monitoring, photonics, and more specifically optical fibers, add great advantages to the equation. Naming just a few, fiber optics offer: immunity to electromagnetic interference, very small size, lightweight, light path control, remote sensor deployment, high transmission rate, multiple sensors working in different wavelength regions just on a single fiber, the use of biocompatible and biofunctionalized materials, inert nature enabling them to have minimal impact on the environment.

Sharp increases of cortisol can indicate that the fishes are under stress. Therefore, the aquaculture industry wants to quantify real time cortisol content in the water, so far this has not been incorporated on any type of POC solution. Cortisol measurements are still based on blood sampling and analyses of plasma cortisol after fish capture and termination, an invasive method where the results may be doubtful from the stress caused by the sampling method. In addition, the analytic method (via chromatographic techniques - laborious and expensive alternative) is time consuming and the results normally are only available long after the sampling of the fish. A successful technology for real time sensing of cortisol in water will represent a significant contribution to fish welfare and management, which has both an ethical (welfare) and economical rational (via the way stress influences mortality and growth). Such technology is particularly important in RAS farms. RAS are particularly suitable farming environments for such sensors, due to low water exchange causing higher accumulation and easier sensing of cortisol compared to flow through systems.

In parallel, the monitoring and control of ammonia levels has high priority in intensive land-based fish farms, since ammonia is toxic to fish at very low concentrations. The toxicity varies with other water quality parameters and accumulation of ammonia is the limiting factor for producing fish in RAS. This parameter is also an indicator of the biological filters performance to ensure good water quality. Hence, better control of ammonia is crucial to reduce risk, improve fish welfare and increase production and revenue from intensive farming. The existing sensors for ammonia are generally used in sewage treatment plants and do not give consistent values in saltwater systems. Also, analytical lab techniques are available such as spectrophotometry methodologies but these are time consuming and require organic solvents. Moreover, there is a lack of available technology to add sensor elements directly in water tanks and to have real time measurements.

Those are the reasons for the choice of these substances as wellbeing indicators. It is crucial to be able to control and regulate the intricate water chemistry, to maintain optimal equilibrium, which is so sensitive in RAS, due to high fish biomass, high growth rate and low water use.

(Continued on next page)

As regards data collection and processing, a wireless technical platform will be developed to be flexible and easy to adapt to different tank environments and setups and minimize interference with daily fish and tank management. A cloud-based data handling, processing and storing platform, via a user-friendly graphic interface on e.g. mobile phone and PC, will secure a unique overview of critical parameters, and give quick time response and high probability event predictions and allow for consequent corrective actions and regulation. Moreover, this type of continuous logging and accumulation of a broad set of environmental data (ammonia and cortisol) may be linked together with other environmental parameters (e.g. O2, pH, CO2, Redox, temperature collected by aquaculture industry) and production data (growth, mortality, % feeding, feed conversion ratio, stocking density, etc.) to develop strong management and planning tools based on multi-modal data analyses and artificial intelligence.

As is well known by the aquaculture industry, large databases are increasingly common and are often difficult to interpret. However, in data sets with many variables, there may be the possibility to create subgroups of these that have a common effect. This is possible as there may be more than one variable that may be measuring the same driving principle that governs the behavior of the entire system. A proper investigation should not aim to only identify these driving forces but also detect redundancy of information and simplify data analysis by replacing a group of variables by just one [3].

Currently, the absence of such POC sensors or the use of unsuitable sensors, preventing accurate visualization and treatment of data in real time, make it difficult to construct predictive models. This can be done with the implementation of the smart POC in-water non-invasive solutions, which will follow the global evolution associated with Internet of Things (IoT), allowing real time and wireless data communication from the tanks to the control center and online platforms.

In this paper we will review the state-of-the-art done in this matter to have the first smart multi-modal sensing platform (cortisol, ammonia and new progress in bacteria and nano-plastics detection) [4-8] for monitoring the wellbeing and quality of farmed fish in tanks in a noninvasive/nonintrusive way allied with predictive analytics tool.

References

1. https://ec.europa.eu/environment/eussd/ (last accessed: 10/02/2021)
2. K. Ogawa, F. Ito, M. Nagae, T. Nishimura, M. Yamaguchi & A. Ishimatsu , Effects of acid stress on reproductive functions in immature Carp, CYPRIMUS CARPIO, Water Air Soil Pollut 130, 2011.
3. L. Silva, B. Lopes, M. Pontes, I. Blanquet, M. Segatto, C. Marques, Fast decision-making tool for monitoring recirculation aquaculture systems based on a multivariate statistical analysis, Aquaculture 530, 735931, 2020.
4. C. Leitão, A. Leal-Junior, A. R. Almeida, S. O. Pereira, F.M. Costa, J. L. Pinto, C. Marques, Cortisol AuPd plasmonic unclad POF biosensor, Biotechnology Reports, 29, 2021, e00587.
5. C. Leitao et al., "Cortisol in-fiber ultrasensitive plasmonic immunosensing", IEEE Sensors J., 21, 2021.
6. A. Sharma, B. Kaur, C. Marques, Simulation and analysis of 2D material/metal carbide based fiber optic SPR probe for ultrasensitive cortisol detection, Optik 218, 164891, 2020.
7. S. Kumar, Z. Guo, R. Singh, Q. Wang, B. Zhang, S. Cheng, F. Liu, C. Marques, MoS_2 Functionalized Multicore Fiber Probes for Selective Detection of Shigella Bacteria based on Localized Plasmon, Journal of Lightwave Technology, 99, 2021.
8. A. Leal-Junior, A. Frizera, C. Marques, Low-cost Fiberoptic Probe for Ammonia Early Detection in Fish Farms, Remote Sensing 12 (9), 1439, 2020.

ADVANCES ON MEAGRE (*Argyrosomus regius*) REARING: TEMPERATURE VS PROTEIN, WHICH IS MORE IMPORTANT?

Cátia L. Marques*, Ana Candeias-Mandes, Sara Castanho, Marisa Barata, Sara Sousa e Brito, Margarida Gamboa, Leticia Luján, Florbela Soares, Laura Ribeiro, Ana C. Matias, Pedro Pousão-Ferreira

IPMA - Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station; Av. Parque Natural da Ria Formosa, s/n 8700-194 Olhão, Portugal
E-mail: catia.marques@ipma.pt

Introduction

Meagre (*Argyrosomus regius*) has been considered, in last decade, as one of the most promising fish species for aquaculture, in Mediterranean countries, and has even been described as the "Southern salmon" by some, due to its fast growing and processing potentials[1,2]"ISSN":"00448486","abstract":"A performance trial (88days.

Since 2009 several research projects have been developed, at the Aquaculture Research Station of Olhão (EPPO), to address questions related to meagre rearing, including the capture in the wild, adaptation to captivity, breeding procedures, larval feeding protocol development and larval and juvenile rearing [1,3–6]"ISSN":"00448486","abstract":"A performance trial (88days. One of the most important questions raised along the years was the optimal rearing temperature for this species, and although the normal spawning temperature rounds the 20ºC, the optimal rearing temperature, for larvae and juvenile, seems to be situated a couple degrees above, which makes meagre a suitable species to rear in recirculation systems (RAS), at higher and controlled temperatures. Another relevant question that has been addressed in trials conducted over the last years, was the optimisation of protein requirements for meagre farming.

In this work we present an overview of the trials conducted on meagre, tackling optimal protein and temperature requirements for this species.

Materials and methods

Meagre breeders were kept in 10m^3 or 50m^3 tanks in a flow through water system with controlled temperature, constant aeration, under a regime of 10 h light:14 h dark. Fish were manually fed *ad libitum*, with a commercial feed (SPAROS) and supplemented every two days with frozen seafood (mackerel and squid). Larval trial: eggs were distributed immediately after hatching in 250L tanks, with a density of 1 larvae/L. Temperatures were kept at 18, 21 and 24 ºC. Fish were fed *ad libitum*, with a commercial diet (Caviar®, BernAqua) until they reach 12mm. Growth and survival parameters were taken daily and samples for larval ontogeny, IGF1 expression and digestive enzymes were collected; Post-larval trial: fish were distributed at the density of 1 fish/L in 300 L tanks. Temperatures were kept at 20 and 24ºC and fish were fed *ad libitum* with a commercial diet (SPAROS). Biometric samplings took place every five days and density was adjusted to 1.5 Kg/m^3, except for the final sampling, that occurred when the fish reach the density of 5 Kg/m^3. Fish size dispersion was accessed and samples for IGF1 gene expression, histology and microbiology were collected. For the juvenile trials fish were distributed in homogenous groups (triplicates) in tanks of 1500L: 1) temperature of the tanks were kept at 20 (LT) and 24ºC (HT) and fish from the two groups were fed, *ad libitum* 3x a day, with isolipidic diets containing either 48 (LP) or 52% (HP) of protein (SPAROS) until doubling their initial weight; 2) temperature of the tanks were kept at 22 (LT) and 26ºC (HT) and fed, *ad libitum* 3x a day, with isolipidic diets with different protein content and origin: CP50 (24.5% fish meal – FM – and 25.5% vegetable origin proteins – VP), CP55 (28% FM and 27% VP) and CP55 ALT (7% FM, 21% VP and 27% poultry meal – PM) (SPAROS), combined in 4 treatments (LTCP55, HTCP50, HTCP55 and HTCP55 ALT). Both juvenile trials were conducted until fish doubled their initial weight. Growth parameters, survival and feed conversion rate were calculated and samples for fatty acids, gene expression, skeletal development and histology, were collected.

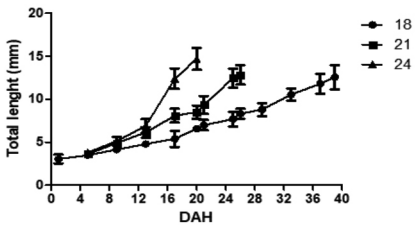


Figure 1 -Growth rate of meagre larvae reared at 18 (*circle*), 21 (*square*) and 24ºC (*triangle*). DAH, *days after hatching*.

	Treatments		ANOVA (*p-value*)[1]
	LT CP55	HT CP55	T[a]
Survival (%)	98,61 ± 0,96[a]	97,78 ± 2,10[a]	0,564
IBW (g)	30,21 ± 5,52[a]	29,81 ± 5,83[a]	0,702
FBW (g)	109,16 ± 20,48[a]	134,53 ± 23,03[b]	0,001
SGR(% day^{-1})	2,29 ± 0,06[a]	2,69 ± 0,06[b]	0,001
FCR	0,85 ± 0,00[a]	0,80 ± 0,01[b]	0,002
HSI	1,90 ± 0,16[a]	1,74 ± 0,04[a]	0,183
PR (% ingestion)	36,57 ± 0,91[a]	40,19 ± 0,99[b]	0,010

Table I – Growth performance and feed use of meagre juveniles reared at 22 and 26ºC. IBW, *initial body weight*, FBW, *final body weight*, SGR, *specific growth rate*, FCR, *feed conversion rate*, HIS, *hepatosomatic index*, PR, *protein retention*

(Continued on next page)

Results and Discussion

For both larvae and juveniles, it was shown that the optimal rearing temperature for meagre is around the 24ºC with fish presenting a fast growth at this temperature (Figure 1). Additionally, a higher rearing temperature had no negative impact on the normal development, with fish presenting a proper external morphology and organ development.

Specific growth rate (SGR) and protein efficiency ratio (PER) were significantly higher in the HPHT treatment with the higher temperature (24ºC) promoting feeding ingestion (Table I). The protein increase in the diet showed a tendency for fish protein retention with that not being affected by a lower temperature. Fatty acids content in the liver was not affected by the temperature nor the protein, however there is a decrease on muscle lipid content in the LTHP treatment. These results showed that growth and feeding efficiency were maximized at higher temperatures and that temperature is prevalent over the source and protein content in the diet, as fish growth performance was not affected by these factors when reared at higher temperatures (Table I).

CAROTENOID TISSUE DISTRIBUTION IN MALE AND FEMALE SEA URCHIN, *Paracentrotus lividus,* FED EXTRUDED DIETS WITH INCREASING LEVELS OF β-CAROTENE

Alexandra Marques[1*], Inês Garrido[1,2], Helena M. Amaro[1], A. Catarina Guedes[1,3], Tânia Tavares[4], Isabel Costa[3], F. Xavier Malcata[4,5], Luísa M.P. Valente[1,2]

[1]CIIMAR, Interdisciplinary Centre of Marine and Environment Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[2]ICBAS, Abel Salazar Biomedical Sciences Institute, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3]ISS, Ínclita Seaweed Solutions, CIIMAR, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[4]LEPABE, Laboratory for Process Engineering, Environment, Biotechnology and Energy, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[5]FEUP, Faculty of Engineering of University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
E-mail: amarques@ciimar.up.pt

Introduction

Gonads of sea urchins are considered a delicacy due to their organoleptic characteristics. The demand for this product has been rising, mostly in Europe, and consequently increasing fisheries. Aquaculture can minimise the overexploitation of natural populations, whilst assuring its availability during the whole year. Baião et al. (2019) reported high gonad yield by feeding a formulated extruded diet to *P. lividus* in captivity. Since colour is the first stimuli presented to a consumer, and a major marketability influencing factor, this characteristic is a relevant quality trait in sea urchin gonads. Echinenone is the most abundant carotenoid in the gonads and depends on availability, uptake and bioconversion of β-carotene from dietary sources. When ingested, β-carotene is transformed into echinenone, however, its metabolisation site is still controversial; Symonds et al. (2007) hypothesised a β-carotene metabolisation into echinenone in the gut wall, and then mobilisation to the gonads. Echinenone is reported as being responsible for the intense colouration in the gonads of the sea urchin (Shpigel et al. 2005). The present study aimed to investigate the response of *P. lividus* to increasing dietary levels of natural sources of β-carotene. Carotenoid distribution was hence evaluated in gut and gonads of both female and male.

Materials and methods

A control diet (CTRL) was formulated and compared with four isonitrogeneous and isoenergetic experimental diets containing two levels of *Dunaliella salina* (0.75% or 1.5%, in diets D1 and D2, respectively) as a natural rich source of β-carotene. In two of these *D. salina* supplemented diets, the commercially available macroalgae mix was totally replaced by *Porphyra* (D1P and D2P). All diets were cold extruded (<30 °C) and softly dried (<45ºC), distributed every 48h, for 8 weeks, to quadruplicate groups of sea urchins, placed in plastic mesh cages in a saltwater recirculation aquaculture system with a stocking density of 3.5 $kg.m^{-2}$; temperature 18 ºC, salinity 35‰, and a 10h L/14h D photoperiod. At the end of the growth trial gut and gonads of 8 animals per tank were sampled for carotenoid quantification by high performance liquid chromatography (HPLC).

Results and Discussion

Carotenoid content in *P. lividus*' gut differed significantly between sexes and diets. The gut content of β-carotene and echinenone increased proportionally to β-carotene dietary inclusion levels, in both sexes, but was always higher in males (**fig.1 A, B**). The gut echinenone content increased linearly with gut β-carotene content in both sexes (**fig.1 C**). Sea urchins fed diets D2, D1P and D2P resulted in significantly higher concentrations of β-carotene and echinenone in the gut than those fed D1 and CTRL. Contrarily to the gut, the dietary treatments did not affect carotenoid content in gonads, but males had always higher levels than females. Comparing both tissues, the gut had higher levels of β-carotene than gonads (10-38 μg/g *vs* 1.7-4.7 μg/g*),* irrespectively of the sex. In males, echinenone levels in gonads were higher than those detected in the gut; female echinenone levels were similar between gonads and gut, and consistently lower than males in both tissues. Echinenone gonad content presented a positive correlation with gonad's β-carotene, and in female a very significant linear relationship could be established (**fig.1 D**).

(Continued on next page)

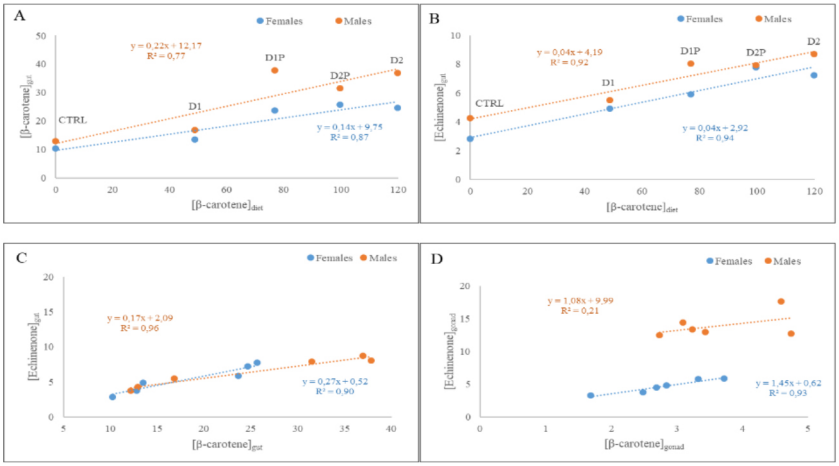


Figure 1 – Regression line between pigment concentration in diets and/or tissues in *P. lividus*. **A** – diet β-carotene vs. gut β-carotene. **B** – diet β-carotene vs. gut echinenone. **C** - gut β-carotene vs. gut echinenone. **D** – gonad β-carotene vs. gonad echinenone.

Conclusion

The inclusion of increasing levels of β-carotene in diets for *P. lividus* resulted in an effective accumulation of β-carotene in the gut, and of echinenone in a lesser extent. The deposition of echinenone in gonads was not affected by the dietary treatments and may depend on the ability of sea urchin to either mobilise pigments from the gut, or to synthesise them in the gonads from β-carotene. So the ability of sea urchins to either mobilise or synthesise those pigments in the gonads will have an important role in defining gonad quality.

Acknowledgments

Work supported by Project CAVIAR - Market valorisation of sea urchin gonads through dietary modulation (FA_05_2017_015), financed through programme Fundo Azul.

References

Baião, L.F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture. 506, 127-138.

Symonds, R.C., Kelly, M.S., Caris-Veyrat, C., Young, A.J., 2007. Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9'-cis-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 148, 432-444.

Shpigel, M., Schlosser, S.C., Ben-Amotz, A., Lawrence, A.L., Lawrence, J.M., 2006. Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. Aquaculture. 261, 1269-1280.

HOW TO SELECT THE CORRECT GENOMICS TEST FOR YOUR BREEDING PROGRAM

E. Marques*, J. Stannard, D. Plouffe, J. Buchanan

Center for Aquaculture Technologies. 8395 Camino Santa Fe, Suite E. San Diego, California, United States, 92129. E-mail: emarques@aquatechcenter.com

Introduction

The aquaculture industry is ripe for the uptake of DNA-based technologies also known as genomics. The sector has observed and, therefore, learned lessons from the livestock industry where DNA tools are heavily applied, and genetic gains are no longer simulations on a computer screen.

Many aquaculture producers have questions and are seeking guidance to improve their genetics and implement new tools in their breeding programs. Before making recommendations, it is important to emphasize that the use of genomics will not make the need for collecting phenotypes or the need to improve a management system go away.

Economically important traits are complex in nature, which means that part of their outcome originates from improvements in genetics - through the observation of measurable traits and other breeding records - and part from improvements in management (i.e., health, nutrition), so investing in one while ignoring the other will not solve all your problems.

Genomics is just one tool in the box and does not replace good husbandry practices and a commitment at all levels of an organization to supporting the genetic improvement program.

Below are the top 3 tools (genomics and bioinformatics) that any aquaculture producer should consider investing in:

The top 3 AquaArray genomics tools:

1- Low Density (LD): for those dipping their toes in genomics without any knowledge of which populations they have (i.e., species or strains), we recommend a panel with less than 200 SNPs. It provides insights into the genetic architecture of the population and a basis for relatedness and inbreeding. This panel also allows for parentage testing, an important step in the calculation of EBVs (estimated breeding values), which is another way of saying genetic merit. Each species has its own LD panel.

2- Medium-Density (MD): If you have been investing in high-density (HD) genotyping (see below) for a while and you have a solid knowledge of how your population is structured (i.e. level of linkage disequilibrium), then you should start thinking about a medium-density panel that can be imputed to your HD dataset to save cost while maintaining precision.

3- High-Density (HD): The most powerful tool for a full-throttle, high-speed genetic improvement program. These panels have around 50,000 markers and can be used for parentage testing, genomic selection, marker-assisted selection, etc. Producers that have been, and are committed to, collecting phenotypic data are in the sweet spot for the use of this HD tool.

The top 3 bioinformatics tools:

The bioinformatics tools provide actionable insights needed to move ahead, and without them, the tools I listed above are just a bunch of As, Ts, Cs, and Gs.

Below is a list of analysis and application coming from these genomics tools:

1. A Genetic Overview (GO) Analysis: A powerful analysis that provides insights on the diversity and level of inbreeding between animals of a population. When combined with a strain ID analysis - it enables the differentiation between animals used in different production systems and markets. For example, Tilapia (the Nile vs Mozambique), Largemouth Bass (Florida vs Northern).

(Continued on next page)

2. Parentage Analysis: This analysis defines the pedigree of your animals, and it is - after you have been collecting phenotypic data – the first step for the calculation EBVs (genetic merit). It requires samples from the broodstock and its progeny.

3. Genomic Selection: At this stage, the pedigree information and phenotypic data have been collected over many generations. Here we assign a value (+ or -) for each of the thousands of markers obtained from the HD SNP panel and that information is then added to the genetic evaluation. You may have heard of the term GE-EBVs (genomic-enhanced estimated breeding values). This is a more precise estimate of the genetic merit because of the power added by the DNA information.

What's next?

Once you have decided which genomics tool best fits your needs, or you may also want to email us at info@aquatechcenter.com to discuss more, it is time to collect a biological sample. This step cannot be ignored, and a misstep here can often mean low quality DNA yield and, therefore, no results. Fin clips (for fin fish) or pleopods (for shrimp) are a great source of DNA. We guide our clients throughout sample collection and submission to ensure that we generate usable results.

What's your weakest link?

A genomic tool will only be as good as your weakest link. So, if you have not already done so, there will come a time when you need to upgrade your management program (i.e., invest in better feed, monitor for pathogens, buy bigger or more tanks, modify your layout). To put it into layman's terms, do not expect to reach the finish line by putting Porsche tires (HD tool) on your Volkswagen Beetle.

If your weakest link right now is in pathogen detection and monitoring, the best way is to couple one of the genomic tools above (LD, MD, or HD) with a panel for pathogen detection where you can choose which pathogen to test for. You may not need all of them, so it is important that you have an à al carte option rather than an 'all you can eat' buffet.

And, if you are noticing that you need help in multiple levels of your business (health, nutrition, genetics, genomics), an annual breeding contract that carries custom made recommendations would likely serve you better and qualified professionals are out there to help new entrants wade through all their options!

SUSTAINABLE AND NATURE-BASED PRODUCTS TO POTENTIALLY MITIGATE CILIATE OUTBREAKS IN TURBOT AQUACULTURE

A. Carvalho[1], M. Válega[2], A.M.S. Silva[2], I. Domingues[1], A.M.V.M. Soares[1], C.R. Marques[1,*]

[1] Centre of Marine and Environmental Studies (CESAM) & Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal
[2] Laboratório Associado para a Química Verde (LAQV) - REQUIMTE, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal
Email to: crmarques@ua.pt

Background:
Philasterides dicentrarchi is a facultative parasite which causes scuticociliatosis, a severe systemic disease leading to high mortality rates in turbot aquaculture (Budiño et al., 2001), ending up in severe economic damages. Hence, the stability of fish production in aquaculture depends on viable prevention and control measures against pathogens (Shankar Murthy and Kiran, 2013). Several measures have been attempted to manage this pathogen, with little success, as its virulence makes this task ascendingly difficult (Shin et al., 2014). Therapeutic agents previously assessed for that purpose (*e.g.*, oxyclozanide, niclosamide, and formaline; Cogliano et al. 2005) can be harmful to fish (Dai et al., 2008) and the surrounding environment, risking the health of fish and humans (Ahilan et al., 2010).

Given the side effects often associated with synthetic drugs, plant extracts have become a focus of research as treatment and prevention agents against microorganisms, due to low associated costs, biodegradability, low environmental impact, low likelihood of resistance, and minimal side effects on fish (Shankar Murthy and Kiran, 2013).

No effective measures have successfully been established to mitigate *P. dicentrarchi* growth and pathogenicity, however, it is urgent to find a mitigation plan against it, most importantly, one which would not harm the fish in aquaculture (Shin et al., 2014).

Aim:
Evaluate the bioactivity of six plant extracts as antiprotozoal agents against *P. dicentrarchi*, with the ultimate objective of verifying their potential feasibility for integrating disease control measures in turbot aquaculture. This work is within the project MAXIAQUA - *MAXimization of the strategies of control of a parasite in aquaculture of turbot*.

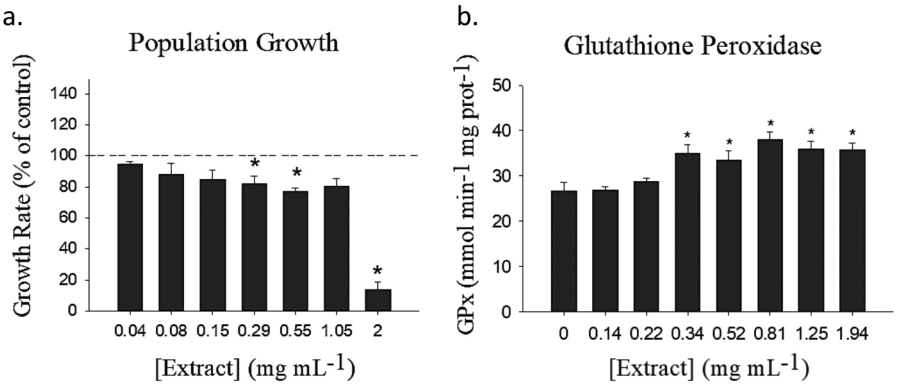


Figure 1 – Graphical representation illustrating the observed effects of one of the plant extracts analysed, on (a.) *P. dicentrarchi* population growth and (b.) glutathione peroxidase enzyme activity. Population growth results are expressed as percentage of control ± standard deviation, with control being 100%, represented by the reference line. Glutathione peroxidase results are expressed as mean ± standard error. * Significantly different from control (One-way ANOVA, Dunnett's test; $p < 0.05$).

(Continued on next page)

Methods:
Plant extracts from six plant species were obtained through the Soxhlet method. The extracts were tested against *P. dicentrarchi* and their effects on parasite populational growth, oxidative stress, and protease activity were assessed. Population growth was assessed by cell counting, the antioxidant enzyme glutathione peroxidase was used as a biomarker for oxidative stress, and protease activity was assessed using FITC-casein. Data analysis involved the application of a one-way ANOVA followed by the *post-hoc* multicomparison Dunnett's test, in order to identify treatments that induced a response significantly different than the control ($\alpha = 0.05$).

Results and Discussion:
All plant extracts, particularly at high concentrations, hindered *P. dicentrarchi* population growth (*e.g.*, Fig. 1a). Glutathione peroxidase activity was significantly affected by all plant extracts, in a non-dose dependent pattern and most extracts caused a decrease in *P. dicentrarchi* protease activity (*e.g.*, Fig. 1b).

Plant extracts affected different sub-lethal responses of the parasite, having hindered its reproduction and virulence, showing their potential as control strategies in turbot aquaculture. These findings further evidence the possible effectiveness of using plant extracts as a prevention strategy against *P. dicentrarchi* growth and pathogenicity, allowing fisheries to avoid antibiotics or such compounds which could have a toxic impact on farmed fish and their environment, which, considering these fish are produced for human consumption, would be detrimental in both health and economic levels (Voon et al., 2012).

References:
Ahilan, B., Nithiyapriyatharshini, A., Ravaneshwaran, K., 2010. Influence of certain herbal additives on the growth, survival and disease resistance of goldfish, *Carassius auratus* (Linnaeus). J. Veternary Anim. Sci. 6, 5–11.

Budiño, B., Leiro, J., Cabaleiro, S., Lamas, J., 2012. Characterization of *Philasterides dicentrarchi* isolates that are pathogenic to turbot: Serology and cross-protective immunity. Aquaculture 364–365, 130–136.

Cogliano, V.J., Grosse, Y., Baan, R.A., Straif, K., Secretan, M.B., Ghissassi, F., Working Group for Volume 88*, 2005. Meeting Report: Summary of IARC Monographs on Formaldehyde, 2-Butoxyethanol, and 1-tert-Butoxy-2-Propanol. Environ. Health Perspect. 113, 1205–1208.

Dai, J.R., Wang, W., Liang, Y.S., Li, H.J., Guan, X.H., Zhu, Y.C., 2008. A novel molluscicidal formulation of niclosamide. Parasitol. Res. 103, 405–412.

Shankar Murthy, K., Kiran, B.R., 2013. Review on usage of medicinal plants in fish disease. Int. J. Pharma Bio Sci. 4, 975–986.

Shin, S.P., Han, S.Y., Han, J.E., Jun, J.W., Kim, J.H., Park, S.C., 2014. Expression and characterization of cathepsin L-like cysteine protease from *Philasterides dicentrarchi*. Parasitol. Int. 63, 359–365.

Voon, H.C., Bhat, R., Rusul, G., 2012. Flower extracts and their essential oils as potential antimicrobial agents for food uses and pharmaceutical applications. Compr. Rev. Food Sci. Food Saf. 11, 34–55.

BACTERIAL DIVERSITY IN NON-DEPURATED *VS*. DEPURATED BIVALVES FOR HUMAN CONSUMPTION – 1st OUTCOMES OF THE SEEBug PROJECT

C.F. Lourenço[1], A.R. Almeida[1], A.C. Abreu, A.M.V.M. Soares and C.R. Marques*

[1]First authorship shared; Department of Biology & CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
E-mail: crmarques@ua.pt

Introduction

Worldwide, and particularly in Portugal, the production and capture of bivalve mollusks are both activities with high cultural, commercial, and economic potential (Oliveira et al., 2013; DGAV, 2020). According to FAO (2020), world aquaculture bivalve production was of 17.7 million Ton in 2018. Due to the intensification of anthropogenic activities, it has been observed an increasing abundance of chemical and biological contaminants in the marine environment (Borja *et al*., 2011). Therefore, in the view of its filtering characteristics, bivalve species are highly exposed to harmful agents (Silva & Irineu, 2008). Moreover, since these species are commonly consumed raw or low cooked by humans, they may represent an important via of pathogens exposure to humans, leading to health concerns (Anacleto *et al*., 2014). Considering this, the goal of our work was to detect and/ or identify cultivable pathogenic bacteria associated with two commercially valuable bivalve species, before and after being depurated. This preliminary study 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 intendsto identify other relevant bacterial pathogens that should eventually be considered withinbivalves microbiological monitoring programs.

Materials and methods

This study focused on *Cerastoderma* sp. and *Ensis* sp. bivalves, which were collected in Ria de Aveiro during the summer season (in July). In order to detect and isolate pathogenicbacteria two protocols were applied based on: (i) the procedures outlined in International Organization for Standardization (ISO) 16649-3, ISO 6887-1, and ISO 6887-3 standards for *Escherichia coli* detection – Protocol I; and (ii) the use of selective (Salmonella Shigella (SS), MacConkey (MC), and TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar) and universal (Trypticase Soy Agar (TSA)) culture media to isolate and determine the cultivable bacterial diversity colonizing bivalves – Protocol II. In Protocol I the MPN(Most Probable Number) of *E. coli* per 100g of bivalve was computed, whilst in ProtocolII the number of colony forming units (CFUs) per 100g of bivalve was counted. Isolated clones were subjected to molecular identification through sanger sequencing of 16S rRNAgene.

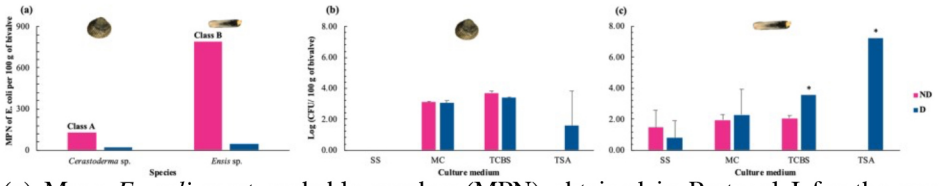


Fig. 1 - (a) Mean *E. coli* most probable number (MPN) obtained in Protocol I for the non-depurated (ND) and depurated (D) *C*erastoderma sp. and *Ensis* sp. bivalves. Logarithm (Log) of colony forming units (CFU) determined by Protocol II for (b) *Cerastoderma* sp. and (c) *Ensis* sp. Error bars represent standard deviation. (*) represent statistically significant differences ($p \leq 0.05$) between ND*vs*. D within each media.

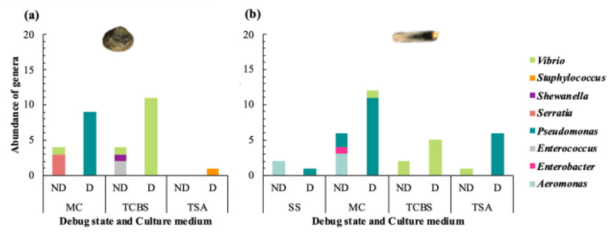


Fig. 2 - Abundance and distribution per genus of the bacterial isolated in different culture media (Protocol II) before and after the depuration process (a) *Cerastoderma* sp., for which there no growth was observed on SS agar and (b) *Ensis* sp.

(Continued on next page)

Results and discussion

Overall, it was possible to detect cultivable bacteria by using the two protocols in both organisms. Through Protocol I, according to EU (2019), the MPN of *E. coli* detected on *Cerastoderma* sp. (Figure 1(a)) before depuration revealed to be within the valuesconsidered acceptable to human consumption (130 MPN/ 100 g – class A areas). The MPNof *E. coli* present *Ensis* sp. non-depurated was within the legal limit set for Class B areas (790 MPN/ 100 g). As expected, after the depuration process a significant decrease of MPN/ 100 g was observed (Figure 1(a)), revealing the efficiency of the depuration method for *E. coli*.

Regarding Protocol II, it was also observed a decrease of CFUs after bivalves' depuration,except for *Ensis* sp., where it was verified a significant increase of CFUs in TCBS and TSA media (Figure 1(b, c)). Moreover, it was isolated other bacteria genera (Figure 2), some of them indicated as human pathogens (*e.g.*, *Pseudomonas* and *Vibrio*), which mayrepresent a public health problem. Hence, our results revealed that, although the depuration process seems to be efficient to reduce *E. coli* MPN, to other bacteria genera itmay not be the best method. Therefore, further studies are needed to detect and characterize in detail pathogenic bacteria present in bivalve mollusks.

References

Anacleto, P., Barrento, S., Nunes, M.L., Rosa, R., & Marques, A. (2014). Portuguese consumers' attitudes and perceptions of bivalve molluscs. *Food Control*, *41*, 168-177.

Borja, Á., Belzunce, M.J., Garmendia, J.M., Rodríguez, J.G., Solaun, O., & Zorita, I. (2011). Impactof pollutants on coastal and benthic marine communities. Ecological Impacts of Toxic Chemicals. Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.). *Bentham Science Publishers Ltd*, 165-186.

DGAV (General Direction of Alimentation and Veterinary) (2020). Surveillance of bivalve mollusks deseases - Annual report (November 2019/ June 2020). Portuguese Republic, Agriculture.

Europe Union (EU) (2019). Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption.

FAO (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

Doi: http://www.fao.org/documents/card/en/c/ca9229en

ISO (2015). ISO/ FDIS 16649-3. (2015) Microbiology of the food chain – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 3: Detection and must probable number technique using 5-bromo-4-chloro-3-indloy-β-Dglucuronide. Geneve,Switzerland: International Organization for Standardization (ISO).

ISO (2017) ISO 6887-1. (2017) Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rulesfor the preparation of the initial suspension and decimal dilutions. Geneve, Switzerland:International Organization for Standardization (ISO).

ISO (2017). ISO 6887-3. (2017) Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products. Geneve, Switzerland: International Organization for Standardization (ISO).

Oliveira, J., Castilho, F., Cunha, Â., & Pereira, M. J. (2013). Bivalve harvesting and production in Portugal: an overview. *Journal of Shellfish Research*, *32*(3), 911-924.

Silva, H.A., & Irineu, B. (2008). Production, healthiness and commercialization of mollusk bivalve in Portugal. Single publications of IPIMAR (Institute for Research in Fisheries and the Sea).

Cofinanciado por:

mar 2020 PROGRAMA OPERACIONAL MAR 2020 PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu dos Assuntos Marítimos e das Pescas

OCCURRENCE OF MYXOSPOREAN PARASITES IN WILD SARDINES (*Sardina pilchardus*) MAINTAINED IN CAPTIVITY

Marcelo Livramento, Cátia Lourenço Marques*, Marisa Barata, Márcio Moreira, Pedro Pousão-Ferreira, Florbela Soares

Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO), Av. Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal

*e-mail: catia.marques@ipma.pt

Introduction

Pelagic fish such as sardines (*Sardina pilchardus*) is the main target of the Iberian fisheries reaching more than ten times the amount of gilthead seabream (*Sparus aurata*) or seabass (*Dicentrarchus labrax*) catches. In Portugal, there has been a decrease of the sardine stock due to prolonged low recruitment and high catch levels (Monteiro, 2017) and the impact in the economy is very serious. Thus, it is very important to find solutions to preserve and maintain the renewal of this valuable resource and the aquaculture production is one of them. Due to being one of the most commercially important species in the canning industry (INE, 2020) the interest in sardine aquaculture is growing (Bandarra, 2018) and the recent publications allows a better understanding of its physiological and ecological requirements (Silva, 2019). The increase of marine aquaculture production allows the frequent report of diseases outbreaks due to different parasites. Under aquaculture conditions, endoparasites such as the myxosporea class, are a potential harmful for marine fishes being present in several tissues (Alvarez-Pellitero, 1993). Little parasitological studies have focused on sardine and there is a lack of information about the problems that can result from a myxosporean infestation. The present study aims to report and characterize for the first time the presence of a myxosporean specie parasitizing the gall bladder of European sardine, *S. pilchardus*, maintained in captivity at the Aquaculture Research Station of Olhão (EPPO).

Material and Methods

One stock of *S. pilchardus* was obtained from commercial purse seiners and adapted to captivity at EPPO facilities. After a quarantine period, sardines were maintained in an open-system water circulation and kept under a natural light regime. After 1 year in captivity, the gall bladder of 56 sardine with an average weight of 43.8 ± 6.8 g and length of 16.6 ± 0.6 cm, was thoroughly examined for the presence of myxosporean infection in a cleaned slide by light microscopy. Free fresh spores were observed at 400x and photographed using a light microscope equipped with a digital camera. Morphometric analysis was performed from the observation of mature spores in 10 microscope fields per slide and the infestation was classified by ranks: 0 – no parasites; I – 1 to 10; II - 11 to 20; III – 21 to 100; IV – 101 to 500; V – more than 500 (parasites per slide). Infected gall bladders were preserved in 96% ethanol and stored at -20ºC for molecular analysis. The genomic DNA was extracted using a Qiagen® QIAamp DNA micro kit, following the manufacturer's instructions. The PCR was carried out in using the extracted genomic DNA and three set of primers, to amplify distinct rRNA regions. The resulting PCR product were excised from gel, purified, and sequenced.

Results and Discussion

Some mature myxospores were ellipsoidal with a length between 25-100μm and the two pyriform polar caps were oriented towards the center of the myxospore (Figure 1).

Myxospores prevalence in the sampled fish was 80%, with an infection level of II. Detection of the myxospores species DNA by PCR can be more effective than microscopic examination. Using 3 set of primers, it was possible to identify 3 distinct regions of the SSU rDNA (Figure 2). With the DNA sequence of the amplified regions, it can be possible to compare the molecular identity of the observed parasite with other myxosporean sequences, resulting in the positioning in a phylogenetic tree.

The infestation level of mature myxospores can be ranked and this classification will provide a better understanding of the pathogenic effects of this type of parasites.

(Continued on next page)

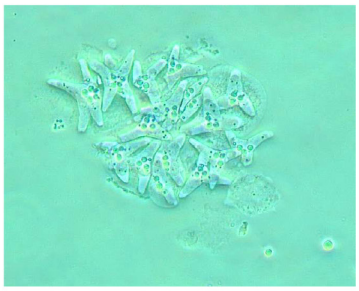


Fig. 1 –Myxosporean infestation level IV in sardine (*Sardina pilchardus*) gallbladder (400x magnification).

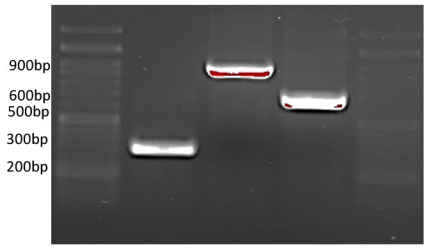


Fig. 2 – Polymerase chain reaction analysis of mature myxospore DNA from the gallbladder of *Sardina pilchardus*, comparing amplification product of a 300bp region using SSU rDNA specific primers.

Acknowledgements

The present work was financed by the projects DIVERSIAQUAII (MAR2020-P02M01- 0656P) and SAUDE&AQUA (MAR-02.05.01-FEAMP-0009).

References

Alvarez-Pellitero, P., Sitjà-Bobadilla, A. (1993) Pathology of Myxosporea in marine fish culture. *Diseases of Aquatic Organisms*, 17, 229-238.

Bandarra, N. M., Marçalo, A., Cordeiro, A. R., Pousão-Ferreira, P. (2018) Sardine (*Sardina pilchardus*) lipid composition: Does it change after one year in captivity? *Food Chemistry,* 244, 408-413.

Monteiro, P. V. (2017) The Purse Seine Fishing of Sardine in Portuguese Waters: A Difficult Compromise Between Fish Stock Sustainability and Fishing Effort, Reviews in Fisheries Science & Aquaculture, 25:3, 218-229

Silva, A., Garrido, S., Ibaibarriaga, L., Pawlowski, L., Riveiro, I., Marques, V., Ramos, F., Duhamel, E., Iglesias, M., Bryère, P., Mangin, A., Citores, L., Carrera, P., Uriarte, A. (2019) Adult-mediated connectivity and spatial population structure of sardine in the Bay of Biscay and Iberian coast. Deep-Sea Research Part II, 159, 62-74.

PROBIOTIC INGESTION PREVENTS TAIL-ROT IN sENEGALESE SOLE EARLY CULTURE

I. Martín[1], J. M. Martínez-Vázquez[1], H. Sanjurjo-González[2], I. Rasines[1], D. G. Valcarce[1*]

[1] Spanish Institute of Oceanography (IEO-CSIC). Monte-Corbanera, 39012, Santander, Spain.
[2] University of Deusto. Dpt. of Information Technology, Electronics and Communication, 48007, Bilbao, Spain
*Corresponding author e-mail: david.garcia@ieo.es

Introduction

The current work focuses on an unexpected observation during the execution of a long-term experiment in which the effects of probiotic supplementation on *Solea senegalensis* culture was being tested. The initial main goal of the experimental design was to evaluate the long-term effects of prolonged intake of probiotics from the first stages of larval development to the juvenile stage. In a periodic routine sampling of the culture on day 45 after hatching, the presence of tail-rotting in some sampled animals was observed. Here, we present the results of the evaluation carried out at this experimental point after this observation, focusing on the potential beneficial effect of the tested probiotic on Senegalese sole early culture to prevent tail rot.

Material and methods

All protocols involving animals were approved (authorization number 2021-02) by the Spanish and institutional bioethical guidelines of the Animal Welfare Service following European Union Directive 2010/63/EU for the protection of animals for experimental uses and Spanish regulations (RD/2013). The initial batch of embryos for the experiment was obtained by in vitro fertilization (IVF) from F1 broodstock following the protocol described by Rasines (Rasines et al, 2013). To ensure the genetic origin of the larvae, maximizing uniformity of the experimental replicates, gametes from only one female and one male were used for IVF. The progeny was split at 1 day post hatching (dph) into six 200 L round tanks (3 per experimental condition) at a 50 larvae/L density. General culture protocol and feeding regime were based on Cañavate and Fernández-Díaz, 1999 with some modifications. Two experimental groups were created: the control group (CTRL) in which live food was enriched with a commercial product (Easy Dry SELCO®, Inve Aquaculture) and the experimental group (PROBIO) in which live food was enriched (10^{11} CFU/mL) with *Pediococcus acidilactici* MA 15/5M (Bactocell®, Lallemand). Immediately after the observation of tail rot in the 45 dph sampling, a prophylactic treatment with hydrogen peroxide was provided to all tanks to avoid deterioration of the specimens.

Cumulative mortality was evaluated daily along the experiment. Thirty fish per experimental group (10 fish/replicate) were sampled for weight at 45 dph. SGR $((\ln W_f - \ln W_0)*100/T_f-T_o)$ was used as parameter to monitor growth. After biometrical analysis, each animal was placed under a stereomicroscope and dorsal images focusing on the tail were captured. Tail areas were measured using Adobe Photoshop CC 2020. In addition to the quantitative approach, the tails were evaluated subjectively by five different people. Evaluators were given an illustrated scale with examples of severity of tail rot starting from 0 (no lesion), 1 (low; a reduction of 25% of fin tissue), 2 (moderate; 50% of tissue reduction) to 3 (high incidence, practically absence of tail fin rays). The evaluators blindly assigned each image a level of affection based on the scale with a margin of 0.5 points for each of the 60 images. The mean of the five data for each image was considered as the tail rot incidence value for the specimen.

In order to analyse the immune response generated in the fish, total RNA was extracted from 9 fish of each group (3 per tank). Each fish body was homogenized (T25 Ultra Turrax ®, IKA) in an initial volume of 1 mL of TRI Reagent® (Merck). After cDNA synthesis (2 µg; High-Capacity RNA-to-cDNA™ Kit, Applied Biosystems), the following genes were evaluated by qPCR using SYBR green master mix (Applied Biosystems): hepcidin (HAMP1), complement c3 (C3), leucocyte cell-derived chemotaxin 2 (LECT2), non-specific cytotoxic cell receptor (NCCRP1), tumor necrosis factor a (TNFA) and interleukin 1 beta (IL1B). Three technical replicates were used in the qPCR analysis. The levels of the expression of these genes were normalized to eukaryotic elongation factor 1 alpha (eEF1A) levels using the formula $2^{-(\Delta\Delta Ct)}$ (Livak and Schmittgen, 2001).

Statistical analysis was performed using SPSS 21.0. Non-parametric variables were analyzed using Mann-Whitney test. Normally distributed variables were analyzed using the student's t-test. Values with $p < 0.05$ were considered to be statistically significant. Data are expressed as mean ± SEM.

(Continued on next page)

Results and Discussion

Mortality data were globally very low at 45 dph. In both experimental groups for this time point, survival was similar and above 98%. Taking into account specific growth rate values, the statistical analysis revealed significant difference in SGR ($p = 0.0161$) indicating a slightly higher growth rate in PROBIO group. Regarding the tails, the statistical analysis showed a strong significant difference ($p < 0.0001$) between the fin area of both groups. While the CTRL group presented a mean area of 0.1893 ± 0.0518 cm^2, the PROBIO group showed a mean value of 0.3240 ± 0.0090 cm^2. These quantitative values were in line with the severity differences ($p < 0.0001$) issued by the evaluators. While in the CTRL group, the mean value of the incidence was scored with 1.8530 ± 0.0993 a.u.; in the PROBIO group the mean was 0.2500 ± 0.0528 a.u., since almost any fish showed signs of tissue reduction. Gene expression analysis corroborated that the animals in the CTRL group were undergoing an activation of their immune system with an overexpression of C3 ($p = 0.0034$) and LECT2 ($p = 0.0012$). Taking these data together, the observations recorded in this experiment indicate that the bioencapsulation of the probiotic strain *Pediococcus acidilactici* MA 15/5M in rotifers and artemia metanauplii may be a useful biotechnological tool to prevent the appearance of tail rot in the early culture of Senegalese sole.

References

Cañavate J.P. and Fernández-Díaz C. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. *Aquaculture*. 174 (3-4), 255-263.

Livak K.J. and Schmittgen T.D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25-4, 402-408

Rasines I., Gómez M., Martín I., Rodríguez C., Mañanós E., Chereguini O. 2013. Artificial fertilisation of cultured Senegalese sole (*Solea senegalensis*): Effects of the time of day of hormonal treatment on inducing ovulation. *Aquaculture* 392–395, 94-97

Acknowledgments

Authors would like to acknowledge FCJ2018-037566-I grant, PROBISOLE project (Fundación Biodiversidad; PLEAMAR 2020-FEMP), Lallemand Animal Nutrition S.A. and STOLT Sea Farm S.A.

ENVIRONMENTAL PERFORMANCE OF ITALIAN MUSSEL FARMING - LIFE CYCLE ASSESSMENT (LCA) AND BIOGENIC CARBON COMPUTATION FOR THREE CASE STUDIES

Arianna Martini*, Domitilla Pulcini, Fabrizio Capoccioni, Marco Martinoli, Luca Buttazzoni, Giacomo Pirlo

CREA - Council for Agricultural Research and Economics, Research Centre for Animal Production and Aquaculture, Via A. Lombardo 11, 26900 - Lodi (LO), Italy
E-mail: arianna.martini@crea.gov.it

Introduction

Mussel farming has been identified as one of the most promising food sectors that can help to meet the nutritional requirements of a growing human population while providing other ecosystem functions and services [1]. Italy, with 92,564 t in 2018, contributed to the 15% of the EU mussel production [2]. This makes Italy the third most important European producer of mussel, after Spain and France.

Mussels are filter-feeders of naturally occurring phytoplankton. As such, mussels represent the functional and trophic connection between pelagic and benthic processes, contribute to the fundamental nutrient storage and cycles, and play a key role in regulating incipient eutrophication phenomena through the top-down control on phytoplankton biomass [3].

Recently, several authors have investigated the possible contribution of farmed bivalves in mitigating the effects of climate change (i.e., increased carbon stock in seawater, CO_2) [4]traditionally considered a waste of aquaculture activities, have recently acquired an interest under the current framework of zero waste circular economy. Shell CaCO3 is a sustainable biomaterial that could partly replace the presently dominating non-renewable mineral sources in some applications. Although the carbon footprint of powdered CaCO3 production from biological or mineral sources are about the same, the environmental impact is notably different. Furthermore, bivalve CaCO3 contributes to sequester anthropogenic carbon dioxide (CO2. Mussels build their shells through the biocalcification process, incorporating chemical carbon species, i.e., hydrogen carbonate (HCO_3^-), in the form of calcium carbonate (CaCO3-) while, at the same time, releasing CO_2, according to the following equation: Ca_2^{2+} $2HCO_3^-$ $\rightleftharpoons$ $CaCO_3 + CO_2 + H_2O$. However, the role of mussel farming as a carbon sink is controversial and a scientific consensus on this topic is far from being achieved due to an open-ended debate about the definition of the criteria for the estimation of the biogenic carbon flux (e.g. mussel respiration) [5], [6]Italy. Yet, according to the Kyoto Protocol, CO_2 fluxes resulting from photosynthesis and animal respiration should not be taken into account when calculating greenhouse gasses (GHG) emissions, as they are part of the short C cycle and are balanced.

In this study, the Life Cycle Assessment (LCA) methodology has been applied to three case studies of mussel farming in the north Adriatic Sea, Italy. This study examines all the relevant fluxes of materials and energy across the systems and explores the potential role of biocalcification processes in sequestering carbon from the seawater during shell formation.

Materials and methods

The case studies investigated in this work are referred to as Class_A1 and Class_A2, and Class_B (one case study). Class_A case studies document the environmental performance of mussel farms located in Class A rearing areas, Class_B case study concerns the evaluation of a mussel farm located in Class B rearing area. Unlike mussels reared in Class A areas, those reared in Class B areas must undergo a depuration process before being placed on the market (Reg. EU 2017/625). The LCA approach follows the guidelines of [7]. *Goal and scope*: the objective of this study is to assess the environmental performance of two mussel supply chains, through the analysis of material and energy flows of the only production phase for Class_A mussels, and through both the production and treatment plant phases of Class_B mussels. In the analysis of the CO_2 flows through the systems, the flows of biogenic CO_2 resulting from the biocalcification process are also considered and computed. For this calculation, data on the shell:meat ratio, specific for each site, have been provided by the farmers. Environmental data are according to the literature [8]. A cradle-to-gate analysis has been carried out, considering the following processes: 1) mussel seeding and growing, 2) mussel harvesting and transport to land, and, only for Class_B, 3) mussel depuration in Italy and 4) in France. For all case studies, the system boundaries include the above processes and all material and energy inputs and outputs to and from the systems. The functional unit chosen is 1 kilogram of fresh mussels, including shell, suitable for sale. The *Life Cycle Inventory* is based on data provided by farmers through questionnaires and interviews (foreground data). Ecoinvent 3 database has been used to gather data about production of electricity, fuel, raw materials and transport (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.

(Continued on next page)

Results and conclusions

Carbon footprint (CF, i.e., global warming impact category) of Class_A farms amounts to 0.07 and 0.13 kg CO_2 eq (case studies Class_A1 and Class_A 2, respectively). CF of Class_B farm is 0.53 kg CO_2 eq. These values do not consider the carbon sequestration potential of biocalcification. The difference between Class_A and Class_B results can be attributed to the depuration process required for the sale of Class_B mussels. For all three case studies, the factor contributing most to the environmental impacts is fuel consumption.

When considering biogenic carbon fluxes, the CO_2 sequestration associated with shell formation, net of the CO_2 released, contributes to lowering the CF. This contribution is approximately equal to the overall GHG emissions produced by Class_A farms (CF corrected for the biocalcification process = 0.01 and 0.06 kg CO_2 eq, Class_A1 and Class_A2, respectively), while it reduces the emissions of Class_B mussel production by 25% (CF corrected = 0.43 kg CO_2 eq).

The CF (including the biocalcification process) associated with the production of 1 kilogram of protein is 1.06 and 1.92 kg CO_2 eq for Class_A1 and Class_A2, respectively, and 9.87 kg CO_2 eq for Class_B. These values are much lower than those for beef production (45-210 kg CO_2 eq/kg protein) [9]aquaculture and fishery have major impacts on the environment. In order to identify the range of impacts and the most important factors thereof, as well as to identify what are the main causes of the differences between products, we analysed 52 life cycle assessment studies (LCAs, and comparable or slightly higher than those of vegetable meat substitutes (6-17 kg CO_2 eq/kg protein), potatoes (11.2 kg CO_2 eq/kg protein) and soybean (1.9 kg CO_2 eq/kg protein) [10].

In conclusion, mussel farming proves to be a sector with low environmental impacts. The positive effect of the biocalcification in incorporating CO_2 within the shell allows for an annulment (Class_A) or a substantial reduction (Class_B) of farms' GHG emissions. Given the tight correlation of shell CO_2 sequestration with the ratio of shell to meat, when this ratio increases, the overall CF potential may even assume negative values.

Moreover, since the ratio between CO_2 emitted and sequestered during the biocalcification process depends on both environmental (pH, salinity, temperature and partial pressure of CO_2) and trophic conditions, further studies could clarify whether the choice of farming site can contribute to making this ecosystem service, typical of shellfish farming, more efficient, thus improving the sustainability of the sector.

References

[1]FAO, 2020.
[2] Eurostat (2018) Production from aquaculture excluding hatcheries and nurseries – fish_aq2a. https://ec.europa.eu/eurostat/data/database. Accessed 20.10.20.
[3] Smaal et al., 2019, Springer Nature.
[4] Alonso et al., 2021, J. Clean. Prod., 279.
[5] Munari et al., 2013, Mar. Environ. Res., 92, 264–267.
[6] Aubin et al., 2018, Int. J. Life Cycle Assess., 23 (5), 1030–1041.
[7] C. E. JRC, 2010.
[8] Frankignoulle et al., 1994, Limnol. Oceanogr., 39 (2), 458-462
[9] Nijdam et al., 2012, Food Policy, 37, (6), 760–770.
[10] González et al., 2011, Food Policy, 36, (5), 562–570.

OLEIC ACID DECREASES FEED-INTAKE IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

Nicole Martins*[1,2], Lúcia Vieira, Ana Couto, Cláudia R. Serra, Aires Oliva-Teles, Helena Peres, Carolina Castro

[1]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, Edifício FC4, 4169-007 Porto, Portugal
[2]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões. Avenida General Norton de Matos, S/N, 289; 4450-208- Matosinhos, Portugal
E-mail: nicole.pires@fc.up.pt

Introduction
Regulation of feed intake (FI) and reducing fat deposition, namely visceral fat deposition, is of extreme relevance in fish aquaculture, from an economic, environmental, and consumer perspective. Indeed, feeding represents more than 50% of operating costs in intensive fish farming. Thus, reducing FI without affecting growth performance may have a significant economic impact and contribute to reducing the environmental impact associated with feeding. Modifying fillet fatty-acid profile will also have a considerable implication on fish flesh quality to consumers.

Oleic acid (OA) is a monounsaturated fatty acid that has been receiving special attention as a functional ingredient in mammal diets, due to the beneficial effects in weight gain and fat deposition control by interfering with FI. Recent research in fish also provided evidence that FI is affected by OA (Librán-Pérez et al., 2014).

OA is abundant in some vegetable oils that are becoming used as an alternative to fish oil in aquafeeds. Therefore, novel aquafeeds may have altered OA levels compared to traditional aquafeeds.

However, up to now, the interplay between dietary OA and FI, fat deposition, and metabolism regulation in fish was not explored. Thus, the present study aimed to evaluate the effect of dietary OA supplementation on FI and lipid metabolism of European seabass (*Dicentrarchus labrax*) fed high-fat aquafeeds.

Material and methods
Six isoproteic diets (45% crude protein) with two different dietary lipids levels (16 and 22%) were formulated with fishmeal and cod liver oil as protein and lipid sources and supplemented with 0, 1, or 2% of OA (diets 16L:0OA; 16L:1OA; 16L:2OA and 22L:0OA; 22L:1OA; 22L:2OA). Triplicate groups of 22 European seabass (initial body weight: 21.4g) were fed with these diets twice a day to apparent visual satiation, six days a week, for 10 weeks. At the end of the growth trial, fish were bulk weighed following one day of feed deprivation. Fish continued to be fed for 3 more days, then hypothalamus and liver from 3 fish per tank were collected 3 hours after the morning meal and stored until analysis. Visceral fat and liver from another 3 fish per tank, were collected to evaluated fat deposition mechanisms.

Results
The growth of European seabass was not affected by dietary treatments. However, regardless of dietary lipid levels, OA supplementation enhanced feed efficiency by decreasing feed intake (Table 1).

Regarding lipid metabolism, dietary OA supplementation increased the hepatic G6PDH and decreased malic enzyme activity (Table 2). However, no effects on other key enzymes of fatty acid anabolism or catabolism were noticed. In the hypothalamus, the activity of lipid metabolism enzymes was not affected either by dietary OA supplementation or dietary lipid levels (data not shown).

Conclusions
Our data showed that supplementation of 1-2% DM of OA to fishmeal and fish oil-based diets lead to a reduction of feed intake by 12% in fish fed 2%OA diets and to an improvement of feed efficiency around 8%, without major impacts on the growth performance of the European seabass. Nevertheless, further analyses are needed to better understand the effect of OA in the mechanisms involved in feed intake regulation of European seabass.

(Continued on next page)

Table 1. Growth performance and feed utilization of European seabass-fed experimental diets.

	Diets						
	16L:0OA	**16L:1OA**	**16L:2OA**	**22L:0OA**	**22L:1OA**	**22L:2OA**	**SEM**
IBW (g)[1]	21.4	21.4	21.4	21.4	21.3	21.4	0.01
FBW(g)[2]	57.4	56.5	56.2	57.0	57.9	55.7	0.74
WG (g kg ABW^{-1} day^{-1})[3]	15.5	15.2	15.2	15.4	15.6	15.1	0.17
DGI[4]	1.83	1.80	1.79	1.82	1.86	1.77	0.03
FI (g kg ABW^{-1} day^{-1})[5]	19.0	16.5	16.7	18.7	17.6	16.5	0.30
FE[6]	0.80	0.87	0.87	0.82	0.89	0.90	0.01
PER[7]	1.77	1.93	1.90	1.83	1.95	1.99	0.02
Survival (%)	98.5	95.5	97.0	100	100	98.5	0.65

Two-way ANOVA						
	Variance Source			**OA level**		
	L	**OA**	**LxOA**	**0%**	**1%**	**2%**
IBW (g)[1]	ns	ns	ns			
FBW(g)[2]	ns	ns	ns			
WG (g kg ABW^{-1} day^{-1})[3]	ns	ns	ns			
DGI[4]	ns	ns	ns			
FI (g kg ABW^{-1} day^{-1})[5]	ns	**	ns	**b**	**a**	**a**
FE[6]	ns	***	ns	**a**	**ab**	**b**
PER[7]	ns	***	ns	**a**	**ab**	**b**
Survival (%)	ns	ns	ns			

[1]Values are presented as mean (n=3). SEM: Pooled standard error of the mean. L: dietary lipid level (22 and 16%); OA: Oleic acid supplementation (0,1 and 2%). ns: non-significant; *p<0.05; **p<0.01; ***p<0.001.
[1]Initial body weight; [2]Final body weight; [3]Weight gain ((FBW-IBW) x 1000)/(((IBW+FBW)/2) x days); [4]Daily growth index ((FBW$^{1/3}$ – IBW$^{1/3}$)/time in days) × 100; [5]Feed intake ((1000 x total ingestion)/((FBW+IBW)/2))/days)); [6] Feed efficiency (wet weight gain/ dry feed intake); [7] Protein efficiency ratio (wet weight gain/ crude protein intake).

Table 2. Hepatic enzymatic activity (mU/mg protein) of enzymes involved in lipogeneses and fatty acid oxidation in European seabass fed the experimental diets[1].

	Diets						
	16L:0OA	**16L:1OA**	**16L:2OA**	**22L:0OA**	**22L:1OA**	**22L:2OA**	**SEM**
G6PDH[a]	265.6	235.9	356.5	280.5	263.0	287.3	12.1
ME[b]	15.7	15.7	14.9	20.2	15.2	12.9	0.69
FAS[c]	1.59	2.50	1.76	2.01	2.38	1.42	0.14
CPT1[d]	5.98	6.93	5.99	6.45	6.56	5.84	0.22
ACLY[e]	3.35	4.62	3.41	3.77	3.33	2.89	0.18

Two-way ANOVA						
	Variance Source			**OA level**		
	L	**OA**	**LxOA**	**0%**	**1%**	**2%**
G6PDH[a]	ns	*	ns	**ab**	**a**	**b**
ME[b]	ns	*	ns	**b**	**ab**	**a**
FAS[c]	ns	ns	ns			
CPT1[d]	ns	ns	ns			
ACLY[e]	ns	ns	ns			

[1]Values are presented as mean (n=9). SEM: Pooled standard error of the mean. L: dietary lipid level (22 and 16%); O: Oleate level (0,1and 2%). ns: non-significant; *p<0.05; **p<0.01. [a]Glucose-6-phosphate dehydrogenase; [b]Malic enzyme; [c]Fatty acid synthase; [d]Carnitine acyltransferase I; [e]ATP-citrate lyase.

References

Librán-Pérez, M., Otero-Rodiño, C., López-Patiño, M.A., Míguez, J.M., Soengas, J.L., 2014. Central administration of oleate or octanoate activates hypothalamic fatty acid sensing and inhibits food intake in rainbow trout. Physiol. Behav. 129, 272–279. https://doi.org/10.1016/j.physbeh.2014.02.061

Acknowledgments

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) and by the project ATLANTIDA - Platform for the monitoring of the North Atlantic ocean and tools for the sustainable exploitation of the marine resources (CCDRN-Norte2020 - Integrated Projects ICDTInstitutions). Nicole Martins was supported by a FCT Grant SFRH/BD/ 137919/2018.

MICROBIOME COMPARISON BETWEEN ECONOMICALLY IMPORTANT BIVALVE SPECIES AT RIA DE AVEIRO LAGOON (PORTUGAL)

J.C.Martins[*1], M. Semedo[1], A. Alex[1], D. Alexandrino[1], F. Carvalho[1], P.R. Costa[2], A. Campos[1], V. Vasconcelos[1,3]

[1]CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208, Matosinhos, Portugal
[2]IPMA – Instituto Português do Mar e da Atmosfera, Lisbon, Portugal, 3 I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[3]Department of Biology, Faculty of Sciences, Porto University, Rua do Campo Alegre, 4069-007 Porto, Portugal
E-mail: jmartins@ciimar.up.pt

Despite the crucial ecological and economic roles of mollusk bivalves, little is known about their associated microbiota members. In this context, high-throughput 16S rRNA gene amplicon sequencing was applied to assess the microbial community diversity among three tissues (intestine, hepatopancreas and gills) of shellfish species living in sympatry at Ria de Aveiro lagoon. This lagoon is one of the major shellfish harvesting sites in Portugal. The study comprehended five different bivalve species with economic value, namely the clams *Ruditapes philippinarum*, *Ruditapes decussatus*, *Venerupis corrugata* and *Solen marginatus* (razor clam), the cockle *Cerastoderma edule* and the mussel *Mytilus edulis*.

Preliminary results show that microbiota structure differed among tissues, with intestine harboring the highest number of microbial ASVs for all bivalve species. Both Chao1 richness estimates and Shannon diversity levels for gut microbiota were significantly higher in comparison with the other tissues for almost all species, with exception of *C. edule*. No significant differences in species richness were found between bivalves for hepatopancreas and gills. On the other hand, results show significantly lower Shannon diversity levels for *V. corrugata* in these organs. Gut microbiota β-diversity for the five bivalves is not only distinct compared to the other organs, but also among the mollusks themselves. Results also show a clear microbial community distinction between gills tissues of *V. corrugata* and *S. marginatus* compared to the other bivalve species. A small set of core ASVs was identified in different tissues - gills (1 ASV), hepatopancreas (2 ASVs) and intestine (43 ASVs)- suggesting a possible role of the core taxa for the ecological fitness of the hosts. Concerning exclusive genera observed, the highest numbers are presented by *M. edulis* in intestine (50), *S. marginatus* (14) and *C. edule* (12) in hepatopancreas and again *C. edule* (51) in gills. Overall, our results indicate that bivalve-associated microbes are host- and tissue-specific among sympatric bivalve species. The resulting data contribute to the deep characterization of the taxonomic diversity of the bivalves' microbial communities which is essential for the understanding of host-microbe association and its impact on bivalve physiology.

GROWTH PERFORMANCE, FEED UTILIZATION, AND TAURINE SYNTHESIS OF COMMON CARP FED DIFFERENT LEVELS AND SOURCES OF DIETARY METHIONINE

Karthik Masagounder[a*], Yuanyuan Zhou[b], Sarah He[c], and Chaoxia Ye[b]

[a]Evonik Operations GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany
[b]Institute of Modern Aquaculture Science and Engineering, South China Normal University, Guangzhou 510631, People's Republic of China
[c]Evonik (China) Co., Ltd. Guangzhou Branch, China
*email: karthik.masagounder@evonik.com

Introduction

Methionine is involved mainly in the body protein synthesis and is a precursor for various metabolites which are essential for DNA methylation (e.g., S-Adenosylmethionine) and antioxidant defense (glutathioine, taurine). Commercially common carp feed is formulated primary with plant ingredients where methionine is often the first limiting amino acid. DL-methionine (DL-Met) and methionine hydroxy analogue (DL-2-hydroxy-4-methylthiobutyrate or DL-HMTBA and its calcium salt) are the two commonly used supplemental methionine sources in the feed production. However, their biological efficiency as a source of methionine is not the same and therefore, the correct nutritional value needs to be considered in the feed formulation to avoid any production loss. The objective of the study was to evaluate the effects of DL-methionine (DL-Met) and a methionine hydroxy analogue (MHA-Ca) on the growth performance, feed utilization and the expression of genes related to taurine and protein synthesis in juvenile common carp.

Material and methods

The experiment involved 9 diets. A basal diet was formulated to meet amino acid requirements for juvenile common carp according to NRC (2011) and Evonik recommendations, except for Met (0.49%) and Met+Cys (0.94%). For diet 2 to 4, the basal diet was supplemented with increasing levels of DL-Met at 0.1% increments (0.1-0.4%), and for Diet 6 to 9, the basal diet was supplemented with 0.1% increment levels of MHA-Ca (0.1-0.4%). The analyzed content of total and supplement Met levels were close to the planned values in all the diets. Each diet was randomly assigned to 5 replicate tanks. Each tank contained 30 fish with an average initial body weight of 3.7g. Fish were fed twice daily to apparent satiation for 8 weeks. Growth performance, feed conversion ratio (FCR), and muscle taurine content of fish were evaluated. In addition, at the end of the trial, fish liver samples (n = 6 fish per tank; 5 tanks) were analyzed for the expression of genes of enzymes (cysteine dioxygenase, CDO; cysteinesulfinate decarboxylase, CSD) involved taurine synthesis and genes of protein involved in the mTOR pathway.

Results and Discussion

Basal diet deficient in Met (0.49%) and M+C (0.94%) showed significantly lower growth rate, final body weight, FCR, protein efficiency ratio, and muscle taurine content ($P < 0.01$, one-way ANOVA). Increasing methionine supplementation significantly improved performance for both the methionine sources. Two-ANOVA test revealed the main effects due to methionine levels and sources on the performance parameters without any interaction effect between the two. Results showed that DL-Met produces better growth, body weight, FCR, protein efficiency and muscle taurine content than does MHA-Ca ($P < 0.01$). Between the two methionine sources, DL-Met fed fish exhibited higher expression genes of enzymes (CDO, CSD) responsible for taurine synthesis in the liver compared to the MHA-Ca fed group, corroborating the taurine levels found in the plasma and muscle tissue. We also found higher expression of mTOR related genes (S6 and eIF4E) by DL-Met vs MHA-Ca in the liver of fish, supporting the better growth and protein efficiency observed with the DL-Met fed fish than with the MHA-Ca fed fish.

Finally, we used multiple exponential or linear regression analysis, depending on the response of the performance parameter (body weight, growth, FCR and muscle taurine content), to compare the biological efficiency of the two methionine sources. Based on the analysis, biological efficiency of MHA-Ca was found to be only 43-52% relative to DL-Met on weight-for-weight basis (51-62% on an equimolar basis), depending on the parameters (Table 2). Overall, results demonstrate that DL-Met is a more efficient Met source compared to MHA-Ca in common carp.

(Continued on next page)

Table 1. Growth performance, feed utilization and taurine content of common carp fed with different methionine levels and sources over an 8-week experimental period[¥].

Diet	Final body weight, g	SGR, %/d	FCR	Protein efficiency ratio %	Muscle taurine %
Basal	17.8	2.8	1.45	1.89	0.21
Supp. level					
0.10%	18.4^{a}	2.87^{a}	1.33^{a}	2.04^{a}	0.26^{a}
0.20%	20.9^{b}	3.09^{b}	1.21^{b}	2.24^{b}	0.30^{a}
0.30%	20.2^{b}	3.01^{ab}	1.25^{ab}	2.17^{ab}	0.42^{b}
0.40%	19.3^{ab}	2.98^{ab}	1.28^{ab}	2.12^{ab}	0.45^{b}
Met sources					
DL-Met	20.3^{x}	3.04^{x}	1.24^{x}	2.20^{x}	0.42^{x}
MHA-Ca	19.3^{y}	2.94^{y}	1.30^{y}	2.10^{y}	0.30^{y}
Two-way ANOVA (*P-value*)*					
Supp. level	<0.01	<0.01	<0.01	<0.01	<0.01
Met sources	0.01	0.02	0.02	0.02	<0.01
Supp. level x Met sources	0.27	0.33	0.2	0.22	0.03

[¥]Mean values with different superscript letters are significantly different ($P < 0.05$); SGR = specific growth rate; FCR = feed conversion ratio

*basal diet was excluded in the two-way ANOVA analysis; one-way ANOVA and contrast analysis showed basal diet was significantly lower than other treatments.

Table 2. Biological efficiency (%) of MHA-Ca relative to DL-Met on a weight-for-weight basis for various performance parameters

Parameters	Equations	DL-Met	MHA-Ca
Final body weight, g	FBW = 17.515 + 3.238 [1-exp(-0.0468 (DL-Met + 0.462 MHA-Ca))]	100	46
Specific growth rate, %/d	SGR = 2.7867 + 0.2952 [1-exp(-0.0439 (DL-Met+0.425 MHA-Ca))]	100	43
Feed conversion ratio	FCR = 1.450 - 0.209 [1-exp(-0.0874 (DL-Met + 0.503 MHA-Ca))]	100	50
Muscle taurine %	Muscle Taurine = 0.19+0.005 (DLM + 0.52 MHA-Ca)	100	52

[*]Data analysis by including all the replicates (n=5).

Reference

NRC (National Research Council) (2011) Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington DC

RESPONSE OF WHITELEG SHRIMP TO DIETARY PROTEIN LEVELS BALANCED FOR AMINO ACIDS USING SUPPLEMENTAL SOURCES

Karthik Masagounder[1*], Tran Thi Thanh Hien[2], Le Quoc Viet[2], Tran Le Cam Tu[2], Tran Thi Tuyet Hoa[2], Nguyen Van Tien[3], Chuong Ngo Tien[4] and Pham Minh Duc[2]

[1]Evonik Operations GmbH, Hanau-Wolfgang, Germany
[2]College of Aquaculture and Fisheries, Can Tho University, Vietnam
[3]Evonik Vietnam Limited Liability Company, Vietnam
[4]Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Office, Hanoi, Vietnam
*email: karthik.masagounder@evonik.com

Introduction

Cost of shrimp feed is largely dictated by dietary protein levels and sources. Commercial shrimp feed typically contains 35-40% crude protein (CP), depending on feed type. Shrimp, like other farm animals, don't have a true requirement for protein, but for a well-balanced dietary amino acid level. In the commercial feeds, the bulk of shrimp requirements for essential amino acids (EAA) are met through intact sources. Following ideal protein concept in formulating shrimp diets for EAA requirements using supplemental sources is slowly gaining momentum in shrimp feeds due to its potential to spare the dietary protein sources such as fish meal and thus, the dietary protein content and feed cost. The objective of this study was to evaluate the performance of whiteleg shrimp to varying levels of dietary protein balanced for amino acid profile using supplemental amino acids.

Material and methods

A positive control diet containing high dietary protein level (40%), mimicking the industry standard diet with 14% fish meal level, was formulated (D40). A set of treatment diets containing 38% (D38), 36% (D36), 34% (D34) and 32% (D32) crude protein levels was formulated by reducing the inclusion of intact protein sources such as fish meal and wheat gluten meal, while meeting our recommended amino acid levels using supplemental sources including AQUAVI® Met-Met, L-Lys and L-Thr. With this approach, the formulation cost of feed was gradually reduced with the increasing protein reduction, up to 20% between D40 and D32. All the diets were produced by a commercial feed mill in Vietnam. Analyzed crude protein and amino acid levels were close to the planned values for the diets. Four tanks (800 L water volume) were randomly allotted to each dietary treatment. Each tank was stocked with 80 shrimp (2.5 ± 0.04 g, mean ± SD). A water temperature of ~30 °C and a salinity of ~15 ppt were maintained throughout the experiment. Shrimp were fed with the respective diets to apparent satiation, 4 times daily over 56 days. Mortality was daily recorded and accounted for survival. All the shrimp were bulk weighed at the beginning and end of the experiment, and the mean weight was calculated based on the counts of shrimp. At the end, four shrimp per tank were pooled and used for body composition analysis. From the initial population, 20 shrimp were pooled and used for initial body composition.

Results and Discussion

After 56 days, survival varied from 95% to 97% with no significant differences detected among treatments. Final body weight, growth rate, feed intake, and FCR did not differ among dietary treatments (Table 1). Net protein utilization (protein gain x 100 / protein intake) showed significant improvement in shrimp fed with the diet containing 34%CP versus those fed with the diets containing 36-40% CP, showing the benefits of feeding shrimp with the amino acid balanced reduced protein diets. Diets did not affect body composition of shrimp (Table 2). We also examined intestinal morphology (n = 3 shrimp per tank) of shrimp. Villus height, villus width (D40 > others), crypt depth (D36> others) and the ratio of villus height to crypt depth (D36>D38) showed significant differences, with no clear trend to the dietary CP levels. Our study results overall demonstrate the opportunity to reduce dietary protein levels in commercial shrimp feed using supplemental amino acids.

(Continued on next page)

Table 1. Growth performance and feed utilization of shrimp fed the experimental diets over an 8-week experimental period.

Diet	Final body weight (g)	SGR (%/day)	Feed intake (g)	FCR	Net protein utilization %	Survival %
D40	17.67	3.47	17.83	1.18	42.0 b	97.2
D38	17.84	3.50	17.94	1.17	42.0 b	96.9
D36	17.30	3.43	18.24	1.24	41.3 b	95.3
D34	18.19	3.56	18.24	1.16	46.3 a	95.3
D32	17.47	3.48	18.21	1.22	45.3 ab	95.3
SEM	0.36	0.04	0.41	0.03	1.27	1.12
P-value	0.51	0.35	0.92	0.36	0.04	0.56

SGR = specific growth rate; FCR = feed conversion ratio; SEM, standard error of the means
*Duncan mean separation was used

Table 2. Whole-body composition (%, wet weight) of shrimp fed the experimental diets over an 8-week experimental period.

Diet	Moisture	Protein	Lipid	Ash
D40	75.4	19.1	1.5	2.8
D38	76.3	18.2	1.7	2.8
D36	76.4	18.0	1.4	2.8
D34	75.5	18.5	1.5	3.1
D32	76.1	17.7	1.4	3.1
SEM	0.39	0.33	0.10	0.11
P-value	0.26	0.10	0.18	0.12

EFFECT OF SEAWEED BIOMASS (*Ulva ohnoi*) AND CHLOROPHYLL ON LIGHT EXTINCTION COEFFICIENT IN TANKS WITH OPAQUE WALLS

I. Masaló[1*], A. Carrascosa[1], J. Bringué[1], J. Oca[1]

[1]Departament d'Enginyeria Agroalimentària i Biotecnologia. Universitat Politècnica de Catalunya - BarcelonaTECH. Esteve Terrades 8, 08860 Castelldefels, Catalunya, Spain

E-mail: ingrid.masalo@upc.edu

Introduction

One of the bottlenecks in multitrophic recirculation culture systems (IMTA-RAS) of fish and macroalgae is to increase the productivity and reduce the land surface required for seaweed production. The knowledge of the effect of the fluctuations in the irradiance received by the algae along their trajectory inside the tanks on the processes of photoinhibition and growth, will contribute to increase the productivity and the elimination of nutrients by surface unit in fish-seaweed multitrophic systems.

In tanks with opaque walls, where light received by the seaweed comes from the incident light in the water surface, light attenuation along a vertical axis depends on algal biomass. The incident irradiance to the water surface (E_0) attenuates through the water columns and declines exponentially with depth. According to Lambert-Beer law the irradiance at a depth Z (E_Z) below the surface will be: $\mathbf{E_Z=E_0 \cdot exp^{(-K'Z)}}$. K' ($\mathbf{K'=K_0+K_a \cdot B_V}$) is the light attenuation constant (m^{-1}), which will be determined by the water light extinction coefficient (K_0 in m^{-1}), the seaweed biomass per unit volume (B_V in g/m^3) and the seaweed light extinction coefficient (K_a in m^2/g). In tanks with seaweed K_0 has a small relative contribution to the K' value.

The aim of this work was to determine the effect of the *Ulva* biomass and chlorophyll content on the light extinction cofficients in seaweed tanks with opaque walls.

Material and methods

Three circular tanks (64 cm diameter, 30 cm height) with bottom air injection (6 l min^{-1}) and opaque walls that received the irradiance through the water free surface were used. A height of water equal to the radius of the tank was always maintained to ensure the formation of two rotating flow cells placed, in the vertical section of the tank, at both sides of the aeration inlet. The incident irradiances in the surface of the tanks were: 1090, 476 and 183 μmol m^{-2} s^{-1} in tanks 1, 2 an 3.

Experiments were carried out with three *Ulva ohnoi* biomass (256, 512 and 768 g, which correspond to stocking densities SD 0.8, 1.6 and 2.4 kg m^{-2}). For each biomass, five PAR measurements were made every 5 cm from the surface to the deepest point of the tank (30 cm). K_0 was calculated without seaweed at different days in the tanks, and the relation between turbidity and K_0 was established (Fig. 1).

The seaweed used in each tank was acclimated during 1 week at SD 2.4. kg m^{-2}, before the experiments were made. Chlorophyll content was determined after the period of acclimation in 10 fronds of each tank with a portable chlorophyll optical meter using the method described by Masaló and Oca (2020). To carry out the PAR measurements the biomass was extracted, centrifuged and consecutively the biomass was increased from 256 to 768 g. With each biomass the PAR along the vertical axis was measured (5 measures at each depth) in the three tanks.

The chlorophyll content in Tanks 1, 2 and 3 were 102.2, 119.8 and 120.1 μmol m^{-2}, respectively (Table 1).

K' increased (Fig. 1), as it was expected, with seaweed biomass. Calculated K_a (from K_0 and K') where higher in Tanks 2 and 3 and was highly correlated with chlorophyll content (Table 1).

Results presented show that light extinction coefficients decreased with a decrease in chlorophyll concentration, indicating that tanks with a low chlorophyll concentration received more irradiation than the ones with higher chlorophyll concentration and could be photoinhibited and productivity would be negatively affected.

The knowledge of K_a for specific seaweed condition will allow to determine the light extinction coefficient in funcition of *Ulva* biomass and to estimate the PAR received by the alga fragments in each position of the tank.

(Continued on next page)

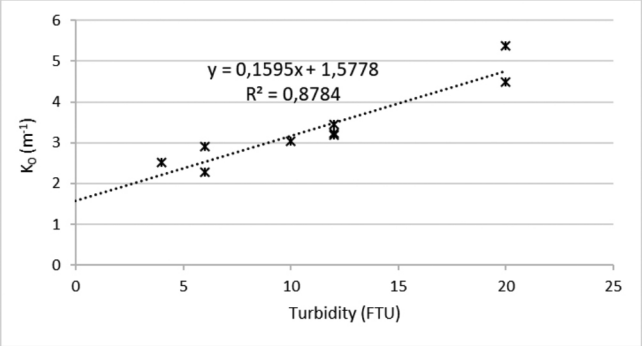


Fig. 1. Relationship between the water light extinction coefficient K_0 and turbidity.

	Tank 1	Tank 2	Tank 3
E_0 (µmol m^{-2} s^{-1})	1090	476	183
Chlorophyll (µmol m^{-2})	102.2±29.4	118.8±16.2	120.1±19.2
K_a (m^2 g^{-1}FW)	$1.65x10^{-3}$ $\pm3.5x10^{-4}$	$1.81x10^{-3}$ $\pm4.9x10^{-4}$	$1.86x10^{-3}$ $\pm3.1x10^{-4}$

Table 1. Experiments conditions and results. K_a is the average of the coefficient obtained in each tank with the three biomass used.

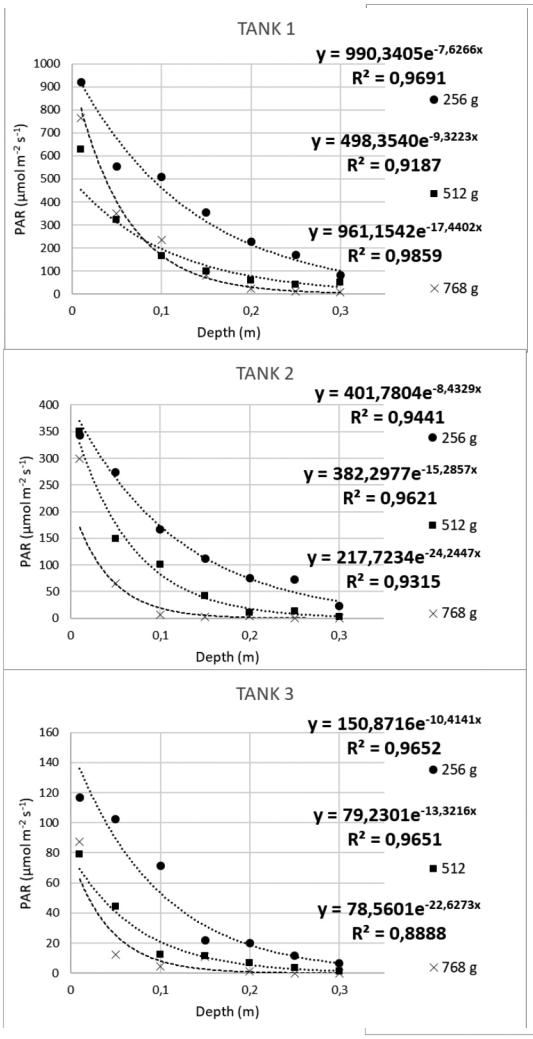


Fig. 2. Relationship between PAR and depth in tanks 1, 2 and 3.

Acknowledgments: Work funded by Spanish MICIU (RTI2018-095062-A-C22).

References

Masaló I, Oca J (2020) Evaluation of a portable chlorophyll optical meter to estimate chlorophyll concentration in the green seaweed *Ulva ohnoi*. J Appl Phycol 32: 4171–4174

THE GONADAL SOMA-DERIVED FACTOR (GSDF1) AS MARKER OF PRECOCIOUS PUBERTY IN MALE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Alessia. Mascoli *, Berta. Crespo, Joan Pizarro, Felipe Espigares, Cinta Zapater, Silvia Zanuy, Ana Gómez.

Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (CSIC) 12595 Ribera de Cabanes, Castellón, Spain.
E-mail: alessia.mascoli@iats.csic.es

Introduction

In nature, European sea bass males reach puberty by their second year of life; however, under intensive culture conditions, the sex ratio male:female increases (3:1), males generally are smaller than females and the precocious puberty occurs as a phenotypic response to enhanced growth conditions and feed availability (Espigares *et al*., 2015). Around 20-30% of farmed male European sea bass enter puberty at one year of age (Carrillo *et al*., 1995). Puberty is associated with a progressive reduction of the growth rate with age (Taranger *et al*. 2010), that is even more marked in precocious males compared to their non-precocious counterparts during the second year (Felip *et al*., 2006), resulting in considerable economic losses in fish farms.

The gonadal soma-derived factor (Gsdf), which belongs to the transforming growth factor beta (TGFβ) superfamily, is exclusively found in teleosts and it is apparently involved in the proliferation of type A spermatogonia, with expression levels decreasing as spermatogenesis progresses (Schulz *et al*., 2010; Skaar *et al*., 2011). Therefore, it could be considered a decisive player in the onset of puberty and a possible marker for the early detection of precocious males.

Materials and methods

All the animals used for experiments were kept under natural photoperiod and temperature conditions at the Instituto de Acuicultura Torre de la Sal (IATS) facilities. Juvenile (9-months-old) European sea bass males were subjected to hemigonadectomy in September, the period when spermatogonial proliferation towards differentiation may occur in precocious males (Molés *et al*., 2011). The left testicle was extracted from each animal, frozen in liquid nitrogen and stored at -80 °C until molecular analysis. Hemigonadectomized animals were kept until the spermiation period (February), when they were sacrificed and the right gonad collected for histological analysis.

One-year-old male European sea bass kept in natural conditions were sampled every two weeks from August to November. In each sampling only the smallest 15% (Small group) and the largest 25% (Large group) fish were selected for analysis. Gonads were sampled for histological analysis or frozen in liquid nitrogen and stored at -80 °C to perform gene expression assays.

Adult specimens of male sea bass (5-years-old) kept in natural conditions were sampled monthly during an entire annual reproductive cycle.

Testes from all fish (9-months-old hemigonadectomized, 1-year-old and adult animals) were staged, according to Begtashi *et al*. (2004). Total RNA was extracted from whole testes and reverse-transcribed, and cDNAs were used as templates for quantitative real-time RT-PCR (qPCR) assays.

Results

The hemigonadectomized 9-months-old animals were classified as “non-precocious” or “precocious” when right testes remained in stage I, or reached stage IV, respectively. The *gsdf1* gene was found to be significantly downregulated in the left testes of precocious animals in September ($p < 0.05$) (Crespo *et al*., 2013).

One-year-old males from the Small group did not arrive to full spermiation and showed higher *gsdf1* expression than the ones of the Large group during all the experiment time, with significant differences in August and October. Most of the animals of the Large group spermiated the next winter.

During the annual cycle of adult sea bass, *gsdf1* showed maximum expression levels in premeiotic (immature) testis, that decreased as spermatogenesis progressed, with a slight increase in stage VI (post-spawning) in preparation for the next reproductive cycle.

(Continued on next page)

Discussion

In juvenile 9-months-old sea bass males, precocious puberty is negatively correlated with *gsdf1* expression. According to Begtashi *et al*. (2004), during their first year of life, precocious males are significantly larger than non-precocious one. Thus, in the group of 1-year-old large fish there are significantly more precocious animals than in the group of small specimens. In accordance with these results, small males (supposed not precocious) showed higher *gsdf1* expression than the large ones.

In adult males, high expression levels of the gene are only detected in premeiotic stages, suggesting a role for *gsdf1* in spermatogonial stem cells, or in proliferation of undifferentiated type A spermatogonia. Once spermatogenesis starts, a number of undifferentiated type A spermatogonia halt the self-renewal process and enter the differentiation pathway toward meiosis, which would explain the lower expression levels of *gsdf1* in adult males after premeiotic stages and in juvenile precocious animals (Crespo *et al*., 2013).

In summary, this study confirms *gsdf1* as marker of precocious puberty and as a potential target for puberty manipulation.

Acknowledgments

Funded by MICINN grants CSD2007-00002 and RTI2018-094667-B-C22, and by EU project LIFECYCLE (FP7-222719-1). A.M. is supported by a PhD contract from GV (GRISOLIAP/2020/129).

References

Espigares, F., Rocha, A., Molés, G., Gómez, A., Carrillo, M., Zanuy, S. New insights into the factors mediating the onset of puberty in sea bass. Gen Comp Endocrinol 2015; 224:176-185

Carrillo, M., Zanuy, S., Prat, F., Cerdá, J., Ramos, J., Mañanós, E., Bromage, N., 1995. Sea bass (Dicentrarchus labrax). In: Bromage, N., Roberts, R. (Eds.), Broodstock Management and Egg and Larval Quality. Black Science, Oxford, pp. 138–168.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., Hansen, T., 2010. Control of puberty in farmed fish. Gen. Comp. Endocrinol. 165, 483–515.

Felip, A., Zanuy, S., Carrillo, M., 2006. Comparative analysis of growth performance and sperm motility between precocious and non-precocious males in the European sea bass (Dicentrarchus labrax, L.). Aquaculture 256, 570–578.

Schulz, R.W., de Franca, L.R., Lareyre, J.J., Le Gac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411.

Skaar, K.S., Nobrega, R.H., Magaraki, A., Olsen, L.C., Schulz, R.W., Male, R., 2011. Proteolytically activated, recombinant anti-mullerian hormone inhibits androgen secretion, proliferation, and differentiation of spermatogonia in adult zebrafish testis organ cultures. Endocrinology 152, 3527–3540.

Molés, G., Rocha, A., Espigares, F., Gómez, A., Carrillo, M., Zanuy, S., 2011. FSH plasma levels during testicular recrudescence of precocious and non precocious male European sea bass using a newly developed species specific enzymalinked immunosorbent assay (ELISA). Indian J. Sci. Technol. 4, 58–59.

Begtashi, I., Rodríguez, L., Molés, G., Zanuy, S., Carrillo, M., 2004. Long-term exposure to continuous light inhibits precocity in juvenile male European sea bass (Dicentrarchus labrax, L.). I. Morphological aspects. Aquaculture 241, 539– 559.

Crespo, B., Gomez, A., Mazon, M. J., Carrillo, M., & Zanuy, S. (2013). Isolation and characterization of Ff1 and Gsdf family genes in European sea bass and identification of early gonadal markers of precocious puberty in males. General and comparative endocrinology, 191, 155-167.

OCEANOGRAPHIC AND ECOLOGICAL INTERACTIONS WITH AN OFFSHORE, LONG-LINE MUSSEL FARM

Llucia Mascorda Cabre1*, Phil Hosegood1, Martin Attrill1, Emma Sheehan1

School of Biological and Marine Sciences, Faculty of Science and Engineering, University of Plymouth, Plymouth, UK
E-mail: llucia.mascordacabre@plymouth.ac.uk

Introduction

Bivalve aquaculture has traditionally been established in shallow, sheltered waters in inshore areas generating notable negative environmental impacts due to the accumulation of waste products. However, the recent global expansion of the offshore industry is perceived to have a lower environmental impact coupled with a higher growth potential. Mussels can positively contribute to marine ecology as carbon storage, nutrient remediation and coastal defence. Hence, the development of offshore aquaculture has the potential to provide the most sustainable source of protein to feed our growing population.

In 2013, the University of Plymouth commenced a robust annual monitoring study of the UK's 1st large scale offshore mussel farm in Lyme Bay, south west UK. Using a range of underwater survey vehicles and sampling techniques, the study has been valuable in showing ecological interactions such as the potential to increase habitat value and increase commercially valuable mobile species (Figure 1).

Aims

This PhD aims to assess the overall footprint of this offshore mussel farm, focusing on the effects that the farm has on the oceanography and plankton, while maintaining the long-term ecological surveys during three more survey seasons. The study will focus on how currents and waves are modified by the structure and the consequent impact on the surrounding ecology. The focus is specifically on sediment transport and plankton depletion as well as the functional change of benthic and commercially targeted species.

Methodology

For the oceanographic study, current velocity data was collected with a boat mounted Acoustic Doppler Current Profiler (ADCP) through an entire tidal cycle during spring tides. A fixed bedframe ADCP will also be placed on the farm to further study water column profiles. For the ecological study, benthic epifauna is being quantified through Towed HD video array, ROV and Baited Remote Underwater Video (BRUV) while pelagic epifauna is being quantified using Non-baited Midwater Video rigs (NMV). Sediment and infauna communities are being quantified by sediment grabs and laboratory analysis whereas plankton communities have been sampled using a plankton mesh throughout the entire water column and identified to species level at the laboratory.

Results

Oceanographic and ecological data is being analyse and will be shown during the oral presentation. Preliminary observation of results show that the presence of the mussel farm is having an effect on the speed and direction of the flow, both at ebb and flood tides (Figure 2) as well as the abundance and type of zooplankton (Figure 3).

(Continued on next page)

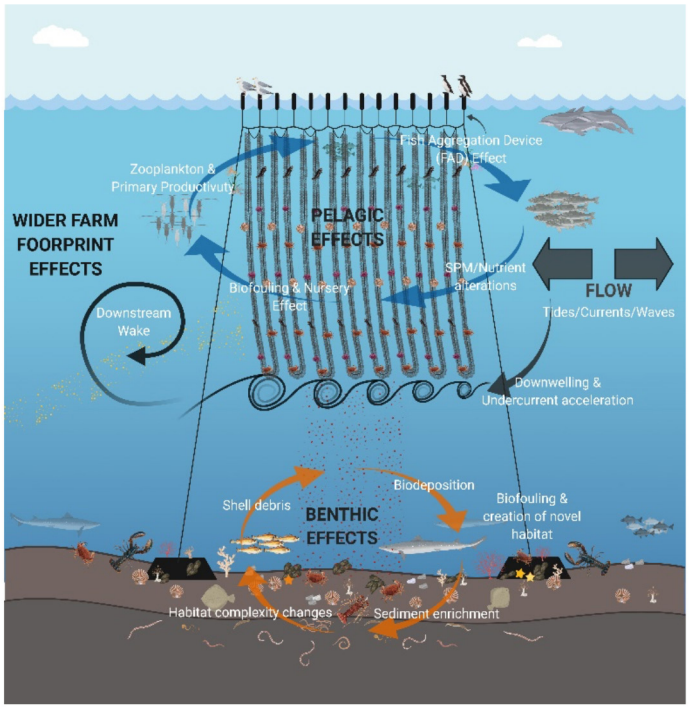


Figure 1 Main potential ecological and oceanographic effects of a longline mussel farm (Mascorda Cabre, 2020).

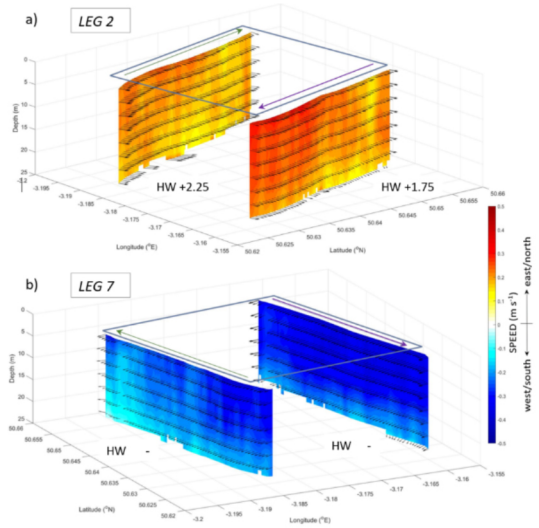


Figure 2 Current velocity profiles from two representatives transect legs of the ADCP survey in the study area (a) Leg 2 and (b) Leg 7 travelling north to south on the east side of the farm (purple arrows) and south to north on the west side of the farm (green arrows).

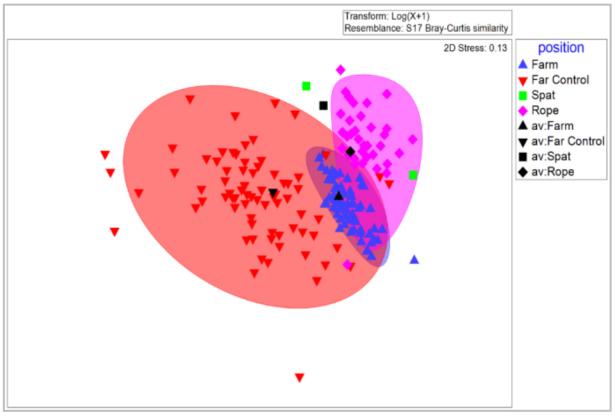


Figure 3 Bootstrap average analysis shows Bray-Curtis similarities for 2019 plankton samples by position. The bootstrap average uses non-metric Multi-Dimensional Scaling (nMDS). Data was pre-treated with a log transformation (PRIMER 7).

TOOLBOX FOR GOOD MARINE LITTER MANAGEMENT IN THE AQUACULTURE SECTOR

Mata-Lara M.*[1], Vidal, M.[2], Alomar, C.[2], Deudero, S.[2], Devriese, L.[3], Sandra, M.[3], De Raedemaecker, F.[3], Altvater, S.[4], Zorgno, M.[5], Gin, I.[6], Lheureux, G.[6], Vale, M.[7] and Hipolito, C[7].

[*1] Geonardo Environmental Technologies Ltd., Zahony utca 7, Budapest, 1031 (Hungary)
Email: mariana.mata.lara@geonardo.com
[2] Spanish Institute of Oceanography. Moll de Ponent, s/n. 07015, Palma de Mallorca (Spain)
[3] Flanders Marine Institute. Wandelaarkaai 7, 8400 Oostende (Belgium)
[4] Sustianable Projects. Kärntener Str. 20, 10827 Berlin (Germany)
[5] EurOcean Foundation. Avenida Dom Carlos I, 126-3°, 1249-074, Lisboa (Portugal)
[6] Nausicaä Centre National de la Mer. Boulevard Sainte-Beuve, 62200, Boulogne-sur-Mer (France)
[7] Regional Fund for Science and Technology. Rua do Mercado 21, 9500-326, Ponta Delgada (Portugal)

Introduction

Ocean based sources account for 20 % of the plastic pollution such as overboard discharges from ships and abandoned, lost or otherwise discarded fishing and aquaculture gear (ALDFG). Considering that ALDFG is estimated to compose less than 10 percent of total marine debris by volume at a global scale, and even though the overall contribution to ALDFG from aquaculture is probably limited due to its static nature; intensive aquaculture still has the potential for lost cages, longlines, poles and other floating and fixed items to escape the system, being transformed in sources of plastic debris.

Considering that Aquaculture is the fastest growing food-producing sector, accounting already for 50 percent of the world's fish that is used for food, and with an European goal of doubling its production by 2030, AQUA-LIT's goal is to avoid that an increase in aquaculture production imply an increase in marine litter input. Thus, the project main objective was to provide the aquaculture sector with a Toolbox that can provide existing, upcoming and already implemented tools, case studies, best practices, a database and links between stakeholders for addressing the 3 main components of marine littering from aquaculture activities: prevention & reduction, monitoring & quantification, and removal & recycling.

Metholodgy

The AQUA-LIT Toolbox is the result of the compilation of available literature and litter databases (e.g. OSPAR, HELCOM, Marine Litter Watch), the information provided by the stakeholders in the frame of the project, the state of play regarding the aquaculture marine litter management in 2019 and 2020 and the input of the experts that have been part of or have work closely together with the AQUA-LIT team. The assessment is based on the proposed tools and governance approaches developed during the AQUA-LIT Learning Labs in the Mediterranean Sea, North Sea and Baltic Sea regions, and at the same time built on AQUA-LIT's comprehensive review including: D2.2 Knowledge Wave on Marine Litter from Aquaculture Sources, D2.3 Available Tools and Measures, D2.4 Potential Future Impacts and D3.5 Learning Labs outcomes (portfolio of best practice fact sheets).

Results

The toolbox accessible online and as an App, provides more than 400 of ideas and solutions to tackle marine litter that can be filtered by stage (prevention and reduction, monitoring and quantification, removal and recycling), by type of aquaculture (shellfish, finfish, seaweed), by sea basin (Baltic Sea, Mediterranean Sea, North Sea), and by category of measure (support, knowledge, responsibility, legislation).

Furthermore, the toolbox also includes information on which ports have the facilities to receive waste, a database of funding opportunities to create a project on marine litter, as well as a marine litter inventory that provides an overview of the available knowledge on marine litter originating from the aquaculture sector, a set of policy recommendations for the EU and lastly, specific action plans for outermost regions.

(Continued on next page)

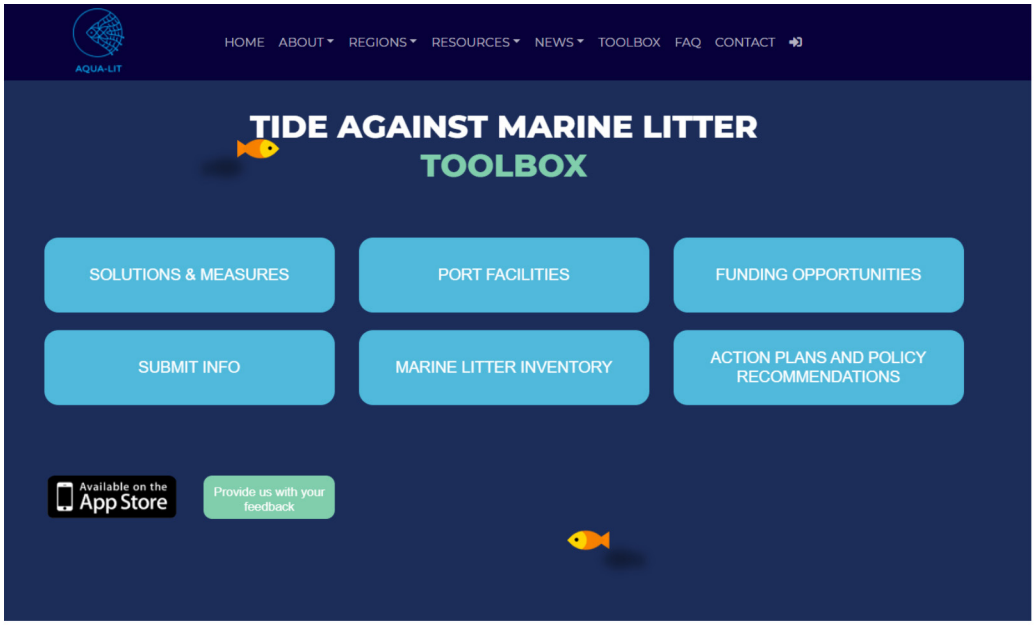


Figure 1. AQUA-LIT toolbox platform

Conclusions

The AQUA-LIT toolbox aims to be considered the most important knowledge repository for aquaculture-mairne litter related information across Europe and the starting point for the development of new European policies regarding the marine litter coming from aquaculture.

This Toolbox can be used as a guidance for the management of the marine litter that comes from aquaculture in the European context. The targeted audience includes aquaculture farmers; professional clusters, associations and platform representatives; policy makers; port authorities; aquaculture gear and equipment producers; engineering, system design and construction companies; plastic manufacturers; waste managers; researchers; environmental and social consultancies; NGOs; classification and certification bodies; communicators and any other interested person.

COLLABORATIVE LAND-SEA INTEGRATION PLATFORM

M. Mata-Lara*[1]

*[1] Geonardo Environmental Technologies Ltd., Zahony utca 7, Budapest, 1031 (Hungary)
Email: mariana.mata.lara@geonardo.com

Introduction

More than 90 % of the EU territory is covered by rural areas, where roughly half the EU population[1] lives and works. Rural development in the EU faces major, highly-dynamic challenges including global competition, decreasing population densities, lack of employment, desertification, land abandonment, and climate change. These significant challenges are addressed by the EU Strategic Guidelines for Rural Development (2006/114/EC), which aim is to improve the competitiveness of the agriculture and forestry sector in these areas, taking into consideration the natural environment, quality of life and simultaneously ensuring a diversity of the rural economy. At the same time, though, in the EU alone, the coastal regions contain half the population as well as half the GDP, and 23 of 28 EU countries have a coastline. Aquaculture, being part of the Blue economy, creates jobs and economic development opportunities in the EU's coastal and rural communities. This sector can also help: decarbonise the economy; fight climate change and mitigate its impact; reduce pollution; contribute to better preserving ecosystems (in line with the objectives of the Biodiversity strategy and the Zero-pollution ambition for a toxic-free environment); and be part of a more circular management of resources[2].

We argue that coastal and rural regions and their related economic activities operate as a coupled system. Economic development in coastal areas can contribute to rural development as addressed by the Guidelines for Rural Development, providing ecosystem goods and services and business opportunities applicable in the hinterland as well[2]. Thus, COASTAL aims to improve the rural-coastal synergies in strategic business and policy decision making and collaboration between coastal and rural actors. This will be achieved by developing, demonstrating, and applying a generic toolset and performance indicators by combining a multi-actor approach with system dynamics modelling. Specific attention is given to aquaculture and to understand the interactions with market, demographic, environmental and climate forecasts, and quantify the positive and negative externalities.

Methodology

COASTAL is a unique collaboration of coastal and rural business entrepreneurs, administrations, stakeholders, and natural and social science experts. Local and scientific knowledge are combined to identify problems and develop practical and robust business road maps and strategic policy guidelines, aimed at improving land-sea synergy. A multi-actor approach is followed to analyse the social-environmental and economic land-sea interactions in a collaborative System Dynamics (SD) framework, taking into consideration the short-, mid- and long-term impacts of decision making and feedback mechanisms on coastal and rural development. The project is organised around interacting Multi-Actor Labs (MALs), combining tools and expertise for six case studies representing the major coastal regions in the EU territory. Most of the case studies have the presence or link with aquaculture. In each MAL local actors and experts participate in collaborative exercises to analyse problems, analyse the causes, propose and discuss solutions, and validate and interpret the impacts of simulated business and policy decisions. The MALs are connected into a durable platform for collaborative knowledge exchange – COASTAL – which is underpinned by a generic set of tools and performance indicators. The COASTAL platform and synergistic tool set will be further exploited and developed beyond the project lifetime.

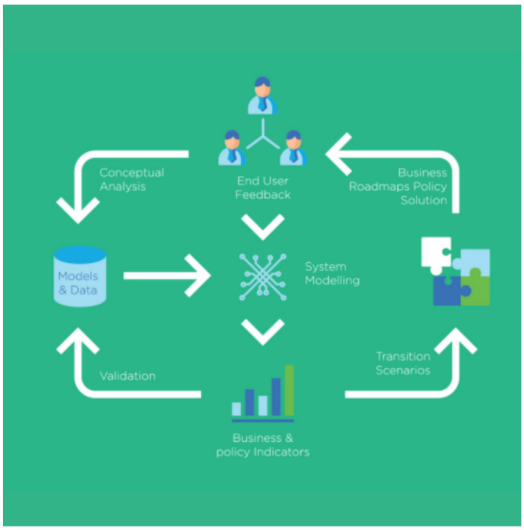
Results

A total of fourteen system models have been developed for themes ranging from coastal tourism, eutrophication, eco farming and decommissioning of offshore wind parks to shellfish farming. The quantification of systemic interactions is based on peer-reviewed published and reported data and modelling approaches, expert judgement and field samples obtained as part of the project. All models address land-sea interactions and capture socio-environmental interactions which were identified earlier in the project by coastal and rural stakeholders. In this project two specific case studies are dealing in depth with aquaculture as a regional spearhead. In the French case-study the long-established oyster culture in the Charente region is being studied. While in the Romanian case study models are made concerning the freshwater aquaculture in the Danube delta as well as for the potential upcoming marine aquaculture in the Black Sea.

Conclusions

Results from the project could lead to policy adjustment in these coastal-rural zone, enabling aquaculture to develop as a sustainable blue economy in those regional zones and contributing to the EU Green deal objectives. The methodology could be transferred to other regions where aquaculture can also play an important role in a sustainable regional development.

(Continued on next page)



1) Agriculture and Rural Development. http://ec.europa.eu/agriculture/lisbon_en
2) EEA, 2013. Balancing the future of Europe's coasts — knowledge base for integrated management, EEA Report No 12/2013, European Environment Agency, Copenhagen, Denmark.

PROSPECTS OF AQUACULTURE GROWTH IN RUSSIA

G.G. Matishov* and E.N. Ponomareva

Federal Research Centre The Southern Scientific Centre of the Russian Academy of Sciences (SSC RAS),
41 Chekhov Street, 344006 Rostov-on-Don, Russian Federation
E-mail: icd@ssc-ras.ru

The strategy for scientific and technological development of the Russian Federation, approved by Decree No. 642 of the President of the Russian Federation of 1 December 2016, defines (as priorities for the scientific and technological development of Russia for the next period of 10 to 15 years) directions, which will allow to obtain scientific and scientific and technical results and create technologies that are the basis for the innovative development of the domestic market for products and services and Russia's stable position in the foreign market. Such directions should ensure the transition to a highly productive and environmentally-friendly agriculture and aquaculture, the production of safe and high-qualitative, as well as functional, food.

According to FAO, the pace of aquaculture development, as before, is ahead of other sectors of the fishing industry in the world (FAO, 2018). Aquaculture is the future of agriculture. The volume of aquaculture production in the world over the past 10 years has doubled and almost equaled the volume of traditional fisheries.

Russia's share in the world aquaculture production is currently only 0.25%. This situation is due to the fact that both in Russia and the Soviet Union the main fisheries forces were aimed at developing and increasing the volume of fish catches. The total volume of aquaculture production (including aquatic plants) reached 328.6 thousand tons in 2020.

The main limiting factor in the development of aquaculture in Russia and in the world is the lack of inexpensive, effective biotechnologies, technical equipment and means, and environmentally-friendly feeds. The pace and scale of development of commercial fish farming in Russia lags far behind the global ones and much lower than the pace of the leading aquaculture countries (China, Norway, Vietnam, etc.). However, aquaculture production in Russia increased from 90.4 thousand tons in 2001 to 328.6 thousand tons in 2020 (increased by 3.6) of which more than 50% is produced in the South of Russia (Krasnodar and Stavropol Territories, Rostov, Astrakhan and Volgograd Regions).

Meanwhile, the total area of only marine areas in the Russian Federation suitable for mariculture is estimated at 0.38 million km². According to expert estimates, the effective use of existing potential in our country allows to increase the volume of aquaculture production by 25 times. In the Far East alone, natural conditions make it possible to cultivate up to three million tons of aquatic biological resources annually. The intensive development of aquaculture, especially in the southern regions of Russia, is possible only if it is industrialized with scientific and technological solutions being integrated into it – both in terms of reproduction, feeding and maintenance, and in terms of processing. The transition to new technologies and an increase in aquaculture production at least by 10% annually will also allow the development of the production of new technical equipment and means both within aquaculture and related industries.

There is an increase of industrial farms for growing aquaculture objects every year in Russia: there were 27% of them in 2019; however, according to forecasts, the growth of industrial (commercial) aquaculture by 2020 is expected to be up to 35%, and up to 40% by 2022.

On the whole, positive trends are observed in the fishing industry in Russia, the volumes of aquaculture are increasing, and fish farming indicators are growing at a significant pace. It was the sanctions that had a positive effect on this very sub-segment of the agro-industrial complex of the national economy. Since 2015, significant changes have taken place when it comes to the increase of the output of aquaculture products and the development of its (aquaculture's) industrial part. Over the past 30 years, the volume of consumption of fish and fish products per person in Russia has increased, on average accounting for 21.5 kg of fish per year.

INSECT MEALS AS INNOVATIVE FEED COMPONENTS IN JUVENILE IDE *Leuciscus idus* NUTRITION

Jan Mazurkiewicz[1,2*], Marcin Wiśniewski[3], Mateusz Rawski[2], Natalia Homska[1,2], Grzegorz Kujawa[3]

[1]Poznan University of Life Sciences, Experimental Station of Feed Production Technology and Aquaculture, Muchocin 20, 64-400 Międzychód, Poland
[2]Poznan University of Life Sciences, Laboratory of Inland Fisheries and Aquaculture, Department of Zoology, Wojska Polskiego 71c, 60-625 Poznań, Poland
[3]Polish Angling Association, Znanieckiego 9, 60-682 Poznań, Poland
E-mail: jan.mazurkiewicz@up.poznan.pl

Introduction

The ide (*Leuciscus idus*) is native European species of rheophilic cyprinid fish, which wild populations are dependent on conservation efforts, in particular regular restocking. For this reason, it is necessary to develop effective methods of rearing for stocking material. One of the important elements of this process is the nutrition of juvenile stages in controlled conditions, requiring the optimization of diet composition in terms of specific nutritional and behavioral requirements. At the moment restocking facilities raising ide juveniles are using commercial feeds for carps which are not balanced in terms of specific needs of ide. The aim of the experiment was to evaluate the effects of insect meal inclusion into the ide diet on fish growth performance and feed utilization in comparison with fishmeal based diet.

Material and Methods

For the production of feeds three insect species larvae meals were used. Four diets were formulated: HI – diet with 20% *Hermetia illucens* meal inclusion, TM – diet with 20% *Tenebrio molitor* meal inclusion, ZM – diet with 20% *Zophobas morio* meal inclusion. The control group (CON) was a diet based on fishmeal with no insect meals. The growth trial lasted 60 days and 200 individuals of ide with an average body mass of 30 grams were randomly assigned to four experimental groups, five replicates each (10 fish/tank). The fish were kept in a recirculation aquaculture system with controlled conditions (water temperature 22˚C, photoperiod 14h light, 10h darkness). The effects of the diets on the efficiency of rearing of ide juveniles were assessed based on fish growth parameters such as: mean individual body weight gain (BWG), specific growth rate (SGR), percent weight gain (PWG); and feed utilization parameters including: feed conversion ratio (FCR), protein efficiency ratio (PER); and somatic indices: Fulton's condition index (CI), viscerosomatic index (VSI) and gastrointestinal tract to fish total length ratio (GIT:FTL).

Results

The highest values of BWG, SGR, and PWG were observed in the HI and TM groups and they were nearly 30% better than the control group. The decrease of FCR was observed in HI (by 0.62) and TM (by 0.63) groups in comparison to CON. There were no statistically significant differences in terms of PER, CI and GIT:FTL. No mortality of the fish occurred during the experiment. In case of VSI it was highest in the control group and lowest in ZM group.

Conclusions

The results indicated that the use of black soldier fly and mealworm insect larvae meals in the diets for ide juveniles has a positive effects on their growth performance and feed utilization, with no adverse effects on fish condition and gastrointestinal track development. Up to date, there is a very little knowledge regarding ide juveniles nutrition and still further studies are needed to correctly define proper feeding strategy and diet requirements of cyprinid rheophilic fish.

This study was carried out as part of the project entitled: "Innovative feed components in the nutrition of rheophilic fish – optimizing and increasing the efficiency of rearing juvenile stages", no. 00001-6521.1-OR1500001/17/19, Task 2.1 "Innovations" according to EU Regulation No. 508/2014, Priority 2 – "Supporting environmentally sustainable, resource-efficient, innovative, competitive and knowledge-based aquaculture" realized in the Operational Program "Fisheries and Sea".

USING A BLOW-UP TARPAULIN FOR REVERSE OSMOSIS FRESHWATER TREATMENT OF AGD AND SEA LICE IN ATLANTIC SALMON (*Salmo salar*)

Tom Mc Dermott[a]*, Jamie K. Downes [a,] Jack D'Arcy[a], Suzanne Kelly[a], Samantha White[a], Aisling Brenan [a], Michael Sammon[b], Felix Scholz[c], Geoff Robinson[d], Neil M. Ruane[a]

[a] Marine Institute, Rinville, Oranmore, H91 R673 Co. Galway, Ireland
[b] Clear Seas Aqua, Co. Cork, Ireland
[c] FishVet Group, Oranmore, Galway, Ireland
[d] An Bord Iascaigh Mhara, Dun Laoghaire, Co Dublin

* Corresponding author at: Marine Institute, Rinville, Oranmore, Co. Galway H91 R673, Ireland
Tel.: +353 91 387200
E-mail address: tom.mcdermott@marine.ie

Introduction

Amoebic gill disease (*Parameoba perurans)* and salmon louse (*Lepeophtheirus salmonis*) infestations of Atlantic salmon are both susceptible to freshwater treatment (Mc Dermott et al., 2021[1]). The attraction of non-medicinal use and the organic status of farmed salmon in a period of climate change has led to an increased use of hyposaline water from the sea through reverse osmosis (R.O.) or nanofiltration (Mc Dermott et al., 2021[2]). In trials, soft freshwater produced on site from an R.O. plant was used to treat amoebic gill disease (AGD) and reduce sea lice numbers using a blow-up tarpaulin placed within the pen to be treated in an effort to eliminate the practice of second pumping. Water chemistry and efficacy of treatment were determined to assess if this adaptation had the potential to reduce handling and workload.

Materials and methods

Lehanagh Pool is a Marine Institute research site, located 0.25 km from the shore in Bertraghboy Bay, on the West Coast of Ireland. The site is a licensed 23-hectare multi species research site. The location is relatively sheltered with a tidal range approximately 5m, where salinity is typically >32 ppt but can range from 24 to 35 ppt. A floating R.O. plant produced 50m³ low salinity (<2ppt) water that was pumped in 25 minutes to a blow-up tarp deployed within the pen to be treated. The pen was split by raising the net in the middle with all 5,000 post smolts in one side and the blow-up tarp in the other. The fish were then pumped and dewatered into the blow-up tarp and after the last fish was transferred the trials commenced. In Trial 1 the fish were retained for 3h and in Trial 2 for 4.5h. When treatment was complete the fish were simply spilled into their original pen. During treatment the water chemistry was carefully monitored. A full appraisal of the sea lice and AGD status before and after treatment was undertaken by the Marine Institute.

Results

The use of the blow-up tarp reduced the handling and time involved in treating both AGD and sea lice by removing the need to pump fish twice. The fall in pH using low buffered R.O. (~1ppt) water was significant, yet the pH remained above the critical level of 5.7, having reached a plateau just above this level midway through the treatment. Water temperature in the blow-up tarp remained constant and was the same as the ambient sea water in the pen. AGD control using R.O. in Trial 1 and Trial 2 was effective and for both time periods. Reinfection of AGD was observed within 8 days and highlighted the importance of continuous freshwater treatments and the need for a constant supply of suitable soft water. Sea lice levels were low at the site, so treatment efficacy was difficult to determine yet the results show that a 3h freshwater treatment was not sufficient to remove adult ovigerous females and adult males. By increasing the treatment time to 4.5h efficacy improved. Biosecurity and the removal of harmful biological or biochemical substances by R.O. were also important considerations for fish welfare in undertaking these trials.

The BIM project of providing freshwater from reverse osmosis for tarpaulin treatments was co-funded by the Government of Ireland and the European Union, under Ireland's European Maritime & Fisheries Fund Operational Programme for the seafood sector.

References

Mc Dermott, T., D'Arcy, J., Kelly, S., Downes, J.K., Griffin, B., Kerr, R.F., O'Keeffe, D., O'Ceallachain, M., Lenighan, L., Scholz, F., Ruane, N.M. 2021. Novel use of anofiltered nhyposaline water to control sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) and amoebic gill disease, on a commercial Atlantic salmon (*Salmo salar*) farm. Aquaculture Reports Volume 20, July 2021, 100703. https://doi.org/10.1016/j.aqrep.2021.100703

Mc Dermott, T., D'Arcy, J., Kelly, S., Kerr, R.F., O'Keeffe, D., O'Ceallachain, Scholz, F., Ruane, N.M. Investigating factors influencing the efficacy of nanofiltered hyposaline water to control sea lice on a commercial Atlantic salmon (*Salmo salar*) farm. Aquaculture Europe Cork 2020.

DO EFFECTS OF NUTRITIONAL PROGRAMMING IN FRESHWATER CONTINUE DURING SEAWATER REARING OF ATLANTIC SALMON (*Salmo salar*)?

Stuart McMillan*[1], John F. Taylor[1], Brett D. Glencross[1], Xu Gong[1], Douglas R. Tocher[1], Pedro Gómez-Requeni[2] and Mónica. B. Betancor[1]

[1]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK
[2]BioMar Technology Centre, Brande, Denmark
Email: stuart.mcmillan@stir.ac.uk

Introduction

The Scottish farmed salmon industry continues to expand and there is a requirement to reduce the level of finite marine origin raw materials used in traditional feed formulations. One alternative is to produce feeds with higher proportions of plant-derived oils and proteins, but these diets can lead to reduced feed utilization and also have lower levels of essential omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). A novel solution to this problem is applying an early nutritional intervention or "stimulus" where fish are fed a predominantly vegetable-based diet for a short period, to induce more efficient uptake and utilization of nutrients from a similar diet when fed later in development, a concept referred to as "nutritional programming". Previously, it was demonstrated that Atlantic salmon (*Salmo salar*) fed such diets during a three-week stimulus phase from first exogenous feeding, resulted in significantly greater growth and nutrient retention efficiency during freshwater grow out (Clarkson et al. 2017). Associated with the improved growth and feed efficiency, key pathways of intermediary metabolism were upregulated (Vera et al. 2017). The current study aims to elucidate whether the effects of nutritional programming are sustained during post-smolt rearing in seawater.

Materials and methods

Four experimental dietary groups were established in triplicated treatment tanks (Figure 1). Two treatments were fed an experimental vegetable-based diet for a three-week "stimulus" period from first feeding, while remaining tanks were fed a standard marine-based diet (Figure 1). Following stimulus, all groups were then fed a standard marine ingredient diet until week 36. Thereafter, two treatment groups were re-fed a vegetable-based diet for four weeks prior to sea transfer, while remaining tanks remained on marine-based feeds. At week 40, smolts were transferred to sea based tank rearing facilities. Following a two-week acclimatization post- transfer, when fish were fed a standard marine based diet, all fish were transferred to a vegetable-based diet for a 14-week period. Samples were collected at key phases for biometrics, body & tissue composition, and nutrient retention efficiency and utilization.

Results & Discussion

Our hypothesis was that fish subjected to a vegetable-based stimulus diet would better utilize dietary nutrients in later development. The study presents results indicating whether there is evidence to support this theory based on growth, feed intake and body composition. Specifically, attributes of digestibility and retention of essential nutrients will be discussed, with primary focus on levels of n-3 LC-PUFA, DHA and EPA. Greater positive retention and/or a net increase of DHA and EPA accumulation and deposition during seawater challenge, by treatment fish fed a vegetable-based stimulus, is one key indicator of successful and sustained nutritional programming. A net increase of EPA and DHA demonstrates endogenous biosynthesis of these essential fatty acids from precursor α-linolenic acid (ALA; 18:3n-3), which is abundant in the vegetable-based feed. Empirical evidence of nutritional programming upregulating fatty acid biosynthesis to trigger endogenous production of essential EPA and DHA could determine how we formulate more resource-efficient feeding strategies and aquafeeds for Atlantic salmon in the future.

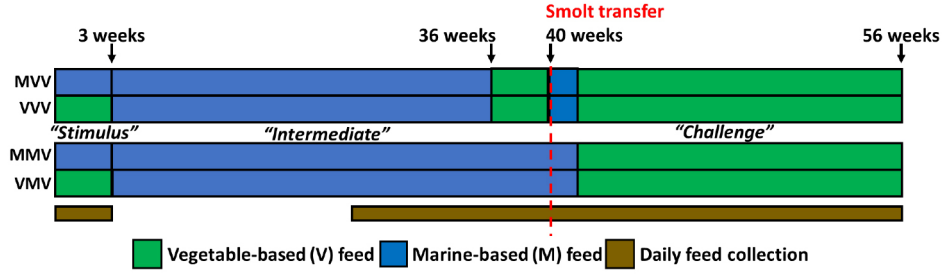


Figure 1. Schematic of trial showing when specific diets were fed and periods when excess feed was collected. All timepoints are cumulative.

(Continued on next page)

References

Clarkson, M., Migaud, H., Metochis, C., Vera, L. M., Leeming, D., Tocher, D. R. and Taylor J. F. (2017) Early nutritional intervention can improve utilisation of vegetable-based diets in diploid and triploid Atlantic salmon (*Salmo salar* L.), British Journal of Nutrition, doi:10.1017/S0007114517001842

Vera, L. M., Metochis, C., Taylor J. F., Clarkson, M., Skjærven, K. H., Migaud, H., and Tocher, D. R. (2017) Early nutritional programming affects liver transcriptome in diploid and triploid Atlantic salmon, *Salmo salar*, BMC Genomics (2017) 18:886

Acknowledgements

This work was completed as part of the biotechnology and biological sciences research council (BBSRC) funded project NUTRIPROG "*Investigating the potential of nutritional programming to improve the utilisation of sustainable feeds in aquaculture*" (BB/R018812/1) in collaboration with colleagues at the
University of Aberdeen and BioMar.

ANTIGEN PERSISTENCE AND ANTIBODY RESPONSE THROUGHOUT A COMMERCIAL PRODUCTION CYCLE OF ATLANTIC SALMON (*Salmo salar* L.) FOLLOWING INTRAPERITONEAL INJECTION WITH A MULTIVALENT VACCINE

Marwa. Mechlaoui*, Endre Nordstrand, Ingvill Jensen and Tore Seternes

Norwegian College of Fishery Science, UiT The Arctic University of Norway, Tromsø, Norway
E-mail : Marwa.mechlaoui@uit.no

Introduction

Global salmonid production continues to grow annually and Atlantic salmon (*Salmo salar* L.) is one of the most economically valuable species. However, infectious diseases is a major challenge affecting both fish welfare and economy in a negative manner. 10% of all cultured aquatic animals are estimated to be lost due to infectious diseases. *Vibrio anguillarum* is within the genus vibrio, and it is the etiological agent of classical vibriosis, a deadly haemorrhagic septicaemia disease in marine and freshwater fish species. Disease prophylaxis through vaccination has been found to be the best approach for prevention of infectious diseases. In aquaculture today, oil-adjuvanted multivalent vaccines containing six to seven antigens are routinely used; however, limited information is available regarding the duration of the vaccine-induced immune response in a commercial aquaculture setting.

In the present field study, we have investigated the immune response against the bacterial antigen *V.anguillarum* in Atlantic salmon throughout a commercial production cycle. After intraperitoneal vaccination with a multivalent commercial vaccine, analysis focused on granuloma development, the localization of *V.anguillarum* vaccine antigen at the injection site and the serum antibody response.

Materials and methods

Atlantic salmon were vaccinated with an oil-adjuvanted multivalent vaccine containing six antigens including *V. anguillarum (*Alpha Ject micro 6, Pharmaq). Five weeks after intraperitoneal vaccination, fish were transferred to the sea site at Skogshamn, production area 10, Troms, Norway, and sampling (blood and organs) took place every six weeks from 20 randomly selected individuals counting 14 samplings in total until final slaughtering (87 weeks after vaccination). Serum antibody response against *V. anguillarum* was measured by an ELISA. Vaccine antigen persistence was microscopically evaluated by immunohistochemistry using a polyclonal antibody that recognize *V. anguillarum* O1. Inflammation and granuloma development were assessed using standard histological methods.

Results and conclusion

Vaccine granulomas were located in the adipose tissue surrounding the injection site of the vaccine up to 44 weeks after immunization. *V. anguillarum* bacterial fragments were identified as red immuno-labelling in the vaccine granulomas either in association with oil droplets or in the periphery.

The serum antibody response to *V. anguillarum* was detected and persisted at a high level at all samplings from week 11 to 56 post-vaccination. Following that, there was a major decrease in specific antibody levels, which remained at low throughout the rest of the production cycle. At week 56 post-vaccination, a slight increase in antibody response was found correlated to an increase in seawater temperature as well as the fish specific growth rate. The given commercial vaccine has proved to induce a good immune response of Atlantic salmon to *V. anguillarum*, but further investigations needs to be done to study more the vaccine efficiency.

EFFECT OF PESTICIDES ON EMBRYONAL DEVELOPMENT OF AQUATIC BIOTA

D. Medkova[1,2*], P. Sehonova[2], J. Blahova[2], E. Postulkova[1], V. Doubkova[2], Z. Svobodova[2], J. Mares[1]

[1]Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of Agrisciences, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
[2] Department of Animal Protection and Welfare & Veterinary Public Health, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Palackeho tr. 1, 612 42 Brno, Czech Republic
*Email: H19004@vfu.cz

Introduction

Pesticides, industrial chemicals, and many other types of contaminants have been constantly entering the aquatic environment. Pesticides, which are commonly used in agriculture, can have a negative impact on non-target species as well as human health. In few past decades, water pollution caused by pesticides has become a commonly discussed issue. Pesticides after entering water environment are persistent in the aquatic environment and they have bioaccumulation potential. These properties can have a negative effect on aquatic biota, fish and even their consumers. We tested the toxicity of commonly used pesticides of MCPA in its clear form and as a part of Bofix commercial herbicide on *Danio rerio* and *Xenopus laevis* embryos. In acute tests toxicity, embryos were exposed to environmental concentration (0.1 µg/L) and to relatively high concentration (10; 100; 1 000; 10 000 and 100 000 µg/L) for 96 hours.

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 six different concentrations (0.1; 10; 100; 1 000; 10 000 and 100 000 µg/L) and the control group. Fertilized eggs of *Danio rerio* and *Xenopus laevis* were selected using binocular microscope and distributed into 24 microwell. For each concentration plus control, 24 embryos were used. Bofix was soluble in ISO 7346 (ISO, 1996) and MCPA was soluble in ISO 7346 with ethanol. During the test the temperature was 26 °C for *Danio rerio* and 23 °C for *Xenopus laevis*, photoperiod was 12 hours light/12 hours dark. The solutions were changed every 24 hours and indicators of the toxicity were observed. We observed mortality, malformation, hatching and the hearbeat. Statistical analysis was conducted using Unistat 5.6 (Czech Republic).

Results and discussion

Bofix had a negative effect on *Danio rerio* and *Xenopus laevis* mortality, where mortality of *Danio rerio* at the concentration 10 000 and 100 000 µg/L reached 100%, at the concentration 1 000 µg/L was mortality 38% after 24 hours post fertilization (hpf). At 24 hpf mortality of *Xenopus laevis* at the concentration 100 000 µg/L reached also 100% and at 48 hpf at the concentration 10 000 µg/L mortality was 100%. Johansson et al. (2006) reported LC50 for fish 100 mg/L and for amphibians 3,6 g/L. A 96hours exposure to Bofix at the concentration of 1 000 µg/L revealed increased malformation (100% of *Danio rerio* specimen, 40% of *Xenopus laevis* specimen) and at the concentration of 100 µg/L were observed 36% malformation of *Xenopus laevis*. Bofix did not have effect on hatching. MCPA had a negative effect on *Xenopus laevis* mortality, where mortality was 100% at the highest tested concentration, but did not have any effects on hatching or malformation rate. In case of *Danio rerio* the negative effect on hatching rate and malformation were observed after 96 hpf, where only 23% embryos were hatched at the highest tested concentration. Moreover, these embryos showed significant malformation rate (84%). The most common malformation caused by Bofix and MCPA were heart oedema, blood clot and deformation of spine. Lutnicka et al. (2018) tested the effect of MCPA at the concentration of 100 µg/L on juvenile common carp, changes in the blood differential and morphologic changes in the kidney were observed.

Conclusion

The results of the study show that the commercial preparation Bofix is more toxic than pure MCPA due to the content of additives. Bofix caused a high percentage of mortality and malformation at the concentration 1 000; 10 000 and 100 000 µg/L. But also, negative effect on development of the embryos were observed with MCPA in clear form, where malformations were observed at the highest tested concentration on *Danio rerio.*

(Continued on next page)

Acknowledgments

This research was supported by project PROFISH *[no. CZ.02.1.01/0.0/0.0/16_019/0000869]* and IGA VETUNI 223/2021/FVHE.

References

ISO 7346. 1996. Waterquality – Determination of the acute lethal toxicity of substances to a freshwaterfish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, *Cyprinidae*)] – Part 1: Static metod.

JOHANSSON, M., PAHA, H., KYLIN, H., MERILA, J. Toxicity of six pesticides to common frog (*Rana temporaria*) tadpoles. *Environmental Toxicology and Chemistry*. 2006, vol. 25, no. 12, s. 3164–3170.

LUTNICKA, H., Bojarski, B., WITESKA, M., CHMURSICA-GASOWKA, M., TRYBUS, W., TRYBUS, E., KOPACZ-BEDNARSKA, A., LIS, M. Effects of MCPA on hematological parameters and ultrastructure of hematopoietic tissues of common carp (*Cyprinus carpio* L.). *Fiola Biologica*. 2018, vol. 66, no. 1, s. 1–11.

OECD 2013. Test No 236: Fish Embryo acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

THE USE OF A NUTRIENT-BASED MODEL AS A DECISION TOOL TO SUPPORT THE DESIGN OF NUTRITIONAL EXPERIMENTS

Rodrigo Mendes*, Filipe Soares, Tomé Silva, Ana Nobre, Luís Conceição

SPAROS Lda., Olhão (Portugal)
*Email: rodrigomendes@sparos.pt

Introduction

In aquaculture research fish performance is often evaluated through *in vivo* trials. This allows the assessment of the impact of factors of interest (change between treatments) on fish reared under the same context (constant conditions between treatments). Although this type of approach is effective in theory, in practical terms it may be difficult to implement when several factors and/or multiple levels are of interest, due to constraints such as space available in the experimental facilities and budget. Consequently, when designing an experiment, aquaculture researchers are often confronted with the decision of what set of treatments to include, to meet existing limitations and not to compromise the effectiveness of the research. This means that usually some prior knowledge must be put on the table to support the design of an experiment.

Nutrient-based mathematical models can be great tools to be used in this regard in the framework of fish nutrition research. On one hand, they are developed and calibrated based on large datasets generated in distinct scientific contexts, which enables to encapsulate the knowledge that has been generated by science in different studies into a single tool. On the other hand, since they consider in detail a wide range of factors, such as feed properties (e.g., proximate composition, digestibility, amino acid and fatty acid profile), feeding quantities and temperature, their application has the potential to provide accurate predictions in distinct scenarios and, as such, they can support a more effective design of a wide variety of *in vivo* trials.

In this work, we illustrate how a specific nutrient-based model (FEEDNETICS™) was used in the context of the H2020 project AquaIMPACT (www.luke.fi/aquaimpact/) to support the design of a research experiment. The main objective of this model application was to select two dietary treatments that show *in silico* relative differences within the range of 5-10%, in terms of final body weight and feed conversion ratio, to later test them in an *in vivo* trial to be carried out with two seabass populations (selected vs unselected).

Methods

The dynamic nutrient-based model FEEDNETICS™ (Soares et al., 2018) was used to run predictions for European seabass (*Dicentrarchus labrax*). In general, data inputs (e.g., rearing conditions and feeding regimes) for the model were defined based on the parameters under which the *in vivo* nutritional trial was expected to be carried out. In terms of rearing conditions, an initial fish bodyweight of 20g, a constant temperature of 20ºC and a production period of 180 days, were defined. Regarding the feeding regimes, four dietary treatments (A, B, C and D), composed of two diets with different pellet sizes (2 mm and 4 mm), were considered, being the main difference between them in terms of crude protein (from 45.5% (2 mm) and 39.5% (4 mm) to 50% (2 mm) and 44% (4 mm), in diets A and D, respectively) and gross energy contents (from 17.36 MJ/Kg (2 mm) and 17.56 MJ/Kg (4 mm) to 18.38 MJ/Kg (2 mm) and 18.57 MJ/Kg (4 mm), in diets A and D, respectively). Moreover, three feeding tables (FiT) were applied (80%, 100%, 120% of a reference FiT). Therefore, a total of twelve feeding regimes (four dietary treatments × three feeding levels) were composed. The relative difference (d_r) between the pairs of results was calculated as:

the pairs of results was calculated as: $d_r = \frac{|x-y|}{max(|x|,|y|)}$.

Results

FEEDNETICS™ predicted that diet D presented the best fish performance in terms of final body weight (FBW) and feed conversion ratio (FCR), followed by diet C, B and A, in this order (Table 1).

The treatments to be used in the *in vivo* trial were the ones with d_r within the range of 5-10%, in terms of FBW and FCR. In this sense, only two combinations of dietary treatments, A-C and B-D, matched the pre-defined screening criteria (Figure 1). Since the proximate composition of diet B, was the one more similar to the protein and energy contents used by the industry for European seabass, the combination B-D was selected to be used in the *in vivo* trial.

(Continued on next page)

Table 1 – Final body weight (FBW), feed conversion ratio (FCR), protein efficiency ratio (PER), nitrogen (N) and phosphorus (P) waste estimated by FEEDNETICS™ in each dietary treatment, grouped by feeding table.

	A			B			C			D		
	80%	*100%*	*120%*	*80%*	*100%*	*120%*	*80%*	*100%*	*120%*	*80%*	*100%*	*120%*
FBW (g)	92.71	147.94	215.97	96.63	154.13	226.56	100.77	159.91	238.11	104.37	166.14	250.88
FCR	1.21	1.09	1.05	1.17	1.07	1.02	1.13	1.04	1.00	1.11	1.02	0.97
PER	1.94	2.22	2.35	1.94	2.20	2.33	1.94	2.18	2.31	1.94	2.16	2.29
N waste (kg/ton fish)	53.73	44.10	40.16	53.73	44.73	40.79	53.76	45.44	41.32	54.09	46.08	41.79
P waste (kg/ton fish)	9.10	8.26	8.03	8.73	7.98	7.76	8.38	7.98	7.50	8.09	7.49	7.25

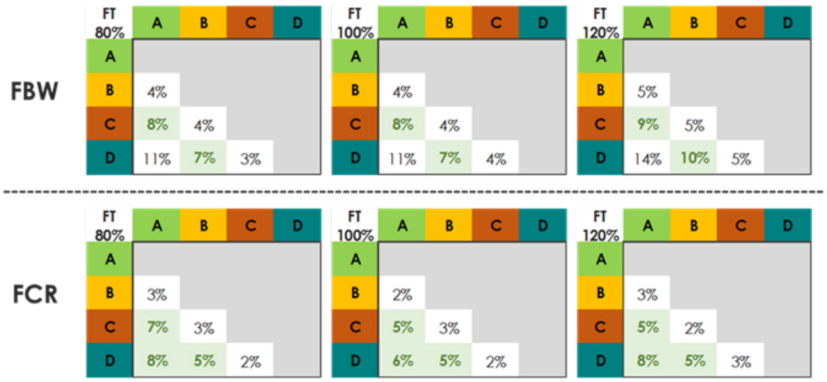


Figure 1 – Relative differences between dietary treatments, grouped by feeding table, in terms of final body weight (FBW) and feed conversion ratio (FCR). The cells highlighted in green show the values where the d_r is between the range of 5-10%.

Conclusions

Nutrient-based models, such as FEEDNETICS™, can be used by aquaculture researchers to support informed decisions in the design of an *in vivo* trial. Screening beforehand the impact of multiple treatments on fish performance with a model, ensures that the experimental design to be adopted is adequate to meet the outlined research objectives and, in addition, it may also contribute significantly to reduce the overall costs of the experiment.

Acknowledgements

This work is part of project 818367_AquaIMPACT supported by the European Union through the Horizon 2020 research and innovation programme.

References

Soares, F. M., Silva, T., Conceição, L. E. C. (2018). FEEDNETICS: a tool to assist fish farms to plan feeding strategies and manage feed stocks. 18th International Symposium on Fish Nutrition and Feeding, 3-7 June, Las Palmas de Gran Canaria, Spain.

DIETARY PHOSPHORUS SUPPLEMENTATION IN CELLULAR-BONED FISH SPECIES DIETS

Mendez-Martinez, L.*[a], Suarez-Bregua, P.[a], Guerrero-Peña, L.[a], Pirraco, R.P[b], Reis, R.L.[b] and Rotllant, J.[a]

[a] Acuabiotec Group, Institute of Marine Research (IIM-CSIC), Vigo, Spain
Email: lmendez@iim.csic.es
[b] 3B's Research Group-Biomaterials, Biodegradables, and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, Portugal

Introduction

Phosphorus is an essential nutrient for skeletal physiology, ionic homeostasis, and energy metabolism in fish. Unlike other minerals, the phosphorus content in water is insufficient to meet fish requirements and therefore the diet is the main external source of phosphorus. In aquaculture, commercial diets commonly have higher phosphorus concentration than the basal requirements in most fish species to ensure growth and prevent skeletal disorders. This practice poses a serious environmental problem as excess phosphorus in water from non-eaten food and urinary/fecal excretions produces eutrophication of aquatic ecosystems. Our previous studies with acellular-boned fish (Suarez-Bregua *et al*., 2021) revealed that a lower phosphorous concentration diet is possible without affecting the fish health and growth. In this study, we wondered if this is also applicable to cellular-boned fish species. Thus, the objective of this study was to analyze the effect of dietary phosphorus concentrations (low, standard, and high phosphorus diets) on bone mineralization of zebrafish (*Danio rerio*), a popular model organism and a cellular-boned teleost species.

Material and Methods

One hundred and eighty zebrafish (30 dpf) were randomly distributed in six plastic tanks of 10 L (30 fish per tank) connected to a recirculation system. Fish from duplicate tanks were fed three times a day with three different diets: low phosphorus diet (LP, 0.47%), standard phosphorus diet (SP, 1.30%), and high phosphorus diet (HP, 3.15%). Dietary trials were conducted at ambient temperature (27ºC) and natural photoperiod (14 h light/10 h dark) for 10 weeks. After this period, 100 dpf zebrafish were randomly sampled, euthanized, weight and length measurements were recorded and fixed for subsequent analysis. Following the fixation, the volumetric bone mineral density (vBMD) was determined by micro computed tomography (CT) scanning. Whole-body mineral content was analyzed by using inductively coupled plasma optical emission spectrophotometry and quantitative real time PCR (qPCR) analysis were also performed to determine the expression of bone mineralization and Pi homeostasis markers (*entpd5*, *sparc* and *pth4*).

Table 1. Growth measurements of zebrafish fed low (LD), standard (SP), and high (HP) phosphorus diet for 10 weeks.

Zebrafish	LP	SP	HP
Length increase (%)	61.18 ± 0.35 [a b]	64.24 ± 0.65 [a]	50.97 ± 2.87 [b]
Weight increase (%)	93.54 ± 0.29 [a b]	94.69 ± 0.26 [a]	81.94 ± 4.13 [b]
Condition factor (K)	0.91 ± 0.02 [a]	0.86 ± 0.02 [a]	0.81 ± 0.06 [a]

Data are presented as means ± SEM (n = 10). Different lower-case letters denote significant differences (Kruskal-Wallis and Dunn's post-hoc test, $p < 0.0005$).

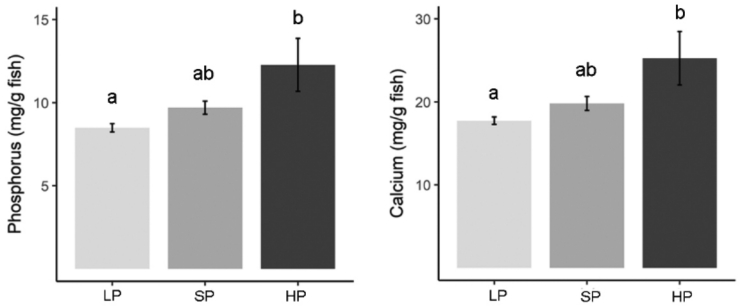


Figure 1. Whole-body phosphorus and calcium concentration determination in zebrafish fed LP, SP, and HP diets.

(Continued on next page)

Results

As shown in Table 1, zebrafish fed the high phosphorus diet were significantly smaller than those fed the standard (SP) or low (LP) phosphorus diet. The whole-body mineral content analysis (fig. 1) showed a significant increase of whole-body phosphorus and calcium contents after HP feeding compared with LP dietary treatment. This suggests that in zebrafish a positive relationship between the dietary phosphorus concentration and whole-body mineral levels exists.

Zebrafish fed the LP diet also exhibited a significantly reduced vBMD in the skull and vertebrae samples compared with fish under SP feeding. This changes in vBMD are supported by the down-regulation of *pth4* and *sparc* gene expression in fish fed the LP.

Conclusion

The dietary phosphorus concentration significantly affects the mineralization of the cellular zebrafish bone in contrast with previous studies with acellular-boned fish (Suarez-Bregua *et al*., 2021). Therefore, a possible differential regulation of phosphate homeostasis depending on the type of bone that a certain fish species has may exist. These results could be taken into account when designing new feed formulations depending on the type of bone of each specific fish species. Nevertheless, further studies with different cellular-boned fish species with commercial relevance should be carried out.

References

Suarez-Bregua, P., Pirraco, R.P., Hernández-Urcera, J., Reis, R.L. and Rotllant, J. (2021), Impact of dietary phosphorus on turbot bone mineral density and content. Aquacult Nutr, 27: 1128-1134.

Acknowledgments

This work was funded by the Spanish Economy and Competitiveness Ministry projects AGL014-52473R and AGL2017-89648P to J. Rotllant. L. Guerrero-Peña was supported by pre-doctoral fellowship of the Spanish Personnel Research Training Program funded by Spanish Economy and Competitiveness Ministry (PRE2018-085475). L. Méndez-Martínez, was supported by pre-doctoral fellowship of the Xunta de Galicia (IN606A-2020/006).

GILL-ASSOCIATED NITROGEN CYCLE BACTERIA CONVERT AMMONIA TO NITROGEN GAS IN COMMON CARP (*Cyprinus carpio*) AND ZEBRAFISH (*Danio rerio*)

Wouter Mes*[1,2], Mike S.M. Jetten[1], Henk Siepel[2], Sebastian Lücker[1], Marnix Gorissen[2], Maartje A.H.J. van Kessel[1]

[1]Department of Microbiology, Radboud Universiteit, Nijmegen, the Netherlands
[2]Department of Animal Ecology and Physiology, Radboud Universiteit, Nijmegen, the Netherlands
E-mail: w.mes@science.ru.nl

Introduction

Ammonia is the main nitrogenous waste produced by teleost fish and its accumulation can lead to toxicity in aquaculture settings where fish are kept at high fish density and are fed protein-rich diets. Controlling ammonia levels is therefore key in recirculating aquaculture systems (RAS). Most ammonia is excreted via the gills of teleost fish, which makes this organ a prime habitat for colonization by nitrogen-cycle bacteria.

Recently, it was shown that excreted ammonia can be converted into dinitrogen gas (N_2) in carp and zebrafish gills through the combined activity of ammonia-oxidizing and denitrifying bacteria, which interestingly seem to reside *inside* gill cells of carp (van Kessel et al., 2016). The role of these bacteria in nitrogenous waste removal by fish is largely unknown. Our project aims to investigate the fundamental characteristics of this novel symbiosis and determine whether it can be used in aquaculture to decrease ammonia excretion of teleost fish. We identified the presence of ammonia-oxidizing bacteria in common carp and zebrafish gills and measured activity of these bacteria *in vivo* in common carp. We additionally examined 16S rRNA sequencing datasets for presence of nitrogen cycle bacteria in other species than carp and zebrafish.

Materials and methods

Common carp (*Cyprinus carpio*) and zebrafish (*Danio rerio*) were grown under control conditions in recirculating systems with nitrifying biofilters. Fish were euthanized and gills were aseptically removed for microbiome analysis.

The gill microbiome of common carp and zebrafish was investigated through molecular methods. Bacterial DNA was isolated from fish gill and PCRs were performed for the ammonia monooxygenase subunit A gene (a functional marker for ammonia oxidizing bacteria) and nitrite reductase (a functional marker for denitrifying bacteria). Additionally, 16S rRNA amplicon sequencing was performed to obtain an overview of bacteria present in fish gill samples. We analyzed and compared the results with publicly available 16S rRNA sequencing datasets from teleost fish that included gill samples using the DADA2 pipeline and amplicon sequencing variants (ASVs) of nitrogen cycle bacteria (ammonia oxidizers, nitrite oxidizers and denitrifiers) were identified using the SILVA database. Phylogenetic relationships were examined, as well as differential abundances of nitrogen cycle bacteria between gill samples and other samples (water, sediment etc.).

Histological analysis was also performed on gills of common carp and zebrafish. Bacteria were localized in gill tissue through fluorescent *in situ* hybridization and electron microscopy.

Common carp (±50-100g) were used for individual nitrogen gas measurements. Carp were placed in 5L tanks that were closed off from the surrounding air. The atmosphere above the water in the tank was replaced by a mixture of argon and oxygen, after which the accumulation of dinitrogen gas in the headspace was regularly measured with gas chromatography/mass spectrometry for 2.5 hours.

Results

Using on molecular methods, we confirmed the presence of *Nitrosomonas*-like ammonia oxidizers in carp and zebrafish gills (fig. 1). Additionally, microscopy indicated that these bacteria seem to be located intracellularly in carp gills.

In our 16S rRNA amplicon sequencing dataset, 2 *Nitrosomonadaceae* ASVs were found in carp and zebrafish gills. These ASVs were different from the *Nitrosomonadaceae* species in the water and biofilter. Analysis of other teleost gill microbiomes revealed the presence of 53 *Nitrosomonas*-affiliated ASVs in gill samples from 6 datasets, indicating that presence of ammonia oxidizers in gills is a widespread phenomenon.

(Continued on next page)

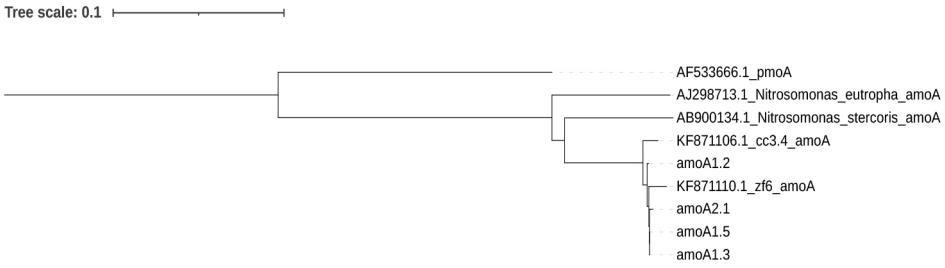


Fig. 1: Phylogenetic tree of the ammonia monooxygenase subunit A genes identified in zebrafish and carp gill tissue.

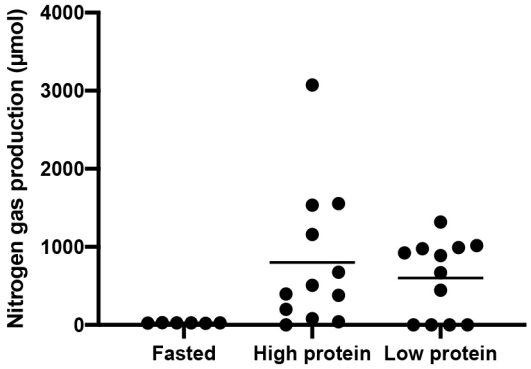


Fig. 2: Production of dinitrogen gas by carp fasted, fed by hand or ad libitum through a pendulum feeder. Mean ± SD, n= 30

Carp that were kept individually in closed tanks produced measurable amounts of dinitrogen gas within 2.5 hours and the amount of dinitrogen gas produced was higher when these fish were fed (fig. 2). This suggests a correlation between the amount of ammonia produced by fish as a waste product and the resulting production of nitrogen gas by the symbiotic bacteria.

Conclusion

Nitrogen cycle bacteria were present and active in fish gills and present a novel symbiosis between vertebrate animals and bacteria. In future experiments, we will study how these bacteria are transmitted, as well as the timepoint of colonization of the gills using germ-free zebrafish.

References

van Kessel, M. A., R. J. Mesman, A. Arshad, J. R. Metz, F. T. Spanings, S. C. van Dalen, L. van Niftrik, G. Flik, S. E. Wendelaar Bonga and M. S. Jetten (2016). "Branchial nitrogen cycle symbionts can remove ammonia in fish gills." Environmental microbiology reports **8**(5): 590-594.

EFFECT OF FRESHWATER RAS ENVIRONMENT ON OSMOREGULATION, GROWTH PERFORMANCE AND ANTIVIRAL RESPONSE IN ATLANTIC SALMON (*Salmo salar*) POST-SMOLTS

Herve Migaud*, Mikey Clarkson, Lynn Chalmers, Chessor Matthew, Pedro Munoz, Ahmed M. Ahmed, Simon MacKenzie, John F. Taylor

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
E-mail: Herve.migaud@stir.ac.uk

Introduction

Within the Atlantic salmon farming sector there is a growing adoption of Recirculation Aquaculture Systems (RAS) for the manipulation and production of smolts. While offering many advantages (e.g. biosecurity, water and waste management, manipulation of physiological windows), RAS systems are operated under relatively intensive and constant conditions (e.g. 24hr light, high temperature) compared to conventional ambient open water/flow through production systems and fish also experience markedly different water chemistry. Under RAS, it has been suggested that de-synchrony between photoperiod and temperature may occur (e.g. "winter" short-day at high temperature) leading to inconsistent smoltification success and suboptimal performance at sea. Osmotic ionic balance & efficiency may also be affected by interactions with rearing water chemistry. Given that the single most critical element of smoltification is the development of hypo-osmoregulatory ability to maintain hydro-mineral balance via excretion of ions, then it is imperative to understand how water chemistry variation between systems (RAS vs FT) interacts with the process of smoltification, and subsequent seawater performance (Kolarevic et al., 2014; van Rijn et al., 2020). In addition, freshwater history is likely to impact immune function and disease resistance at later sea water (SW) stages given immune suppression has been reported in smolt transferred to SW (Johansson et al., 2016), but the potential effects of RAS environment remain to be documented. Therefore, a large collaborative project was launched in 2019, ROBUSTMOLT, to test the hypothesis that environmental conditions experienced in RAS during the freshwater phase (e.g. water chemistry and microbiology, nutrition, temperature and photoperiod) may influence early life history traits of salmon that will subsequently impact the microbiomes, immune barriers, ion regulatory capacity, and ultimately robustness at sea. This communication will present results from several studies which compared growth and osmoregulation in parr reared in either FW RAS and open loch cage and performances following SW transfer including feeding, growth and disease resistance tested through an immune challenge.

Material and Methods

Analyses were done from two main studies using fish from a commercial salmon production company in Scotland reared in either a RAS or loch site during the freshwater phase and transferred to seawater either in the company marine production sites (study 1 – smoltification) or the Machrihanish Marine Environmental Research Laboratory (MERL) at the Institute of Aquaculture (study 2 – SW performance). Sampling in study 1 was performed in RAS between -500 degree.days (DD, June 2019) and 400 DD (late July 2019) from the onset of the spring photoperiod and consisted in water and blood samples for mineral analyses, gill samples for Na+ K+ ATPase (NKA including enzyme activity and gene expression by qPCR), and histological analyses, and physiological assessment (weight/length, condition and smolt index) at regular intervals from parr to smolt. Two different smoltification regimes were compared in RAS using two different identical streams, either photoperiod (RAS-P) or diet manipulations (RAS-D). Loch reared smolt were sampled similarly however SW transfer occurred later in 2019. Additional samples were collected at 1 and 4 weeks post SW transfer. In study 2, smolts produced commercially in either freshwater RAS or open loch cages were transferred to MERL between mid-February and early March 2020 (2 weeks window) with an initial weight of 92.2 g and 101.2 g, respectively. Smolts (480 from each origin) were stocked into 12 x 1.5m^3 (n = 6; 80/tank) and reared for 5 months. Fish were assessed for seawater adaptation (including ATPase, chloride, survival), growth (weight/length, SGR, FCR), feed intake (daily waste feed collection) and immune parameters following an artificial viral challenge using Polyinosinic:polycytidylic acid (poly I:C) I.P. injection (1.25 mg of poly(I:C) tested against PBS and control, n=3, 6 fish/treatment/tank) at two time points post SW transfer (2 and 6 weeks). Fish were marked with panjet (alcian blue, Sigma-Aldrich, UK) to differentiate between treatments. Sections of head kidney, liver and spleen were dissected and preserved in RNA later for gene expression analysis by qPCR of innate immunity gene markers.

(Continued on next page)

Results and Discussion

Results of study 1 showed similar growth profiles between cohorts (RAS-P/D and loch). However, differences were found in the temporal changes in smolt index and NKA in FW and blood ion balance post SW transfer. Gill NKA remained relatively stable in RAS-P fish while diet clearly promoted smoltification with a sharp rise between -500 and 150 DD and a more classic profile was observed in the loch fish. Post SW transfer, RAS fish appeared to be under osmotic stress within the first 4 weeks. Water mineral levels increased in RAS throughout the FW phase and reached levels much higher than in the loch. Further data on gill (and gut) NKA gene expression will be presented.

Study 2 showed clear differences between the RAS and loch cohorts in SW. Thermal growth coefficient was significantly higher in loch fish compared to RAS fish for the first 8 weeks post transfer. Loch fish feed intake (expressed as g/metabolic body weight/day) was significantly higher from the time to transfer indicating a better capacity of loch fish to cope with SW. Mortality remained low however was higher in RAS fish during the first 4 weeks post SW transfer further supporting the challenge experienced by RAS fish. Complement increased sharply in the RAS immuno challenged fish 2 weeks post SW transfer but not in the loch fish. The PolyI:C challenge worked effectively and elicited a strong immune response in fish. In the 6-week post-SW, a greater complement activity response was observed in the loch fish than RAS-reared salmon following the PolyI:C challenge. Expression of anti-viral genes showed significant differences between RAS and loch fish especially for LGP2, a modulator of cellular anti-viral response, in the head kidney and Mx, LGP2 in the spleen.

Conclusions

This study provides new scientific data on the impact of RAS compared to loch on osmoregulation and smoltification in FW and growth, feeding and immune response following SW transfer. Data supported anectodal reports from the industry and recently published data (van Rijn et al., 2020) with regards to RAS fish performance in the weeks following SW transfer. However, given the multifactorial differences between RAS and loch rearing conditions, further studies are needed to identify factors explaining the apparent reduced coping ability of smolts reared in RAS and develop mitigation strategies.
This work was funded by UK Research Council (UKRI), the Scottish Aquaculture Innovation Centre (SAIC) and industry partners.

Bibliography

Christian A. van Rijn, Paul L. Jones, Brad S. Evans, Christine Huynh, Stephen D. McCormick, Luis O.B. Afonso (2020). Characterization of smoltification in the Tasmanian strain of Atlantic salmon (*Salmo salar*) in recirculation and flow-through systems. Aquaculture 516, 734603.

Kolarevic J., Baeverfjord G., Takle H., Ytteborg E., Reiten B.K.M., Nergård S., Terjesen B.F. (2014). Performance and welfare of Atlantic salmon smolt reared in recirculating or flow-through aquaculture systems. Aquaculture 432, 15-25.

Lill-Heidi Johansson, Gerrit Timmerhaus, Sergey Afanasyev, Sven Martin Jørgensen, Aleksei Krasnov (2016). Smoltification and seawater transfer of Atlantic salmon (*Salmo salar* L.) is associated with systemic repression of the immune transcriptome. Fish and Shellfish Immunology 58, 33-41.

EU-CONEXUS EUROPEAN UNIVERSITY FOR SMART URBAN COASTAL SUSTAINABILITY: RECENT ADVANCES IN EDUCATION, RESEARCH AND SOCIETAL CHALLENGES

H. Miliou*[1], I. Baer-Eiselt[3], F. Baltaretu[4], V. Charitou[1], E. Chatzoglou[1], D. Daunys[5], L. Delvaux[3], A. De Luis[2], E. Flemetakis[1], S. Kintzios[1], A. Margineanu[4], E. Malandrakis[1], S. Mavrikou[1], G. Moschopoulou[1], N. Ntalamagka[2], Z. Penezić[6], A. Sancho[2], J. Tena[2], A. Tsopelakos[1], L. Vaucel[3], P. Vidal[2], D. Vlachakis[1], R. Viederyte[5], S. Zjalić [6], J.M. Ogier[3]

[1] Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
[2] Universidad Católica de Valencia Carrer de Quevedo, 2, 46001 València, Spain
[3] La Rochelle Université, 23 avenue Albert Einstein BP 33060 - 17031 La Rochelle
[4] Technical University of Civil Engineering Bucharest, 122-124 Lacul Tei Blvd, Bucharest, Romania
[5] Klaipeda University, H. Manto str. 84, Klaipėda 92294, Lithuania
[6] University of Zadar, Ul. Mihovila Pavlinovića, 23000, Zadar, Croatia
Email: elenmi@aua.gr

Introduction

EU-CONEXUS is a transnational European higher education and research institution that covers the theme of Smart Urban Coastal Sustainability (SUCS) from a global point of view. It was created in the framework of the European universities' initiative (2019), led by the European Commission, with the aim to strengthen strategic partnerships across Europe. EU-CONEXUS is focused on urban and semi-urban coastlines that are very important, for inter alia, aquaculture and fisheries, trade, energy, tourism but at the same time, most vulnerable, to the consequences of climate change. The EU-CONEXUS Alliance includes 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 the University of Klaipeda (LT) and 3 Associated Partners: Waterford Institute of Technology (IE), Rostock University (GE) and Frederick University (CY). The partners, from all European geographical regions, develop teaching, research and innovation programmes that include the totality of social, economic, technical and environmental problems and opportunities that the coastlines are facing today. The recent HORIZON 2020 project RESEARCH FOR SOCIETY (2021) will strengthen the EU-CONEXUS research approach into a fully-fledged university service equally covering research, education and societal needs.

Materials and Methods

The Alliance implements a student-centred service, which relies on the active engagement of students in all activities of this European University, orchestrated by the Student Board. Joint actions have been developed by creating two joint Minor course programmes, by designing joint study programmes for the Master and PhD cycle, launching a joint action for secondary schools to raise awareness on SUCS, and by developing a joint learning platform and a smart campus. A personalised approach and study track system has been chosen by EU-CONEXUS as a guiding principle of its university services in a multilingual and multicultural environment. Virtual mobility options facilitate further the selection of the appropriate course offer for special study interests by benefitting from the best available expertise wherever it is located on the inter-university campus. Several tools and activities have been developed for promoting EU-CONEXUS Joint Research Area and submitting joint 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". In the course catalogues, students have a detailed insight into the curriculum and the opportunities/industries/jobs in the labour market where the knowledge and skills acquired in these courses can be applied. The first graduates were awarded a Minor Certificate and graduation ceremonies have been organized by the partner universities. A Skills Map for SUCS relevant employment was created, aiming to engage with the socio-economic environment. EU-CONEXUS has developed a list of stakeholders from public and private sectors, active in the fields of SUCS. A 2-year transnationally integrated multi- and interdisciplinary joint Master programme in Marine Biotechnology (JMPMB) will be initiated in 2022. The programme has been designed to entirely cover the marine biotechnology pipeline from biodiscovery to market product launch, enabling the student to integrate in a variety of professional careers. The 1st PhD summer school was held in Zadar (July 2021). Students

(Continued on next page)

gained experience working in interdisciplinary research teams, conducted by recognised experts and received a certificate and transcript of records. EU-CONEXUS Joint Research Institutes (JRIs) and research teams represent a multicultural, multilingual and multidisciplinary environment for conducting innovative research and projects, sharing common equipment, research outputs and methodology. An online Research Portal has been developed, based on a comprehensive scientific mapping, which enables the users to look up scientific interests, projects and contacts. Researchers have already benefited from the broad EU-CONEXUS network by submitting joint proposals to the HORIZON 2020 calls, COST Actions and others. Joint Standard Operating Procedures (JSOPs) have been developed, harmonising research protocols and processes between laboratories. A common Multilingual Manual of General Laboratory Safety Procedures is applied in EU-CONEXUS laboratories. In addition, educational material that encompasses audio and visual guidance was produced, to enhance laboratory training. All methodology and educational materials are available at the Protocol Portal, where researchers and students are invited to apply them in their research activities. Accreditation of certain EU-CONEXUS laboratories according to ISO/IEC 17025 validate the status of excellence and also the possibility of external services. To support the EU-CONEXUS Joint Research Area, a Call for Research Staff Mobility among the Alliance has been launched, giving researchers the opportunity for scientific knowledge exchange, life-long training and job shadowing on SUCS topics. EU-CONEXUS supports the development of joint research projects by offering a Project Development Fund supporting partners in the preparation of projects to international calls for proposals. “Research hours” are organized every month, aiming to create teams with common research interests, to identify topics for joint projects, to facilitate partnerships, to inform on upcoming calls, and eventually (?) to advice researchers on writing a good proposal. Schools, teachers and pupils participated in the school contest “Think smart, create green” and were exposed to SUCS problems and developed creative thinking by searching for solutions through scientific methodologies. The Buddy System, an initiative of the EU-CONEXUS Student Board, aims at connecting students within the alliance.

Conclusions

EU-CONEXUS partners are united around common values: sustainability, expertise, bravery and novelty. The EU-CONEXUS study programmes give students of EU-CONEXUS universities the opportunity to participate in flexible, international, multidisciplinary curricula and to acquire up-to-date competences that are in high demand on the labour market. The EU-CONEXUS Joint Research Area aims to brings together researchers and students from the different partner institutions around common fields of interest, developing science and innovation into a hub of excellence on SUCS and creating an attractive career environment for EU-CONEXUS students. Researchers actively invite partnerships with coastal industries, businesses and society actors to ensure the transfer of knowledge and the improvement of quality in and around coastal areas.

Acknowledgments

The European University EU-CONEXUS is funded by ERASMUS+ “Cooperation for innovation and the exchange of good practices” EAC-A03-2018. EU-CONEXUS RESEARCH FOR SOCIETY project is funded by the European Union’s Horizon 2020 research and innovation Programme.

DEEPER *IN VITRO* INVESTIGATION INTO THE POSSIBLE ROLES FOR FOUR MAJOR CORTICOSTEROIDS IN FOM IN RELATION TO THEIR PLASMA KINETICS ALONG THE WHOLE REPRODUCTIVE CYCLE OF FEMALE EURASIAN PERCH *Perca fluviatilis* L

L. El Mohajer[1], C. Chevalier[1], P. Fontaine[1] & S. Milla[1]*

[1] University of Lorraine, INRAE, UR AFPA, F-54000 Nancy, France

*Corresponding author e-mail: Sylvain.Milla@univ-lorraine.fr

Introduction

Eurasian perch, *Perca fluviatilis* L. is a promising candidate for the freshwater aquaculture and is mainly cultivated in intensive monoculture in recirculating aquaculture systems (RAS) (Fontaine and Teletchea, 2019)pikeperch Sander lucioperca. Such systems depend on the artificial control of both photoperiod and temperature to induce synchronous spawning. However, the production of eggs with optimal quality under such artificial conditions proved to be challenging (Rocha de Almeida et al., 2020; Żarski et al., 2011)regardless of the mechanism. The maternal mRNA in fish eggs is crucial for the proper embryogenesis. Our working hypothesis is that modifications of maternal mRNAs may reflect potential genetic and/or epigenetic modifications occurring during domestication and could have consequences during embryogenesis. Consequently, we investigated the trancriptomic profile of unfertilized eggs from two populations of Eurasian perch. These two populations differed by their domestication histories (F1 vs. F7+–at least seven generations of reproduction in captivity. Such deterioration in reproduction might be accredited to four major corticosteroids (11-deoxycorticosterone, 11-deoxycortisol, corticosterone and cortisol). Up to date, neither the basic roles nor the kinetics of these four corticosteroids during the whole reproductive cycle of female perch has been well defined. In previous reports on yellow perch, the involvement of the four main corticosteroids in Final Oocyte Maturation (FOM) as maturation inducing hormones (MIH) had been suggested following some *in vitro* assays (Goetz and Bergman, 1978; Theofan and Goetz, 1983). However, we recently eliminated their involvement in FOM as MIHs using *in vitro* culture assays. In fact, we confirmed using both *in vitro* and *in vivo* injection assays that 17α,20β-dihydroxy-4-pregnen-3-one (DHP) is the MIH in female perch (El Mohajer et al., 2021). The latter finding still cannot entirely eliminate their involvement in FOM mechanisms. In this study, we therefore further investigated other possibilities for their involvement in FOM using *in vitro* assays as well. Additionally, we used *in vivo* assays to better spot their involvement and monitor their kinetics during the whole reproductive cycle. Through both assays, we anticipate to better define the role of the four corticosteroids in female perch as an attempt to better understand the reproductive drawbacks in RAS systems.

Materials and Methods

In vitro assay: the activity of the four corticosteroids, at three different doses, was tested during FOM in combination with the recently realized MIH (DHP) at two different doses. The hormonal combinations were dissolved in Cortland culture medium into which mature female Eurasian perch follicles, at the start of the FOM, were added. The follicles were subjected to the treatment for a 62hrs incubation period.

In vivo assay: domesticated female Eurasian perch were kept in RAS system under strictly controlled photothermal conditions used to initiate and derive the reproductive cycle until spawning. The fish were blood sampled periodically and the plasma levels of corticosteroids were measured for each sampling time.

Results

The results of our *in vitro* combination experiment revealed that we cannot eliminate the possibility of an effect for corticosteroids during FOM where most of corticosteroids revealed a slight inhibitory effect of the MIH activity of DHP suggesting a negative effect for these steroids on the FOM achievement. Additionally, the *in vivo* plasma kinetics for most of the corticosteroids revealed some elevations in their plasma levels around ovulation and spawning. It also revealed some high levels at the initiation of the reproductive cycle. Therefore, our results are in favor of the possibility for the involvement of corticosteroids in FOM and the initial stages of the reproductive cycle. Through becoming closer to defining the roles of these corticosteroids, we believe we would better combat the reproductive drawbacks in perch maintained in RAS systems.

(Continued on next page)

References

El Mohajer, L., Bulteau, R., Chevalier, C., Selmi, S., Fontaine, P., Milla, S., 2021. *In vitro* follicle culture shows that progestagens are the maturation-inducing hormones (MIH) and possible regulators of the ovulation-mediating hormone PGE2 in female Eurasian perch Perca fluviatilis. Fish Physiology and Biochemistry. https://doi.org/10.1007/s10695-021-00946-5

Fontaine, P., Teletchea, F., 2019. Domestication of the Eurasian Perch (*Perca fluviatilis*), in: Teletchea, F. (Ed.), Animal Domestication. IntechOpen. https://doi.org/10.5772/intechopen.85132

Goetz, F.W., Bergman, H.L., 1978. The Effects of Steroids on Final Maturation and Ovulation of Oocytes from Brook Trout (*Salvelinus fontinalis*) and Yellow Perch (*Perca flavescens*). Biology of Reproduction 18, 293-298. https://doi.org/10.1095/biolreprod18.2.293

Rocha de Almeida, T., Alix, M., Le Cam, A., Klopp, C., Montfort, J., Toomey, L., Ledoré, Y., Bobe, J., Chardard, D., Schaerlinger, B., Fontaine, P., 2020. Domestication may affect the maternal mRNA profile in unfertilized eggs, potentially impacting the embryonic development of Eurasian perch (*Perca fluviatilis*). PLOS ONE 14, 1-25. https://doi.org/10.1371/journal.pone.0226878

Theofan, G., Goetz, F., 1983. The *in vitro* synthesis of final maturational steroids by ovaries of brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). General and comparative endocrinology 51, 84-95. https://doi.org/10.1016/0016-6480(83)90101-6

Żarski, D., Palińska, K., Targońska, K., Bokor, Z., Kotrik, L., Krejszeff, S., Kupren, K., Horváth, Á., Urbányi, B., Kucharczyk, D., 2011. Oocyte quality indicators in Eurasian perch, *Perca fluviatilis* L., during reproduction under controlled conditions. Aquaculture 313, 84-91. https://doi.org/10.1016/j.aquaculture.2011.01.032

DIETARY FUMONISINS AFFECT THE IMMUNE RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*)

H. Minarova[1,2*], L. Pojezdal[1], M. Palikova[2,3], J. Mares[3], K. Hlavova[1], K. Stastny[1], M. Faldyna[1], H. Modra[3]

[1]Veterinary Research Institute, Brno, Czech Republic, [2]University of Veterinary Sciences Brno, Czech Republic, [3]Mendel University in Brno, Czech Republic
E-mail: hana.minarova@vri.cz

Introduction

Mycotoxins and their effects have been examined in different animal species; however, more information about their impact on the fish immune response is still required. Fumonisins, produced by several *Fusarium* species, represent the most common mycotoxins in plant meals, which have been more frequently used in fish feed production in recent years. These toxins can cause major health problems in fish (Oliveira & Vasconcelos 2020), including both immunostimulation and immunosuppression (Riley et al. 1996; Pestka et al. 2004). Their effects need to be investigated more, especially in the main aquaculture species.

Materials and Methods

Rainbow trout (*Oncorhynchus mykiss*) kept in recirculating systems were fed fumonisins for a period of 10 weeks. Some of the fish from the fumonisin group and from the control group were vaccinated against *Yersinia ruckeri* at week 6. At weeks 3 and 10, samples of the head kidney were taken after euthanasia of the fish (6–10 fish from each group).

Non-specific mitogen-driven (100 μg/ml phytohaemagglutinin) as well as specific antigen-driven lymphocyte proliferation assay was performed, with leukocytes isolated by density gradient centrifugation. The test was evaluated by an ELISA-based assay using bromodeoxyuridine. Levels of specific antibodies were also determined by an in-house ELISA at weeks 9 and 10.

Results

Non-specific stimulation showed a significant increase in proliferative activity after vaccination against *Y. ruckeri* in the fish from both fumonisin groups (vaccinated and non-vaccinated), indicating a pro-inflammatory immune reaction. Similar results were obtained in the non-vaccinated control. The vaccinated compared to the non-vaccinated fish from the control group showed significantly lower proliferation levels. With specific stimulation, significantly higher values were detected in the vaccinated fish from the fumonisin group compared to the vaccinated control.

Levels of specific antibodies were significantly increased in the vaccinated fumonisin group compared to the non-vaccinated fish at week 9. However, at week 10, the control fish showed similar results with even higher values. The enhanced immune reaction, which occurred very quickly in the vaccinated fish from the fumonisin group, may be indicative of pro-inflammatory changes (Ellis 1999).

Conclusions

Dietary fumonisins caused an activation of the immune system of rainbow trout after vaccination against *Y. ruckeri*, indicating a pro-inflammatory immune reaction. Alterations in lymphocyte proliferative activity and in the production of specific antibodies confirm a negative impact of these toxins on the fish immune response.

Funding

This research was supported by BIOMIN Research Centre Tulln and by the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869. The project is financed by the European Regional Development Fund in the operational programme VVV MŠMT.

References

Ellis, A.E., 1999. Immunity to bacteria in fish. Fish & Shellfish Immunology, 9: 291-308.

Oliveira, M., Vasconcelos, V., 2020. Occurrence of Mycotoxins in Fish Feed and Its Effects: A Review. Toxins (Basel), 12: 160.

Pestka, J.J., Zhou, H.R., Moon, Y., Chung, Y.J., 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicology Letters, 153: 61-73.

Riley, R.T., Wang, E., Schroeder, J.J., Smith, E.R., Plattner, R.D., Abbas, H., Yoo, H.S., Merrill, A.H., JR., 1996. Evidence for disruption of sphingolipid metabolism as a contributing factor in the toxicity and carcinogenicity of fumonisins. Natural Toxins, 4: 3-15.

IMPROVED FERTILIZER MANAGEMENT FOR SMALL TO MEDIUM SIZED COMMERCIAL DECOUPLED AQUAPONIC SYSTEMS

Hendrik Monsees[a], Rebecca John[a], Gösta Baganz[a], Georg Staaks[a], Werner Kloas[a], Daniela Baganz[a],

a Leibniz-Institute of Freshwater Biology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany
*E-mail: h.monsees@igb-berlin.de

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).

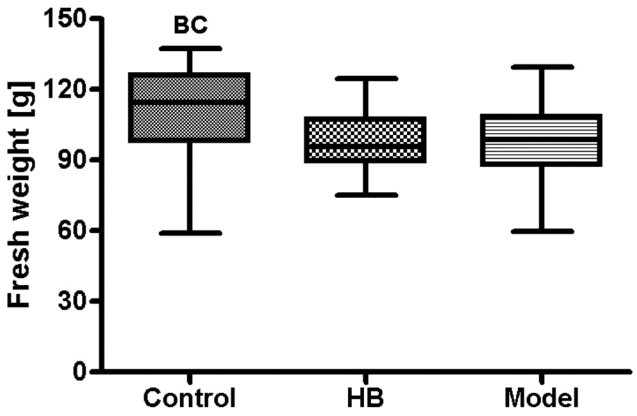


Fig. 1. Lettuce growth in three different hydroponic treatments. C = control, tab water based nutrient solution; HB - aquaponics, fish water based nutrient solution with supplemented nutrients after detailed nutrient analyses; Model - like HB, but without detailed nutrient analyses prior to nutrient supplementation. The three different treatments were applied in triplicates, each with ten individual lettuce plants.

(Continued on next page)

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.

References

Kloas W, Groß R, Baganz D, Graupner J, Monsees H, Schmidt U, Staaks G, Suhl J, Wittstock B, Wuertz S, Zikova A, Rennert B (2015) A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. *Aquaculture Environment Interactions,* 7 (2) 179-192

Monsees, H, Suhl, J., Paul, M., Kloas, W., Dannehl, D., Wuertz, S. (2019). Lettuce (*Lactuca sativa*, variety Salanova) production in decoupled aquaponic systems: Same yield and similar quality as in conventional hydroponic systems but drastically reduced greenhouse gas emissions by saving inorganic fertilizer. *PloS one*, 14(6), e0218368

Monsees, H., Kloas, W., & Wuertz, S. (2017). Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. *PloS one*, 12(9), e0183056.

Suhl J, Dannehl D, Kloas W, Baganz D, Jobs S, Scheibe G, Schmidt U (2016). Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agricultural water management*, 178, 335-344.

NUTRITIONAL INNOVATIONS IN SUPERIOR EUROPEAN SEA BASS (*Dicentrarchus labrax*) GENOTYPES: IMPLICATIONS IN FISH PERFORMANCE AND GUT HEALTH

D. Montero1*; S. Torrecillas1; S. Rimoldi2, A. Serradell1, R. Fontanillas3, F. Acosta1, F. Allal4, P. Haffray5, A. Bajek6 and G. Terova2

1Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Telde, Las Palmas, Canary Islands, Spain
2 Department of Biotechnology and Life Sciences, University of Insubria, Via J.H.Dunant, 3, 21100 Varese, Italy.
3 Skretting Aquaculture Research Centre, Stavanger, Norway.
4 MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34250 Palavas-les-Flots, France
5 SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
6 Ecloserie Marine de Graveline Ichtus, Route des Enrochements, 59820 Gravelines, France

* Corresponding author E-mail: daniel.montero@ulpgc.es

Introduction

A proper development of the aquaculture sector implies: (a) an effective replacement of marine raw ingredients (fish meal, FM; fish oil, FO) by sustainable raw materials and (b) a successful breeding program addressed to improve growth, feed utilization and fish health. Thus, the formulation of diets for genetically selected European sea bass (*Dicentrarchus labrax*) to determine how the interaction of breeding programs and nutrition together determines fish performance and diet utilization is needed, being the aim of the present study.

Materials and methods

During a production cycle a sea bass selected genotype (GS) and an unselected (wild) genotype (NGS) (15 ± 0.5 g initial mean weight), were fed a control diet with a low content of FM/FO based in nowadays commercial formulations and a "future" diet based on low FM/FO contents but formulated to cover the predictable sea bass requirements of the GS and enable them to completely express their growth and feed utilization potential. Fish were fed for nine months in triplicate (3 replicates/diet/genotype). Growth performance and feed utilization were monitored along the feeding trial. Additionally, at the beginning (t=0) and at the end of the feeding trial (t=9 months), fish were sampled for fillet biochemical composition and fatty acid profiles (15 fish/diet/genotype), morphological evaluation of the intestine (9 fish/diet/genotype), microbiota (6 fish/diet/genotype) and gene expression analyses (6 fish/diet/genotype).

Results

Sea bass genotype markedly affected fish performance. After 8 weeks of feeding GS sea bass presented higher weight gain than NGS, regardless of the diet fed (Fig. 1). This marked effect was maintained to the end of the experiment. Fillet biochemical composition results indicated that GS fish present higher ($p<0.05$) lipid and lower ash ($p<0.05$) percentages than NGS fish. Fish fed Future diet presented higher ($p<0.05$) lipid content than fish fed control diet. Besides, a significant interaction ($p<0.05$) between genotype*diet was detected for ash and protein content. Fish fed future diet presented higher ($p<0.05$) liver lipid content in expenses of protein, ash and protein percentages. A significant interaction ($p<0.05$) between genotype*diet was detected for moisture liver content. Fillet and liver fatty acid profiles will be also discussed.

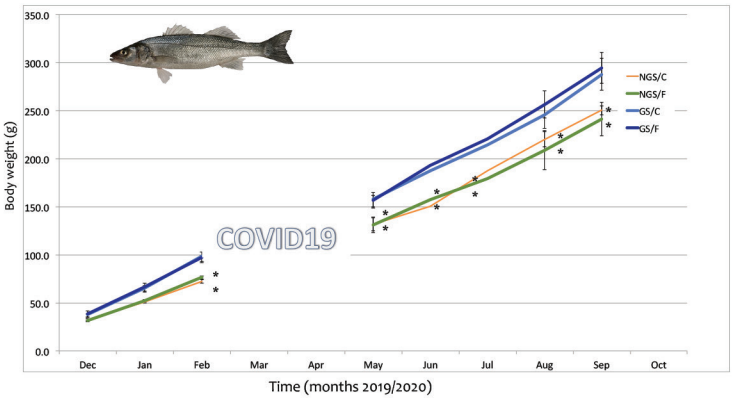


Fig. 1. Sea bass growth in terms of body weight. NGS: unselected genotype; GS: Selected genotype. C: control. F: future diet. *denotes significant differences among groups in a specific month ($p<0.05$)

(Continued on next page)

In terms of intestine gene expression, responsiveness to the feeding trial was more relevant in posterior section, where a significant interaction ($p<0.05$) between strain*genotype was found in the expression of *cd4, il-1β, il-10, mhc ii,* and *tnf-α* genes. Specifically, *il-1β* was up regulated in distal intestine of NGS fish compared to GS Europen sea bass, while *tnf-α* expression was up regulated ($p<0.05$) in GS fish fed future diet compared to fish fed the control diet. The expression of *il-10* was up regulated ($p<0.05$) in GS fish compared to NGS fish. These results were correlated with the morphology patterns observed in both intestinal regions.

The reduction of gut microbiome biodiversity was more evident in GS than in NGS fish.

Concerning gut microbiome analysis, a reduced biodiversity and species richness were found in fish fed experimental diets compared to their T0, but not among them.

At family level, Lactobacillaceae, Streptococcaceae, Staphylococcaceae, and Peptostreptococcales-Tissierellales were significantly less abundant in GS fish than in NGS fish, regardless of the diet. Accordingly, *Lactobacillus* and *Streptococcus* genera were absent in the same fish. In contrast, in NGS fish the presence of *Acinetobacter, Staphylococcus*, and *Pseudomonas* was detected.

In summary, our data indicated a higher influence of the genotype on *D. labrax* growth performance, than the diet formula fed. However, the diet formula influenced the composition of the fillet and the liver as well as their fatty acid profile. On the other hand, the genotype had a slight influence in modulating the resident intestinal microbiota composition compared to the GS strain, but NGS fish showed the presence of opportunistic pathogenic bacteria in their intestine. The NGS strain revealed a trend to show a like-chronic inflammatory status, that was more evident in fish fed future diet.

Acknowledgements
This research was funded by the EU Horizon 2020 project AquaIMPACT (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture); number: 818367.

Cyberlindnera jadinii YEAST INDUCES SYSTEMIC IMMUNOMODULATORY EFFECTS IN THE SPLEEN OF ATLANTIC SALMON EXPOSED TO A DIETARY SOYBEAN MEAL INDUCED CHALLENGE

B. Morales-Lange*, J. Agboola, J. Hansen, O. Øyås, L. Mydland, M. Øverland

Department of Animal and Aquaculture Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway
E-mail: byron.maximiliano.morales.lange@nmbu.no

Introduction

Future growth in aquaculture depends on feed ingredients that can meet nutritional needs and improve overall health of the fish (Agboola et al., 2021). To face this problem, dietary composition of salmon feed has shifted from marine ingredients towards increased use of plant ingredients. Nevertheless, these ingredients can have negative effects such as soybean meal induced enteritis (SBMIE) and changes in gut-microbiome that affect the mucosal immunity. The effects of plant ingredients are not limited only to local targets such as the intestine, but also to systemic effects on active immune organs, since immunity has a wide range of cellular and molecular components that can act in an integrated and systemic way. Based on this, our study evaluates the immunomodulatory potential of novel microbial ingredients (heat-inactivated or autolyzed *Cyberlindnera jadinii yeast*) to control the inflammatory profile on Atlantic salmon exposed to a dietary soybean meal challenge. To achieve this, we propose the spleen as a target organ to characterize the immune response of the fish, as this organ plays a key role in the activation and coordination of the local and systemic immunity, regulating components associated with both innate and adaptive responses, through the antigen presentation process.

Materials and Methods

In this study, four diets were used (previously reported in Agboola et al., 2021), a fish meal based control (FM), a challenging diet with 40% soybean meal (SBM), and two diets containing 40% SBM and 5% of *C. jadinii* yeast exposed to different down-stream processing conditions: heat-inactivated (ICJ) or autolysation (ACJ). Then, using RNA-seq and indirect ELISA, the immunomodulatory effects of the diets were analyzed in the spleen of 10 Atlantic salmon per dietary group (from duplicated tanks) after 37 days of feeding.

Results

The results showed that SBM (compared to FM-group) induced a down-regulation of pathways related to ion binding and transport (Fig. 1), along with an increase at the protein level of CD83 and pro-inflammatory cytokines such as TNFα and IFNγ and (Fig. 2). On the other hand, the inclusion of *C. jadinii* induced an increase in MHC II and a reduction in ZBTB46, compared to both control diets (Fig. 2). However, while ICJ (compared to FM-group) maintain the inflammatory response associated with SBM, with higher levels of TNFα and IFNγ (Fig. 2), and with an upregulation of creatine kinase activity and phosphagen metabolic process (Fig. 1), the inclusion of ACJ modulated the inflammatory response compared to SBM group by the activation of biological pathways related to transporter and ion binding activity (Fig. 1). ACJ also controlled the pro-inflammatory profile of SBM, by increasing IL-10 levels and decreasing TNFα levels, triggering an immune response similar to that of FM (Fig. 2).

Conclusions

Our data suggest that the spleen is a good target to evaluate the immunomodulatory effects of functional ingredients in fish. Moreover, *C. jadinii*, after a down-stream process by autolysis, can serve as a an alternative protein sources with health beneficial effects when formulating sustainable feeds for salmonids, due to its favorable amino acid composition and its ability to control the inflammatory profile in Atlantic salmon fed challenging plant-based diets.

References

Agboola JO, Schiavone M, et al. Impact of down-stream processing on functional properties of yeasts and the implications on gut health of Atlantic salmon (*Salmo salar*). Sci. Rep. (2021) 11:4496.

(Continued on next page)

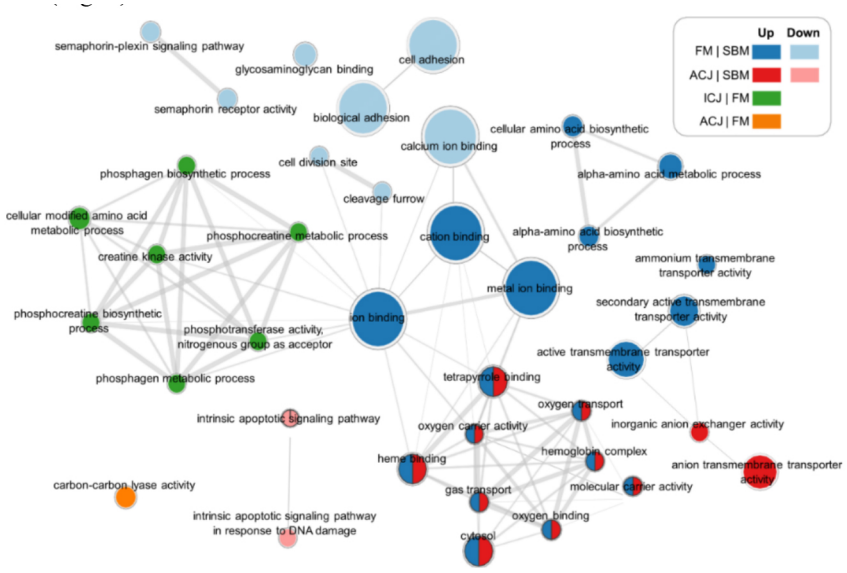


Fig 1. Gene Ontology terms among different diets. FM|SBM: Up (up-regulated in blue), Down (down-regulated in light blue). ICJ|FM: Up (up-regulated in green). ACJ|FM: Up (up-regulated in orange). ACJ|SBM: Up (up-regulated in red), Down (down-regulated in pink).

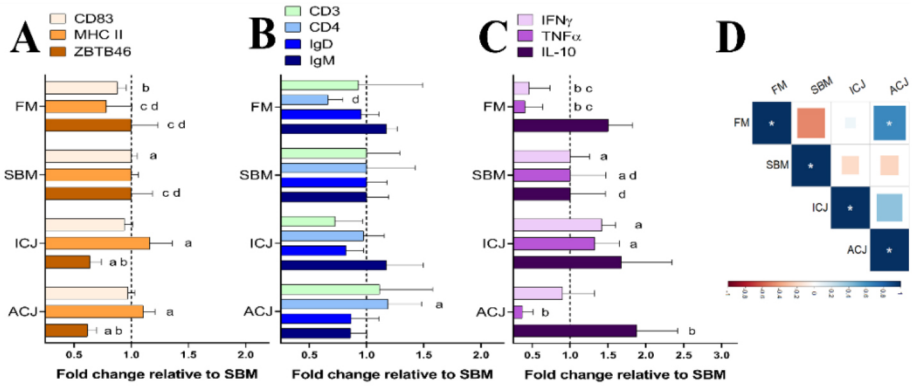


Fig. 2. Immunological markers on spleen. (A) Antigen-presenting cell (APC) markers. (B) Lymphocyte markers. (C) Cytokines. (D) Correlation of markers. In A, B and C: a, b, c and d: significant differences compare to FM, SBM, ICJ and ACJ, In D: * significant correlation ($p<0.05$).

BIOACCUMULATION OF TITANIUM DIOXIDE AND SILVER NANOPARTICLES IN CULTURED MUSSELS AND TURBOT

A. MOREDA-PIÑEIRO*[1], B. ESPIÑA[2], J. LÓPEZ-MAYÁN[1], I. PINHEIRO[2], L. RODRÍGUEZ-LORENZO[2], M. QUARATO[2], M.C. BARCIELA-ALONSO[1], E. PEÑA-VÁZQUEZ[1], M. VÁZQUEZ[3], S. CABALEIRO[3], J. MAGUIRE[4], M. MACKEY[4], P. BERMEJO-BARRERA[1]

[1] Trace Element, Spectroscopy and Speciation Group (GETEE), Strategic Grouping in Materials (AEMAT), Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain

[2] International Iberian Nanotechnology Laboratory (INL), 4715-330 Braga. Portugal

[3] Ctr Tecnol Cluster Acuicultura, Cluster Acuicultura, Punta Couso S-N, Ribeira 15965, Spain

[4] Indigo Rock Marine Research, Gearhies, Bantry, Co. Cork, P75 AX07, Irland

*antonio.moreda@usc.es

Introduction

The novel mechanical, thermal, optical, and antimicrobial properties of nanomaterials (NMs) make them valuable tools in many sectors such as the food industry and also in the aquaculture activity. However, there is great concern regarding the impacts of engineered NMs, mainly metallic nanoparticles (NPs), on environmental and human health. NPs are present in the marine environment, and background levels of TiO_2 and Ag NPs have been reported in molluscs [1,2]. Therefore, studies regarding controlled exposition of NPs to cultured molluscs and fish are important for elucidating the bioaccumulation rate of these new emerging pollutants and the impact on the aquaculture sector.

Transmission electronic microscopy (TEM) and single particle-inductively coupled plasma – mass spectrometry (sp-ICP-MS) has been for studying and quantifying the presence of TiO_2 and Ag NPs in mussels (*Mytilus sp*) and turbot (*Scophthalmus maximus*) after controlled exposure in an aquaculture facility. This communication summarises the preliminary results regarding the bioaccumulation rate of these new pollutants in tissues from mussels (digestive gland, mantel and gill) and turbot (muscle, kidney, intestine and liver).

Methods and results

Mussel and turbot tissues were subjected to microwave assisted acid digestion followed by ICP-MS for assessing total Ti and Ag contents; whereas, an assisted enzymatic hydrolysis procedure and sp-ICP-MS was used for TiO_2 and Ag NPs quantification/characterization. The collected tissues follow a standard procedure for TEM analysis. Shortly, fixation with Karnovsky fixative, post-fixation with osmium tetroxide and finally embed and include on resin.

ICP-MS have shown low bioaccumulation of TiO_2 NPs when exposing mussels to P25 TiO_2 (25 nm) at 0.1 mg L^{-1} [total Ti increase from 0.03 (day 0) to 0.27 µg g^{-1} (day 28)] and at 1.0 mg L^{-1} [total Ti increase from 0.03 (day 0) to 9.0 µg g^{-1} (day 28)]. The presence of TiO_2 NPs were not detected by sp-ICP-MS since the low NPs size (25 nm) but the measurement of dissolved Ti plus TiO_2 NPs (sp-ICP-MS) and total Ti (ICP-MS) were in good agreement.

Regarding Ag NPs (PVP-15 nm Ag), exposes at 0.1 mg L^{-1} showed an increase on the total Ag from 0.003 µg g^{-1} (time 0) to 1.5 µg g^{-1}, value which reached a plateau at sampling day 21st. Low bioaccumulation was also observed for experiments at 1.0 mg L^{-1} (the maximum total Ag concentration was closed to 1.5 µg g^{-1}, value which was obtained after sampling day 7th, being then constant until the end of the experiment). Ag bioaccumulation in mussels appears to be independent on the dose at longer exposure times since the maximum Ag concentrations measured in the tissues has been the same for both Ag NPs concentrations. This finding could be related with the dark scum observed in some tanks after long exposure times and/or high Ag NPs concentrations (the analysis of the dark scum revealed the presence of silver). Similar findings have been found when assessing Ag NPs instead of total Ag: Ag NPs are accumulated at a maximum concentration of 2.37×10^8 Ag NPs g^{-1} after 28 days (0.1 mg L^{-1} Ag NPs) and 2.56×10^8 Ag NPs g^{-1} after 28 days (1.0 mg L^{-1}).

Digestive gland, mantel and gill from mussels were analysed by TEM after 0, and 28 days of exposure. Neither Ag NPs nor TiO2 NPs were observed in any tissue.

(Continued on next page)

Regarding turbot, both TiO_2 and Ag NPs were found to be poorly accumulated and negligible total Ti and Ag concentrations were detected in the muscle. Most of Ti was detected in the faeces, which concentrations ranging from 1.6 (equivalent dose 0) to 40 μg g^{-1} (equivalent dose 1.5 mg Kg^{-1} per fish and day). It seems that Ti is eliminated in faeces. An analysis of the faeces by energy dispersive X-ray spectrometry (EDX)-scanning electron microscopy (SEM) confirmed the massive elimination of the TiO2 NPs by this route. However, liver was found to be a target organ for TiO_2 and Ag NPs, and little bioaccumulation rates were observed: total Ti concentration up to 0.25 μg g^{-1} (dose of 1.5 mg kg^{-1}, day 90) and total Ag concentration up to 6.0 μg g^{-1} (dose of 1.5 mg kg^{-1}, days 30, 45 and 90). Ag was also found to be accumulated in kidney [Ag concentration up to 0.25 μg g^{-1} (dose of 1.5 mg kg^{-1}, days 30, 45 and 90)]. Because of the low concentrations of total Ti and Ag in turbot tissues, the levels of TiO_2 and Ag NPs in were found un-detected in cultured turbot by sp-ICP-MS. TEM analysis of muscle, liver, kidney and intestine after 0 and 90 days of exposure showed that there was not significant bioaccumulation because no NPs were visualized in any tissue, which is in agreement with the sp-ICP-MS results. However, some ultrastructural changes were observed in some of the tissues (e.g. kidney) indicating some damaged that is currently being investigated.

References

[1] M.V. Taboada-López, et al. Ultrasound assisted enzymatic hydrolysis for isolating titanium dioxide nanoparticles from bivalve mollusc before sp-ICPMS. Analytica Chimica Acta, 1018 (2018) 16–25.

[2] M.V. Taboada-López, et al. Determination and characterization of silver nanoparticles in bivalve molluscs by ultrasound assisted enzymatic hydrolysis and sp-ICPMS. Microchemical Journal, 148 (2019) 652–660.

Acknowledgments

The authors wish to acknowledge the financial support of the European Union (Interreg Atlantic Area, project NANOCULTURE, reference EAPA_590/2018), and the *Ministerio de Economía y Competitividad* (project INNOVANANO, reference RT2018-099222-B-100).

SILVER AND TITANIUM DIOXIDE NANOPARTICLES BIOACCUMULATION IN CULTURED CLAMS

A. MOREDA-PIÑEIRO[1], C. SUÁREZ-OUBIÑA[1], P. HERBELLO-HERMELO[1], N. MALLO[2], M. VÁZQUEZ[2], P. BERMEJO-BARRERA[1], S. CABALEIRO[2]

[1] Trace Element, Spectroscopy and Speciation Group (GETEE), Strategic Grouping in Materials (AEMAT), Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain
[2] Centro Tecnológico del Cluster de Acuicultura, Cluster Acuicultura, Punta Couso S-N, Ribeira 15965, Spain
*pilar.bermejo@usc.es

Introduction

The wide spread use of metallic nanoparticles, mainly silver nanoparticles (Ag NPs) and titanium dioxide nanoparticles (TiO_2 NPs) is a current concern due to their increasing presence in the environment and the current doubts about its potential toxicity to living beings. This communication deals with controlled exposure assays of clams (*Ruditapes philippinarum*) to Ag NPs and TiO_2 NPs in order to test the potential bioaccumulation rate of these nanomaterials in species of aquaculture interest. Single particle-inductively coupled plasma – mass spectrometry (sp-ICP-MS) has been used for studying and quantifying the presence of Ag NPs and TiO_2 NPs in enzymatic extracts from exposed and unexposed clams. In addition, we have also studied the effect of exposure of these nanoparticles on the growth and mortality of clams.

Methods and results

Bioaccumulation assays consisted of exposing clams to Ag NPs (PVP Ag, 15 nm) and TiO_2 NPs (P25 TiO_2, 25 nm) at equivalent doses of 0.1 and 1.0 mg L^{-1} for twenty-eight days.

In both assays, 360 clams were distributed in 9 tanks of 50L each (40 clams / tank). Each equivalent dose was tested in triplicate. The water was renewed 3 times a week. Once a week the clams were fed with a microalgae suspension (T-iso) together with the corresponding NPs concentration. During the tests the mean temperature of the water was 18.9 ± 0.7 and 19.9± 0.8 ºC for AgNPs and TiO_2NPs assays, respectively. At days 0, 7, 14, 21 and 28, weight and length of clams shell were measured. Then, 3 clams from each tank were slaughtered (9 clams / dose) and their meat was frozen at -20ºC until analysis. Initial clams weights were 23.3 ± 3.4 and 19.9 ± 2.3 g for AgNPs and TiO_2NPs assays, respectively.

Total Ag and Ti assessment was performed by ICP-MS after a microwave assisted acid digestion sample pre-treatment. The determination/characterization of Ag NPs and TiO_2 NPs was carried out by sp-ICP-MS and an assisted enzymatic hydrolysis procedure with pancreatine and lipase was required for NPs isolation from the fresh clam tissues.

Negligible total Ag contents were assessed in clams at the initial time of the experiment (time 0) and also in the control tanks along a twenty-eight days period. Exposure at 0.1 mg L^{-1} led to a little increase on total Ag concentration in clams which was found to remain constant along the assay [from 322±66 ng g^{-1} (sampling day 7^{th}) to 310±103 ng g^{-1} (sampling day 28^{th})]. A similar result has been obtained when using an equivalent dose of 1.0 mg L^{-1} [similar total Ag concentrations at all sampling days, from 313±18 ng g^{-1} (sampling day 7^{th}) to 352±76 ng g^{-1} (sampling day 28^{th})]. It must be noticed that the increase on the equivalent dose does not imply a higher bioaccumulation rate.

Further analysis by sp-ICP-MS has revealed that the bioaccumulation rate of Ag NPs is dependent on the equivalent dose. Therefore, Ag NPs concentration were found to remain constant along the experiment [from $7.20\times10^{8}\pm4.28\times10^{8}$ Ag NPs g^{-1} (sampling day 7^{th}) to $2.66\times10^{8}\pm7.78\times10^{7}$ Ag NPs g^{-1} (sampling day 28^{th})] when exposing clams to an equivalent dose of 0.1 mg L^{-1}. However, bioaccumulation rates at an equivalent dose of 1.0 mg L^{-1} were found to increase from $1.20\times10^{9}\pm6.32\times10^{7}$ Ag NPs g^{-1} (sampling day 7^{th}) to $1.24\times10^{10}\pm1.68\times10^{10}$ Ag NPs g^{-1} (sampling day 14^{th}), remaining constant until the end of the experiment [$1.03\times10^{10}\pm1.19\times10^{10}$ Ag NPs g^{-1} (sampling day 21^{st}), and $2.57\times10^{10}\pm2.65\times10^{10}$ Ag NPs g^{-1} (sampling day 28^{th})]. During this assay, an increase in the fragility of the shells was observed, correlated with the higher the dose and the longer the exposure time. However, no significant differences were observed in weight and length between the different groups. Neither mortalities associated with the administration of AgNPs were observed.

(Continued on next page)

Regarding TiO_2 NPs, higher bioaccumulation rates than those observed for Ag NPs were obtained. Low total Ti contents in clams were assessed at the beginning of the experiment (time 0), and the total Ti concentration was found to slightly increase in the control (unexposed clams) tanks [from 109±93 ng g^{-1} (sampling day 7th) to 707±156 ng g^{-1} (sampling day 28th)]. Exposure to an equivalent dose of 0.1 mg L^{-1} led to a gradual increase of total Ti concentration from 386±124 ng g^{-1} (sampling day 7th) to 2123±479 ng g^{-1} (sampling day 21st), remaining constant at sampling day 28th (1989±596 ng g^{-1}). A similar trend was observed when using the equivalent dose of 1.0 mg L^{-1}, but the total Ti concentration was found to increase from sampling day 7th (996±392 ng g^{-1}) to sampling days 14th (2974±529 ng g^{-1}) and 21st (2866±856 ng g^{-1}), followed by a fast increase at sampling day 28th (5892±1582 ng g^{-1}).

The assessment of TiO_2 NPs at both equivalent doses has shown a little increase until the sampling day 14th, followed by a decrease on the TiO_2 NPs at the end of the experiment. Therefore, concentrations from $7.18\times10^5\pm7.28\times10^4$ TiO_2 NPs g^{-1} (equivalent dose of 0.1 mg L^{-1}) and $1.51\times10^6\pm3.46\times10^5$ TiO_2 NPs g^{-1} (equivalent dose of 1.0 mg L^{-1}) at sampling day 7th increased to $3.12\times10^6\pm2.35\times10^6$ TiO_2 NPs g^{-1} (equivalent dose of 0.1 mg L^{-1}) and $8.23\times10^6\pm2.73\times10^6$ TiO_2 NPs g^{-1} (equivalent dose of 1.0 mg L^{-1}) at sampling day 14th. Then, TiO_2 NPs concentration decreased at $1.79\times10^6\pm4.95\times10^5$ TiO_2 NPs g^{-1} (equivalent dose of 1.0 mg L^{-1}) and $2.95\times10^6\pm1.21\times10^6$ TiO_2 NPs g^{-1} (equivalent dose of 1.0 mg L^{-1}) at sampling day 21st. TiO_2 NPs concentration at the end of the experiment (sampling day 28th) were $8.66\times10^5\pm3.63\times10^5$ TiO_2 NPs g^{-1} (equivalent dose of 0.1 mg L^{-1}) and $8.17\times10^6\pm5.09\times10^6$ TiO_2 NPs g^{-1} (equivalent dose of 1.0 mg L^{-1}). The slight accumulation of TiO_2 NPs at short times affected the weight gain of the clams, although this effect disappeared when the concentrations of TiO_2Nps were reduced. The mean weight of the clams that received the 0.1mg L^{-1}dose was significantly lower with respect to the control on day 14; while in those that received the higher dose (1.0 mg L^{-1}) the weight increase was significantly less on days 7 and 14. However, no significant differences in shell length growth were detected. Furthermore, as in the AgNPs bioaccumulation test, no significant differences were obtained between the mortality of the control clams and those that received the different doses of TiO_2 NPs.

Acknowledgments

The authors wish to acknowledge the financial support of the Ministerio de Economía y
Competitividad (project INNOVANANO, reference RT2018-099222-B-100), the European Union (Interreg POCTEP, project ACUINANO, reference 07-12-ACUINANO_1_E), and the Xunta de Galicia (Grupo de Referencia Competitiva, grant number ED431C2018/19; and Program for Development of a Strategic Grouping in Materials – AEMAT, grant number ED431E2018/08).

FILTRATION PARAMETERS IN PACIFIC OYSTER (*Crassostrea gigas*): EFFECTS OF FLOW RATE AND MICROALGAE CONCENTRATION

Herrera, M., Ferrer, J.F., Moreno*, O.

IFAPA Agua del Pino, Ctra. El Rompido-Punta Umbría, 21459 Cartaya
oscar.moreno@juntadeandalucia.es

Abstract
It has been reported that the wild oyster populations provide several ecosystem services such as the water quality improvement, thanks to their high filtration capacity. To know the real filtration capacity in natural populations is initially necessary to study the individual filtration at laboratory scale. Therefore the objective of this work was to study the influence of water microalgae concentration and flow on the clearance and ingestion rates in the Pacific oyster (*Crassostrea gigas*). Oysters were collected from the wild, acclimated to captivity and kept in 1 L flow through individual tanks. Several culture conditions were tested: inlet water microalgae concentration (100, 200, 300, 400 and 500 cel/mL) and flow rate (25 and 50 mL/min). Clearance and ingestion rates were calculated every hour for 8 hours, and referred to the dry meat weight (mL/min·g, and cel/min·g, respectively). Both phytoplankton concentration and flow rate affected significantly the clearance and ingestion rates. At higher phytoplankton concentrations, the efforts for collecting feed were lower due to an elevated availability. However the concentrations between 100-200 cel/mL favored the clearance rate.

Introduction
Recently, the study of the ecosystem services in coastal and marine environments is gaining attention. It has been reported that the wild oyster populations provide several ecosystem services as the water quality improvement, seashore stabilization and habitat for fish and invertebrates (Grabowski et al., 2012).

The role of the oyster reefs on the water quality is based on their high filtration capacity. For instance, it is known that, in the late 19th century, oysters in the Chesapeake Bay probably filtered a water volume equivalent to the entire volume of the bay in less than a week (Newell, 1988). In this sense, small scale experiments are necessary to study the individual filtration for extrapolating to natural populations and determining its efficacy as natural depurators (one of their ecosystem services).

Therefore the objective of this work was to study the influence of water microalgae concentration and flow on the clearance and ingestion rates in the Pacific oyster (*Crassostrea gigas*).

Material and methods
Oysters were collected from the El Rompido shore (Spanish South-western coast), and acclimated to captivity during 14 days before starting the experiment. The mean size and wet total weight were 111.15±0.48 cm and 139.67±1,51g, respectively. The essay was carried in individual 1 L-tanks, with a continuous water and phytoplankton supply through peristaltic bombs. Animals were kept in vertical position into the tanks through a specific structure, with the valve aperture at the top. Several flow rates and phytoplankton concentrations were assayed: 25 and 50 mL/min, and 100, 200, 300, 400 and 500 cel/mL. Monospecific cultures of *Isochrysis galbana* were used for reaching those concentrations in the supplied water. *Isochrysis* cell concentration was measured at every tank water inlet (the same for all tanks) and outlet every 60 min during 8 hours for every oyster (8 per treatment). After every trial (8 hours) every oyster was sacrificed and calculated the dry meat weight. Clearance and ingestion rates were calculated every hour, and referred to the dry meat weight (mL/min·g, and cel/min·g, respectively). Data are expressed as mean±SE. A 2-way ANOVA was performed in both variables to study the effects of flow rate and phytoplankton concentration on them ($p<0.05$).

Results and conclusions
The p values obtained after the 2-way ANOVA performance are shown in Table 1. Both phytoplankton concentration and flow rate affected significantly the clearance and ingestion rates. The mean clearance and ingestion rates obtained for every trial is shown in Fig.1.

The best results for clearance rate were obtained for the *Isochrysis* concentration of 200 cel/mL, and it grew as well as the flow rate. The ingestion rate was directly related to the phytoplankton concentration for every flow rate. Therefore, at higher concentrations, the efforts for collecting feed were lower due to an elevated availability. However the concentrations between 100-200 cel/mL favored the clearance rate. This can be due to this range is similar to that found in wild conditions, and this rate can increase if flow rate grow.

(Continued on next page)

Table 1. Results (p values, ns=no significant) coming from the two-way ANOVA (phytoplankton concentration and flow rate as factors) performed on the clearance and ingestion rates.

Factor	Clearance rate	Ingestion rate
Concentration	0.00	0.00
Flow	0.00	0.00
Concentration*flow	ns	0,41

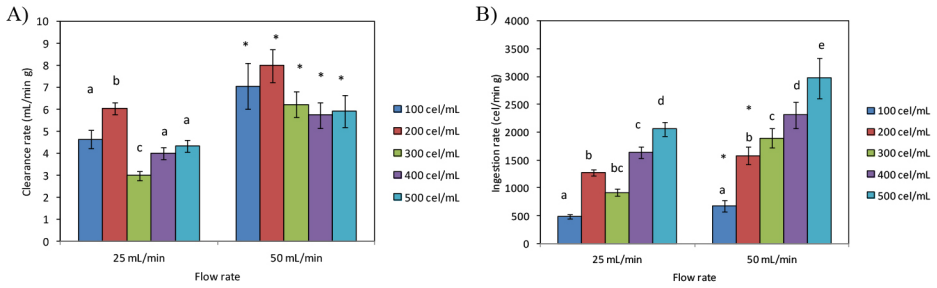


Fig 1. A) Clearance rate (mean±SE) for every treatment. B) Ingestion rate (mean±SE) for every treatment. Different letters indicate significant differences within every flow rate, and asterisks indicate significant differences between flow rates for every concentration.

Acknowledgements

The authors acknowledge to INTERREG V A Espanha Portugal (POCTEP) program for funding through project 0750_AQUA_AMBI_2_5_P and project 0240_AQUA_AMBI_5_P.

References

Grabowski, J.H., R. D. Brumbaugh, R. F. Conrad, A. G. Keeler, J. J. Opaluch, C. H. Peterson, M. F. Piehler, S. P. Powers, A. R. Smyth, 2012. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. BioScience 62, 900–909.

Newell, R.I.E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*?. In: Understanding the Estuary: Proceedings of a Conference, 29-31 March 1988. Chesapeake Research Consortium Publication 129, Baltimore, Maryland

EVALUATING *Lactococcus lactis* STRAIN AS PROBIOTICS FOR GILTHEAD SEA BREAM (*SPARUS AURATA*): EFFECTS ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY, TRANSCRIPTIONAL RESPONSE AND GUT MICROBIOTA

F. Moroni[1]*, F. Naya-Català[2], M. C. Piazzon[3], S. Rimoldi[1], J. Calduch-Giner[2], F. Brambilla[4], J. Pérez-Sánchez[2] and G. Terova[1].

[1]Department of Biotechnology and Life Sciences, University of Insubria, Via J.H. Dunant, 3, 21100 Varese, Italy.
[2]Nutrigenomics and Fish Growth Endocrinology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain.
[3]Fish Pathology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain.
[4]VRM S.r.l. Naturalleva, Via S. Michele,41 - 37044 Cologna Veneta (Verona), Italy.
E-mail: f.moroni@uninsubria.it

Introduction

In aquaculture a great number of bacterial species are used as probiotics (Newaj-Fyzul et al., 2014). The use of these microorganisms is mostly related to the need to decrease or avoid the use of antibiotics, increasing the sustainability of the aquaculture industry. Probiotics can reduce pathogenic bacteria due to direct competition dynamics, producing inhibitory molecules and enhancing the host immune system (Balcázar et al., 2007). In cultured fish, probiotics improve fish growth and feed conversion rates, due to an increase in feed digestibility and absorption of nutrients (Merrifield et al., 2010; Martínez Cruz et al., 2012). Furthermore, the use of probiotics can restore the eubiotic state of the intestinal microbiota or can help maintain gut microbiota homeostasis (Borch et al., 2015; Ringø et al., 2016). Accordingly, the aim of the present research was to evaluate, in gilthead sea bream (*Sparus aurata*), the effects of the administration of the probiotic *Lactococcus lactis* subsp. *lactis* SL242, on growth performance, feed utilization, intestinal morphology, transcriptional response, and gut microbiota.

Materials and Methods

The trial was conducted with sea bream juveniles (70-90 g), individually tagged and reared in nine 500-L tanks with 40 fish/tank. Fish were divided into 3 groups and were fed with a control (diet A) or experimental diets (diets B and C), supplemented with 2.0 E+09 CFU/kg and 5.0 E+09 CFU/kg dose of probiotic, respectively. At the end of the feeding trial, all the animals were weighed, in order to calculate the growth index, and four fish per replicate were sacrificed to collect intestinal samples for morpho-histological evaluation, gene expression and microbiota analysis. The latter performed using Illumina MiSeq platform and a metagenomics pipeline based on VSEARCH and RDP databases.

Results and Discussion

The final biomass of fish fed diet C was significantly higher than the control group (diet A), with intermediate values for fish fed diet B. Indeed, even if without significant differences, the best growth performance tended to be achieved by the animals that have received the higher dose of probiotic. Histological analysis, performed using a quantitative metric system, confirmed that probiotic did not alter the macroscopic morphology of the intestine and did not cause inflammation. In addition, the microscopic evaluation detected an amelioration of the intestine structure, through the increase of the height of the mucosal folds and the number of goblet cells, in fish fed probiotic. These results suggest that *L.lactis* SL242 played an important role improving the potential capacity of digest and absorb nutrients. A customized PCR array was designed to study the transcriptomic response. As discussed by numerous authors (Nayak, 2010), probiotics enhance the piscine immune system. The present results confirmed these effects, as significant changes were found in the expression of key genes involved in innate and acquired immunity (il10, il12 and tlr2), that resulted upregulated in fish fed diet C as compared to the control group. The composition analysis of the gut microbiota showed a higher Firmicutes/Bacteroidetes and Proteobacteria/Firmicutes ratios in the sea bream fed diets containing probiotic compared to control fish. Despite the physiological effects on the host are difficult to assess from genomic data alone, these results could be correlated to their better growth performances (Magne et al., 2020; Rimoldi et al., 2020). Accordingly, although the microbial gut-adherent analysis reveal a lack of colonization of the probiotic in the host's intestinal mucosa, the functional prediction showed that the microbiota of fish that received probiotic was more involved in the digestion and absorption of protein. This confirms that positive modulation of fish gut microbiota can occur without probiotics colonization in the intestinal environment.

Conclusion

Our results highlight the interactions between diet and fish microbiota, suggesting that manipulating fish microbiome, through the use of well-designed probiotics, may represent a promising intervention to improve fish growth performances and digestive capacity.

(Continued on next page)

Bibliography

Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Gironés, O., and Múzquiz, J. L. (2007). In vitro competitive adhesion and production of antagonistic compounds by lactic acid bacteria against fish pathogens. *Vet. Microbiol*. 122, 373–380. doi:10.1016/j.vetmic.2007.01.023.

Borch, K., Pederson, I. E., and Hogmo, R. O. (2015). The use of probiotics in fish feed for intensive aquaculture to promote healthy guts. *Adv. Aquac. Fish. Manag*. 3, 264–273.

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., et al. (2020). The firmicutes/bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients* 12. doi:10.3390/nu12051474.

Martínez Cruz, P., Ibáñez, A. L., Monroy Hermosillo, O. A., and Ramírez Saad, H. C. (2012). Use of Probiotics in Aquaculture. *ISRN Microbiol*. 2012. doi:10.5402/2012/916845.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., et al. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302, 1–18. doi:10.1016/j.aquaculture.2010.02.007.

Nayak, S. K. (2010). Probiotics and immunity: A fish perspective. Fish Shellfish Immunol. 29, 2–14. doi:10.1016/j.fsi.2010.02.017.

Newaj-Fyzul, A., Al-Harbi, A. H., and Austin, B. (2014). Review: Developments in the use of probiotics for disease control in aquaculture. *Aquaculture* 431, 1–11. Available at: http://dx.doi.org/10.1016/j.aquaculture.2013.08.026.

Ringø, E., Zhou, Z., Vecino, J. L. G., Wadsworth, S., Romero, J., Krogdahl, Å., et al. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquac. Nutr.* 22, 219–282. doi:10.1111/anu.12346.

Rimoldi, S., Gini, E., Koch, J. F. A., Iannini, F., Brambilla, F., and Terova, G. (2020). Effects of hydrolyzed fish protein and autolyzed yeast as substitutes of fishmeal in the gilthead sea bream (*Sparus aurata*) diet, on fish intestinal microbiome. *BMC Vet. Res*. 16:118, 1–13. doi:https://doi.org/10.1186/s12917-020-02335-1.

AN IMAGE ANALYSIS PIPELINE FOR MOLLUSCS SHELLS PHENOTYPING: DEVELOPMENT AND VALIDATION ON THE PACIFIC OYSTER *Crassostrea gigas*

A. Jourdan[a,c], J. Bugeon[b], J.-B. Lamy[c], F. Enez[a], L. Degremont[c], P. Boudry[d], P. Haffray[a], F. Chenier[e], E. Vetois[f], R. Morvezen[*,a]

[a] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[b] INRAE LPGP UR1037 (Laboratory of Fish Physiology and Genomics) 35000 Rennes, France
[c] Ifremer, LGPMM (Marine Molluscs Genetic and Pathology Laboratory), 17390 La Tremblade, France
[d] Ifremer, Department of Biological Resources and Environment, 29280 Plouzané, France
[e] Vendée Naissain, 85230 Bouin, France
[f] SATMAR, 50760, Gatteville-le-Phare, France

Introduction

Image analysis has proven to be a powerful phenotyping tool in breeding programs. It allows an objective and numerical measures of color and shape, improving the accuracy of phenotyping these traits when compared to human-based evaluation. In aquaculture, several methods have been used to describe animal shape. Procruste based methods used morphological points of interest on the animal whereas Fourier analysis use a whole contour-based analysis. More recently, machine learning algorithms have been implemented. For shellfish, defining easily identifiable points of interests for Procruste approach is not obvious. Elliptic Fourier transformation has been shown to be more suitable in organism with shapes resembling shellfish, such as seeds or tree leaves (Costa *et al.* 2011). In our poster, we describe the development of an analysis pipeline using standardized photography, automated image analysis for colorimetric evaluation, contour-tracing and elliptic Fourier analysis for multivariate approach to shape.

Material and Methods

1249 photos of 2.5 years old shucked Pacific oyster *Crassostrea gigas*, from Vendée Naissain hatchery (Bouin, France) were taken using a Canon EOS 2000D camera, fixed and under standardized light. Upper face of the upper shell as well as animal in its lower shell were photographed. Luminosity and white balance were standardized using an internal control using Fiji software (Schindelin *et al.* 2012). Mean upper shell color in the L*a*b* color space were measured. Shell positions on images were defined, and contour of the upper shell was reconstructed using package Momocs in R (Bonhomme *et al.* 2014). Contours for all shells were then aligned, centered, and scaled, to remove position and size effects on shape. Shape was then reconstructed by using elliptic Fourier analysis for up to 15 harmonics. Variance of the Fourier descriptors was then analyzed using a principal component analysis.

Results and discussion

Color in le L*a*b* color space was found moderately variable, with coefficient of variation varying from 13 to 53%. However, the analyzed oysters were not representative of all possible color variation that can be found in the Pacific oyster – they were white to slightly orange. We expect this methodology to perform accurately even with different colored oysters such as black or stripped oysters, and more testing is currently being done.

Six harmonics described 95% of shell shape variance, and 12 harmonics described 99% of shell shape variance. We limited the number of harmonics to 6, as adding more help only to described unwanted details like the knife scar made during oysters shucking (figure 1).

The PCA of Fourier descriptors revealed that the two first axes explained 35.9 and 24.1% of variance (Figure 2). Axis one seemed to be driven by the overall roundness of shell, when axis 2 was mainly driven by shell symmetry (or asymmetry).

Conclusion

The pipeline presented here is useful for objective quantification of shape and color variance in any given population of mollusks. The method could be useful for a wide range of application: breeding and genetic improvement, rearing methods impact evaluation, or grow-out site characterization. The pipeline was developed using only open-source software for easier transferability.

(Continued on next page)

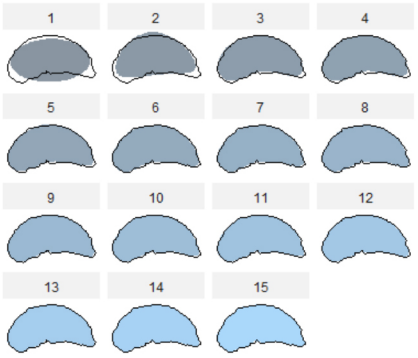
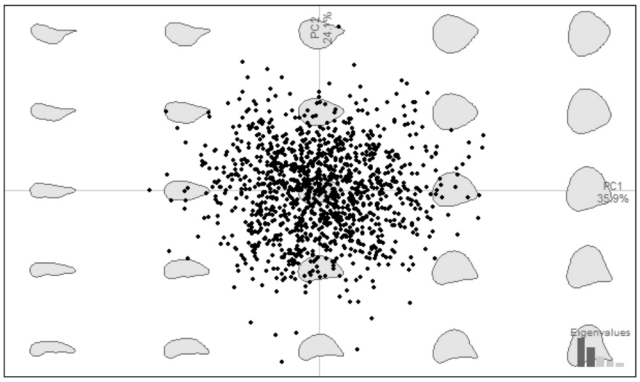


Figure 1: Shell shape reconstruction by Fourier analysis using an increasing number of harmonics. Black line: Sample of a real oyster contour. Blue shape: shape reconstructed by Fourier analysis.

Figure 2: Principal component analysis of Fourier coefficient obtained using 6 harmonics.



Acknowledgement

The data presented here were obtained in the Quality-Huitre project which received funding from the European Maritime and Fisheries Fund (EMFF) research grant number PFEA470018FA1000011.

References

Bonhomme, V., Picq, S., Gaucherel, C., & Claude, J. (2014). Momocs: outline analysis using R. Journal of Statistical Software, 56(13), 24-p.

Costa, C., Antonucci, F., Pallottino, F., Aguzzi, J., Sun, D. W., & Menesatti, P. (2011). Shape analysis of agricultural products: a review of recent research advances and potential application to computer vision. Food and Bioprocess Technology, 4(5), 673-692.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature methods, 9(7), 676-682.

TOTAL DIETARY FISHMEAL REPLACEMENT WITH *Hermetia illucens* PRE-PUPAE LARVAE MEAL: EFFECTS ON GROWTH AND LIVER OXIDATIVE STRESS STATUS OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

S. Moutinho*[1,2], H. Peres[1,2], N. Martins[1,2], C. Castro[1], A. Oliva-Teles[1,2]

[1] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto
[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto
*smoutinho@ciimar.up.pt

Introduction

Aquaculture has an increasingly important role in providing global food security. To support the predicted growth of intensive aquaculture production it is crucial to find alternative ingredients to fishmeal (FM). Insect meals arise as a sustainable alternative to FM since insects are efficient converters of biowaste, require almost no land and water for their production, and possess high amounts of protein, lipids, vitamins, and minerals. Chitin, which is present in the exoskeleton of insects, has also been indicated to possess antioxidant boosting proprieties, indicating that insect meals can also be used as functional ingredients in aquafeeds (1). Therefore, this study aimed to evaluate the effects of dietary FM replacement with increasing levels of defatted black soldier fly, *Hermetia illucens,* prepupae larvae meal (HM), up to 100%, on growth performance and liver oxidate stress response of gilthead seabream juveniles.

Materials and methods

Four isoproteic (43% CP) and isolipidic (18% CL) diets were formulated to include HM (61.8% CP, 6.8% CL, 11.9% ash, 21.6 kJ g^{-1} gross energy, 6.20% chitin) at 0 (HM0), 15 (HM15), 30 (HM30), and 45% (HM45), replacing 0, 22, 60, and 100% of FM protein, respectively. A feeding trial was conducted with triplicate groups of gilthead seabream juveniles (initial body weight of 32 g). Fish were hand-fed twice a day, 6 days a week, for 67 days. At the of the trial, feed consumption was recorded, and fish were bulk weighted for estimation of growth performance. The liver of 3 fish per tank was also sampled for the determination of oxidative stress parameters.

Results and discussion

Present results indicate that HM is well tolerated by gilthead seabream juveniles and can replace all FM protein (corresponding to 79% of the protein in the control diet) without compromising growth performance and feed efficiency (Table 1).

Compared to the control, the dietary inclusion of 15% and 30% HM led to a decrease in the activity of superoxide dismutase (SOD) and glutathione reductase (GR), respectively, while a 45% inclusion of HM decreased the activity of glutathione peroxidase (GPX) and glucose-6-phosphate dehydrogenase (G6PDH) (Figure 1). On the other hand, catalase (CAT) activity and lipid peroxidation (LPO) were not significantly affected by diet composition (Figure 1). These results seem to indicate that dietary HM induced some responses in the hepatic oxidative enzyme response, while not affecting liver oxidative stress.

References

(1) Ngo, D.H., Kim, S.K., 2014. https://doi.org/10.1016/B978-0-12-800268-1.00002-0

Acknowledgments

This work was supported by the R&D&I project "ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources", reference NORTE-01-0145-FEDER-000040, funded by the North Portugal Regional Operational Program for Norte's Regional Operational Program (NORTE2020), through the European Regional Development Fund (ERDF). S.M. and N.M. were supported by the Portuguese Foundation for Science and Technology (FCT) (grant references SFRH/BD/138224/2018 and SFRH/BD/137919/2018, respectively).

TREATMENT WITH ORGANIC ACIDS EXTENDS SHELF-LIFE OF GUTTED EUROPEAN SEA BASS

Angelakopoulos Rafael[2], Ntzimani Athina[1], Semenoglou Ioanna[1], Tsironi Theofania[1,3], Moutou Katerina[2], Taoukis Petros[1]

[1] Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Athens, Greece
ntzimani@chemeng.ntua.gr
[2] Laboratory of Genetics, Evolutionary and Comparative Biology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
kmoutou@bio.uth.gr
[3] Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
ftsironi@aua.gr

Introduction

Fresh fish can easily deteriorate after being captured due to the endogenous enzyme activity and rapid microbial growth naturally present in fish. What is more, changes in composition during fish decay leads to protein degradation and lipid oxidation, as well as changes in fish odor, flavor, and texture. Therefore, it has become imperative to develop effective treatment methods in order to extend the shelf life of fish (Campos et al., 2012). Application of organic acids on fish surfaces, mainly through dipping or spraying, is a widely used and well-known practice due to their antimicrobial properties (Mei et al., 2019). The objective of the present study was to investigate the effect of minimal processing methods, i.e. surface decontamination of fish with organic acids, on the quality and shelf life of farmed European sea bass (*Dicentrarchus labrax*) under controlled isothermal conditions (0°C).

Materials and methods

Whole farmed European sea bass were treated using organic acids. Samples were stored isothermally at 0°C for 6 days after harvesting to evaluate the effect of organic acids on the initial microbial load. Fish was gutted manually and immersed in water for 0-10 min. The incorporation of natural organic acids (0-200 ppm, 0-10 min - lactic acid, citric acid and peracetic acid) at different concentrations in the washing water during gutting was also tested for its efficacy to reduce the initial microbial load and prolong the shelf life. Control (treated with water) and organic acid treated samples were afterwards stored under controlled isothermal conditions (0°C) for shelf-life testing. Microbial enumeration included several spoilage microorganisms, such as total viable count (TVC), *Pseudomonas* spp., *Enterobacteriaceae* spp. and H_2S-producing bacteria (mainly *Shewanella putrefaciens*). Quality evaluation included evaluation of pH, colour and texture measurement and sensory parameters (1-9 scale). A sensory score of 5 was taken as the average score for minimum acceptability.

The activity of major proteases, namely Calpain, Collagenase, Cathepsin B and L responsible for white muscle degradation was also measured; a piece of white muscle was extracted from the treated fillet on day 0, 2, 3, 6, 7, 10, 12 post-treatment, snap-frozen in liquid nitrogen and stored at -80 °C until enzyme extraction. Enzymes were extracted and the activity of these enzymes was assayed as described in Ntzimani et al. (2021). Activity was expressed as fluorescence units (FU) change per minute per mg protein.

Results

Higher washing solution concentrations and longer treatment, led to increased microbial load reduction. Initial surface decontamination in the range of 1.0-2.0 log cfu/g (for total viable count, *Pseudomonas spp. and Enterobacteriaceae spp.*) by the addition of organic acids in the washing water, resulted in 3-4 days shelf-life extension of fish stored at 0°C. Higher reduction of the initial microbial load was observed after treatment with citric acid for TVC, *Pseudomonas* spp. and H_2S-producing bacteria, and with lactic acid for *Enterobacteriaceae* spp. Microbial growth during subsequent refrigerated storage of untreated (Control) and treated fish was modeled using the Baranyi Growth Model. The limit of sensory shelf life of gutted fish (score 5 by the sensory panel for overall impression) coincided with a level of 10^7 cfu/g of *Pseudomonas* spp. for gutted samples, stored at 0° C (Tsironi et al., 2019).

(Continued on next page)

Amongst the treatments all four proteolytic enzymes recorded the lowest activities post-treatment with 200ppm peracetic acid (P200). In contrast, the lower concentration of 150ppm peracetic acid (P150) could not delay an early activation of calpain and collagenase. P200 and P150 groups differed significantly in all four enzymes (p-value<0.05). Following the citric acid treatment, calpain and collagenase shared a similar activation pattern having an early high peak two days after the citric acid was applied. Cathepsins are acid proteases located in the lysosomes. They may be liberated into both the cytoplasm and the intracellular spaces as a consequence of lysosomal disruption occurring after cell death due to a pH fall (Cheret et al., 2007). Cathepsins B & L are two cysteine proteases with an optimal activity pH of 5-7.5 (Larsen et al., 2004). Applying citric acid (200ppm) appeared to influence their activities recording lower activities compared with the control group even 12 days post-treatment (p-value<0.05).

Discussion and conclusion

The results of the study indicated that the application of organic acids, as alternative disinfection approaches to the conventional post-harvest fish processing methods, led to improved stability of flesh quality during subsequent refrigerated storage and significant shelf-life extension, in terms of microbial growth, physicochemical and organoleptic degradation of the fillets. Regarding the endogenous enzymatic activity, applying peracetic acid in the highest, according to the FDA, concentration resulted in a late and low activation on all four enzymes, in favour of the flesh quality. Similarly, the suppression of cathepsin activity following the citric acid treatment (200ppm) is expected to delay the fillet degradation during storage. The results encourage the application of minimal processing of fish to extend shelf life and penetrate new distant markets currently inaccessible to fresh fish products.

Acknowledgment

This research was Co-financed by Greece and the European Union, European Maritime and Fisheries Fund in the context of the implementation of the Greek Operational Programme for Fisheries, Priority Axis “Innovation in Aquaculture”, Project title: “Development and application of novel methods for fish harvesting and processing for quality improvement and shelf life extension” (2018-2021) MIS 5010939, website: slurryfish.chemeng.ntua.gr.

References

Campos, C.A.; Castro, M.P.; Aubourg, S.P.; Velázquez, J.B. Novel Technologies in Food Science; Springer: New York, NY, USA, 2012.

Mei J, Ma X, Xie J. Review on Natural Preservatives for Extending Fish Shelf Life. Foods. 2019;8(10):490. Published 2019 Oct 13.

Ntzimani, A., Angelakopoulos, R., Semenoglou, I., Dermesonlouoglou, E., Tsironi, T., Moutou, K., & Taoukis, P. (2021). Slurry ice as an alternative cooling medium for fish harvesting and transportation: Study of the effect on seabass flesh quality and shelf life. Aquaculture and Fisheries,

Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. Trends in Food Science and Technology, 13(12), 400–421.

Tsironi T., Anjos L., Pinto P.I.S., Dimopoulos G., Santos S., Santa C., Manadas B., Canario A., Taoukis P., Power D. (2019). High pressure processing of European sea bass (*Dicentrarchus labrax*) fillets and tools for flesh quality and shelf life monitoring. J Food Engi, 262, 83-91.

EFFECTS OF EARLY TEMPERATURE EXPOSURE ON MITOCHONDRIAL BIOENERGETICS OF EXERCISED METAMORPHOSING LARVAE OF GILTHEAD SEABREAM (*Sparus aurata*)

Andreas Tsipourlianos[a], Lamprini Tzioga[a], Chara Kourkouta[b], Giorgos Koumoundouros[b], Nikos Papandroulakis[c], Deborah M. Power[d], Zissis Mamuris[a], Katerina A. Moutou[a]

[a] Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece
[b] Biology Department, University of Crete, Vasilika Vouton, 70013, Heraklion, Crete, Greece, Greece
[c] Institute of Aquaculture, Hellenic Centre for Marine Research, AquaLabs, 71500 Gournes, Heraklion, Greece
[d] Group of Comparative Endocrinology and Integrative Biology, Centro de Ciencias do Mar, University of Algarve, Campus de Gambelas, Faro, Portugal
email: kmoutou@bio.uth.gr

Introduction

Temperature is a high impact factor for the performance and wellbeing of poikilothermic organisms and it is gaining importance as climate change and disturbance of seasonal temperatures are expected to impact on organismal fitness. Each animal is adapted to perform within a thermoneutral zone of temperatures and within that range, optimal temperatures for survival, development, and growth are recorded. An important biological function that is implicated in growth and development and is influenced by changes in environmental temperatures is the oxidative phosphorylation (OXPHOS), which produces enough energy to cover up to 90% of the cellular energy needs. Here, we investigate how different rearing temperatures at early development may influence the response of OXPHOS genes to exercise in metamorphosing larvae of gilthead seabream (*Sparus aurata*).

Materials and methods

Gilthead seabream 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). Swimming performance was assessed by estimating the relative critical swimming speed at the middle of metamorphosis. Both rearing and swimming test conditions are described in detail by Kourkouta and colleagues [1]. A total of 68 samples of the caudal region were collected and stored in methanol at -20°C until RNA extraction. Samples represented the three rearing temperatures while for each case, control (not exercised) and exercised individuals were sampled. RNA was isolated and cDNA synthesis was performed. The expression of nine (9) genes of interest was quantified by real-time PCR and it was normalized against two reference genes (rps18, rpl13a). Eight of the target genes are the paralogues coding for subunits of OXPHOS complex III (*uqcrfs1a, uqcrfs1b, uqcrha, uqcrhb, uqcrc2a, uqcrc2b, uqcr11a, uqcr11b*); the ninth one is PPRAG-coactivator-1a (*pprgcg1a*), a marker of mitochondria biogenesis. Significant differences between the expression level of each gene at different temperatures and exercise level were analyzed using Wilcoxon signed-rank test.

Results

Developmental temperature (DT) alone (control samples) affected the expression of neither of the OXPHOS genes nor *pprgc1a* with only exception *uqcr11a* that was significantly lower at 17°C than at 20°C reared fish. Interestingly, DT governed differential responses to swimming in four genes implicated in mitochondrial biogenesis and function (*pprgc1a, uqcrfs1a, uqcrfs1b, uqcrhb*) as compared with the control fish. These increases were common in fish reared at 23°C, while such an increase was also observed in *uqcrfs1a* of fish reared at 17°C. Exercised fish reared at 20°C or/and 23°C exhibited significantly higher expression levels than exercised fish reared at 17°C.

Discussion

The effect of exercise on mitochondrial biogenesis and on OXPHOS regulation and efficiency are well studied in different animals including fish [2]. While the impact of exercise on those procedures is obvious, the way that different factors including the kind and length of the exercise [3], the nutrition uptake [4] and the general state of the exercised individual, the needs of specific tissues [5] and or developmental stages along with environmental factors such as temperature [6] or oxygen availability [7] affect those procedures may vary. In this study we show that while the different DT appeared to have no impact on the mitochondrial bioenergetics of metamorphosing fish under normal circumstances, the exercise challenge revealed a differential capacity to respond as shaped by early DT. Our results suggest that DT changes in a short time window at the beginning of development is sufficient to influence plasticity in OXPHOS gene response later on, under highly energetic demanding processes such as acute exercise. For each gene examined, only one of the gene paralogues was highly affected by DT, indicative of the subfunctionalization occurred following teleost-specific whole genome duplication. The results render further investigation in a fast changing climate and aquatic environment.

(Continued on next page)

Acknowledgements
This research was funded by the EU Horizon2020 Research Framework in the context of the project PerformFISH, Grant nº 727610, www.performfish.eu

Bibliography

[1] C. Kourkouta, A. Printzi, G. Geladakis, N. Mitrizakis, N. Papandroulakis, and G. Koumoundouros, "Long lasting effects of early temperature exposure on the swimming performance and skeleton development of metamorphosing Gilthead seabream (Sparus aurata L.) larvae," *Sci. Rep.*, vol. 11, no. 1, p. 8787, Dec. 2021, doi: 10.1038/s41598-021-88306-4.

[2] A. P. Russell, M. K. C. Hesselink, S. K. Lo, and P. Schrauwen, "Regulation of metabolic transcriptional co-activators and transcription factors with acute exercise," *FASEB J.*, vol. 19, no. 8, pp. 986–988, Jun. 2005, doi: 10.1096/fj.04-3168fje.

[3] E. L. Martins, J. C. Ricardo, E. de-Souza-Ferreira, J. Camacho-Pereira, D. Ramos-Filho, and A. Galina, "Rapid regulation of substrate use for oxidative phosphorylation during a single session of high intensity interval or aerobic exercises in different rat skeletal muscles," *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, vol. 217, pp. 40–50, Mar. 2018, doi: 10.1016/j.cbpb.2017.11.013.

[4] M. Perelló-Amorós *et al.*, "Mitochondrial Adaptation to Diet and Swimming Activity in Gilthead Seabream: Improved Nutritional Efficiency," *Front. Physiol.*, vol. 12, p. 678985, Jun. 2021, doi: 10.3389/fphys.2021.678985.

[5] D. F. Wilson, "Oxidative phosphorylation: regulation and role in cellular and tissue metabolism: Oxidative phosphorylation: role in cell and tissue metabolism," *J. Physiol.*, vol. 595, no. 23, pp. 7023–7038, Dec. 2017, doi: 10.1113/JP273839.

[6] L. Gerber, K. A. Clow, F. C. Mark, and A. K. Gamperl, "Improved mitochondrial function in salmon (Salmo salar) following high temperature acclimation suggests that there are cracks in the proverbial 'ceiling,'" *Sci. Rep.*, vol. 10, no. 1, p. 21636, Dec. 2020, doi: 10.1038/s41598-020-78519-4.

[7] Y. Zhang, M. J. H. Gilbert, and A. P. Farrell, "Finding the peak of dynamic oxygen uptake during fatiguing exercise in fish," *J. Exp. Biol.*, p. jeb.196568, Jan. 2019, doi: 10.1242/jeb.196568.

ERYTHROCYTE TRANSCRIPTOME EXHIBITS FAMILY-SPECIFIC RESPONSES TO PLANT-BASED DIETS IN GILTHEAD SEABREAM

Angelakopoulos Rafael[1], Themistoklis Giannoulis[1], Arkadios Dimitroglou[2], Leonidas Papaharisis[2], Dimitrios Chatziplis[3], Zissis Mamuris[1], Katerina A. Moutou[1]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece
[2] Department of Research & Development, Nireus Aquaculture SA, 341 00 Chalkida, Greece
[3]Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Dept of Agricultural Technology, School of Geotechnical Sciences, International Hellenic University, Alexander Campus, P.O. Box 141, 57 400 Sindos, Thessaloniki, Greece
Email: kmoutou@bio.uth.gr

Introduction

The reliance of European aquaculture on marine feed ingredients continues to be high and the dependence on wild caught fish cannot support the aim for sustainable doubling in production by 2030(Leduc et al., 2018).In the quest for highly nutritive and functional raw materials to efficiently replace fish meal, plant proteins represent a suitable protein alternative to fish meal and are increasingly being used in fish feed (Morais, 2011). However, plant-based diets have also been reported to impact fish metabolism by regulating the expression of genes involved in amino acid metabolism, lipid metabolism, carbohydrate metabolism, and immune function (Xu et al., 2018). In gilthead seabream (*Sparus aurata)* aquaculture, genetic selection has been applied to improve several key features such as growth and to allow the adoption of plant ingredients with minimal impact on growth and health (Perera et al., 2019). In this study, we examine the response of erythrocyte transcriptome to an experimental plant-rich across different genetic backgrounds in gilthead seabream, as shaped during a commercial breeding programme.

Materials and methods

Sampling took place in a commercial fish farm of Nireus Aquaculture SA (member of AVRAMAR Group), in Paleros, Greece. Fish originated from six full-sib families were tagged with an intraperitoneal chip and randomly divided into two sea cages, which were fed on different diets; a high plant-protein containing 85% plant proteins and a high animal-protein containing 30% of marine animal protein. Two samplings were performed at 15 and 30 days after the initiation of feeding trial. Blood was sampled by caudal puncture, transferred into sterile tubes, centrifuged to separate the plasma from erythrocytes, which were stored in RNAlater until RNA extraction.

Total RNA from erythrocytes of selected samples was extracted using the E.Z.N.A.® Total RNA Kit I. Samples were pooled in full-sib families and sent to Novogene Europe for RNA sequencing. In total 24 samples (6 families x 2 Feeds x 2 sampling days) were sent for creating sequencing libraries. The indexed libraries were sequenced on HiSeq 2000 platform (Illumina, USA) employing 150bp paired end reads. Quality control, assembly and annotation of the transcriptome was performed from Novogene Europe. For the validation of a number of differentially expressed genes a real time quantitive PCR was performed individually on the samples used for the transcriptome analysis.

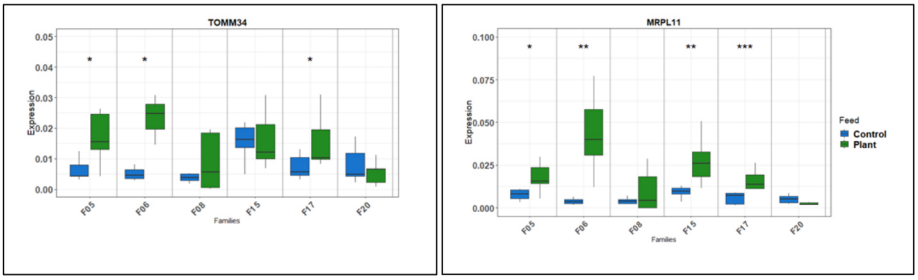


Figure 1. *Family-specific response of mitochondrial ribosomal protein 11 (MRPL11) and translocase of the outer mitochondrial membrane (TOMM34) in erythrocytes 15 days from feeding initiation.*

(Continued on next page)

Results and Discussion

A high number of differentially expressed genes were identified at all pairwise comparisons performed. Specifically, 2618 genes were upregulated, and 1312 genes were downregulated in all families fed on the plant-based diet. Genes with elevate transcription level were participating in biological processes as oxidative phosphorylation, proteasome, ribosomal proteins, and the supplement. On the contrary, a decreased gene transcription was observed on phospholipid synthesis and membrane cytoskeleton. Also, a high number of genes involved in immune responses were detected suggesting inflammatory response.

A differential expression analysis was also performed within each family. In total, 118 genes continued to emerge amongst the comparisons. A selection of genes with the higher p-value and the biggest log-foldchange was made for evaluating their expression through real time quantitative PCR. The detailed expression patterns revealed significant family-specific response of erythrocyte transcriptome to the plant-rich diet (Figure 1). In addition, the expression patterns differentiated between day 15 and day 30 after initiation of feeding, adding an extra dimension to the family-specific response.

Conclusion

Erythrocyte transcriptome offers a non-invasive way to explore the physiological consequences of novel raw materials in aquaculture towards increased sustainability. Moreover, the results of the study verify the potential of genetic selection towards the sustainability of aquaculture production.

Acknowledgements

This research was Co-financed by Greece and the European Union, European Maritime and Fisheries Fund in the context of the implementation of the Greek Operational Programme for Fisheries, Priority Axis "Innovation in Aquaculture", Project title: "Development of a novel method of genetic selection of farmed fish aiming at optimizing food conversion rate " (2018-2021) MIS 5010669.

References

Leduc, A., Zatylny-Gaudin, C., Robert, M., Corre, E., Corguille, G. Le, Castel, H., … Henry, J. (2018). Dietary aquaculture by-product hydrolysates: impact on the transcriptomic response of the intestinal mucosa of European seabass (Dicentrarchuslabrax) fed low fish meal diets. BMC Genomics 2018 19:1, 19(1), 1–20.

Morais, S., Pratoomyot, J., Taggart, J. B., Bron, J. E., Guy, D. R., Bell, J. G., &Tocher, D. R. (2011). Genotype-specific responses in Atlantic salmon (Salmo salar) subject to dietary fish oil replacement by vegetable oil: a liver transcriptomic analysis. BMC Genomics 2011 12:1, 12(1), 1–17.

Perera, E., Simó-Mirabet, P., Shin, H. S., Rosell-Moll, E., Naya-Catalá, F., de las Heras, V., … Pérez-Sánchez, J. (2019). Selection for growth is associated in gilthead sea bream (Sparus aurata) with diet flexibility, changes in growth patterns and higher intestine plasticity. Aquaculture, 507, 349–360.

Xu, W., Jin, J., Han, D., Liu, H., Zhu, X., Yang, Y., &Xie, S. (n.d.). Physiological and transcriptomic responses to fishmeal-based diet and rapeseed meal-based diet in two strains of gibel carp (Carassius gibelio). Fish Physiology and Biochemistry.

ELECTROSTUNNING EVALUATION IN TWO TEMPORAL PERIODS ON FISH FILLET DEGRADATION POST-HARVEST IN GILTHEAD SEABREAM (*Sparus aurata)*

Angelakopoulos R.[1], Dimitroglou A.[2], Kalemkeridou M.[1], Papaharisis L.[2], Moutou K.A.[1*]

[1]Laboratory of Genetics, Comparative and Evolutionary Biology, Department of Biochemistry and Biotechnology, School of Medical Sciences, University of Thessaly, Greece, Viopolis, Mezourlo, 41500, Larissa, Greece
kmoutou@bio.uth.gr
[2]Department of Research and Development, Nireus Aquaculture S.A. Chalkida, 34100, Evvoia, Greece

Introduction
Progressive loss of fish freshness post-mortem is attributed to complex combinations of biochemical and structural changes along with muscle spoilage caused by microbial activity (Tavares et al. 2021). Proteases that hydrolyze the collagen of the connective tissue (collagenases), and the muscle fibril proteins, predominantly cathepsins and calpains, hold a key role in this process (Sriket, 2014). The objective of this study was to evaluate the effect of electrostunning as an effective alternative harvest technique on the flesh quality of gilthead seabream (*Sparus aurata)*. How this effect differentiates with seasonal water temperature was also explored.

Materials and methods
Whole gilthead seabream (*Sparus aurata*) was harvested from net cages in Nireus Aquaculture SA (member of AVRAMAR Group) facilities and stored immediately in ice. Two different electrostunning settings were compared with the conventional harvest method (**CS**) of ice water; **HC**, high electric field 2V/cm and flow 1.6m/sec; **LC**, low electric field 1.8V/cm and flow 1.6m/sec. Sampling was performed in the same fish farm in Astakos (Aitoloakarnania, Greece) in August 2020 and February 2021.

The activity of major proteases, namely Calpain, Collagenase, Cathepsin B and L responsible for white muscle degradation was measured; a piece of white muscle was extracted from the fillet at slaughter (day 0) and on days 1, 2, 5, 7 and 13 post slaughter, snap-frozen in liquid nitrogen and stored at -80 °C until enzyme extraction. Crude enzyme extract was extracted according to Lakshmanan et al. (2005) with slight modifications. The activity of these enzymes was assayed by the Barrett and Kirschke (1981) method with minor refinements. Protein content in enzyme extracts was quantified with the Bradford (1976) method using bovine serum albumin as standard. Activity was expressed as fluorescence units (FU) change per minute per mg protein.

Results
At sampling, the average water temperature was 25°C in August 2020 (Hot) and 15.5°C in February 2021 (Cold). Calpain exhibited the highest levels of activity of all the proteolytic enzymes measured in both seasonal periods. In Cold period, HC group exhibited the lowest enzymatic activity amongst all proteolytic enzymes, whereas LC elicited an early activation. These differences between LC and HC groups were statistically significant (Cathepsin L p-value<0.05 Cathepsin B, Collagenase and Calpain with p-value<0.001). On the contrary, in Hot period a mixed activation pattern was observed. Calpain and Collagenase activity in HC group recorded an early high activation pattern in comparison with the LC group (p-value<0.05). Although, a late peak in enzymatic activity of both cathepsins was observed in HC group in comparison with the LC group, statistically significant differences were observed only in cathepsin B activities (p-value<0.05) A significant correlation amongst enzymatic activities was observed across slaughter methods and water temperatures.

Discussion and conclusion
In both seasonal periods, calpain and collagenase activities showed an early activation post-mortem in accordance with previous studies (Ntzimani et al. 2021; Bao et al. 2020). Significant temporal differences in all enzyme activities were recorded in either water temperature, and different harvest methods. Fish being poikilothermic animals, cannot maintain stable temperature and its physiology is directly affected by water temperature changes. This may be a decisive factor in deciding which electrostunning settings will be used in each temporal period.

(Continued on next page)

Acknowledgements
This research was Co-financed by Greece and the European Union, European Maritime and Fisheries Fund in the context of the implementation of the Greek Operational Programme for Fisheries, Priority Axis "Innovation in Aquaculture", Project title "Development and industrial scale evaluation of an innovative humane slaughter system and assessment of welfare in aquaculture marine fish species" MIS 5010690.

References

Bao, Y., Wang, K., Yang H., Regenstein, J.M., Ertbjerg, P., Zhou, P. (2020). Protein Degradation of Black Carp (*Mylopharyngodon Piceus*) Muscle during Cold Storage. Food Chemistry 308

Barrett, A. J., Kirschke, H. (1981). Cathepsin B, cathepsin H, and cathepsin L. Methods in Enzymology, 80, 535–561. https://doi.org/10.1016/S0076-6879(81)80043-2

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry 72 (1–2): 248–54.

Caballero, M. J., Betancor, M., Escrig, J. C., Montero, D., Espinosa de los Monteros, A., Castro, P., … Izquierdo, M. (2009). Post mortem changes produced in the muscle of sea bream (*Sparus aurata*) during ice storage. Aquaculture, 291(3–4), 210–216.

Lakshmanan, R., Patterson, M., Piggot, J. (2005). Effects of High-Pressure Processing on Proteolytic Enzymes and Proteins in Cold-Smoked Salmon during Refrigerated Storage. Food Chemistry 90 (4): 541–48.

Ntzimani, A., Angelakopoulos, R., Semenoglou, I., Dermesonlouoglou, E., Tsironi, T., Moutou, K.A., Taoukis, P. (2021). Slurry Ice as an Alternative Cooling Medium for Fish Harvesting and Transportation: Study of the Effect on Seabass Flesh Quality and Shelf Life. Aquaculture and Fisheries, March.

Perez-Won, M., Lemus-Mondaca, R., Herrera-Lavados, C., Reyes, J. E., Roco, T., Palma-Acevedo, A., … Aubourg, S. P. (2020). Combined Treatments of High Hydrostatic Pressure and CO2 in Coho Salmon (Oncorhynchus kisutch): Effects on Enzyme Inactivation, Physicochemical Properties, and Microbial Shelf Life. Foods 2020, 9: 273.

Sriket (2014). Proteases in fish and shellfish: Role on muscle softening and prevention. International Food Research Journal, 21(1), 433–445.

Tavares, J., Martins, A., Fidalgo, L.G., Lima, V., Amaral, R.A., Pinto, C.A., Silva, A.M., Saraiva, J.A. (2021). Fresh Fish Degradation and Advances in Preservation Using Physical Emerging Technologies.

Zhang, X., Xie, J. (n.d.). The differential effects of endogenous cathepsin and microorganisms on changes in the texture and flavor substances of grouper (*Epinephelus coioides*) fillets. RSC Advances.

DEEP LEARNING FISH IN TANK BEHAVIOR MONITORING

O. Movchan*, P. Cisar, D. Bekkozhayeva

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, Institute of Complex Systems, Zámek 136, 373 33 Nové Hrady, Czech Republic
Email: omovchan@jcu.cz

Introduction

Fish behavior activity is complex activity, which is traditionally determined by human experts, telemetric tags, flow through chamber of by tracking of the individual fish using computer vision. Several methods of fish behavior monitoring have been developed for different reasons. The most promising and cheap method is computer vision fish tracking. It provides an automated, non-invasive, and cost-effective methods of recording behavioral parameters. Single camera or multi-camera 3D machine vision has been used in behavior analysis or trail tracking (Saberioon and Cisar, 2016). The visible or near infrared light is used for fish monitoring (Farokhi et al., 2016, Pautsina et al., 2015). The output of the monitoring systems is the 3D trajectory of the fish which represent the fish swimming during the given period. The main issue of the monitoring systems is the tracking of the fish in the tanks with high fish density. The fish overlapping enables to monitor individual fish behavior just for very limited time. Therefore, some studies focus on the parametrization of the behavior of the fish school instead of the individuals.

Application of the modern deep learning methods (Nichols et al., 2019) could solve the problem of fish behavior parametrization without the need to detect and track individual fish. To be able to optimize the feeding we have to distinguish (classify) different states of the fish based on the analysis of the behavior. The deep learning concept will be used to automatically parametrize fish behavior and classify it into predefined classes. The research is focused on the feeding optimization in intensive aquaculture and specially on the recirculation aquaculture systems (RAS) represented by the fish cultivation tanks. This focus is given by the possibility to control all conditions of the fish cultivation and precise control of fish feeding and feed distribution in the tank.

Materials and methods

The initial experiments were focused on the classification of Sea bass behavior into 3 classes: normal, stress (caused by knocking on the tanks) and feeding, Fig. 1. The video records of fish swimming in the standard cultivation tanks (3 tanks) were collected. The short videos (4 minutes) of all three types of behavior were collected using 45 fish. Video data were cut into 3 seconds clips and resized to 228 by 228 pixels. Only the inner area of fish tank was used for processing. Totally 210 sequencies were obtained for each class and 100 randomly selected sequencies were used for classification validation. for The GoogleNet feature extractor (Szegedy, 2015) was used for data processing.

Each frame of video was transformed into 1024 features and stored in vectors in sequence of 45 rows (3 second of 15 fps video). After preprocessing, feature vectors sequencies were feed to Long Short-Term Memory (LSTM) network to process the sequential data and perform the classification.

Results

The classification accuracy after 30 epochs of training was 99%, such high accuracy shows that classes are significantly different and easily distinguishable by the network. On validation dataset accuracy was 99% that also show that our network was not overfeed.

Discussion

In this study we proved that we could distinguish between different types of fish behavior using the concept based on deep learning. The concept avoids the detection and tracking of individual fish in the tank. The disadvantage of the approach is generalization of the solution. The system is specific for the fish species and for the cultivation tanks used for network training. The similarity of cultivation tanks (mainly circular shape) and possibility to make specific models for fish species will enable to use the concept for general fish behavior monitoring. We started new experiments for common carp with more classes of fish behavior to study the possibility of feeding optimization and fish welfare determination.

(Continued on next page)

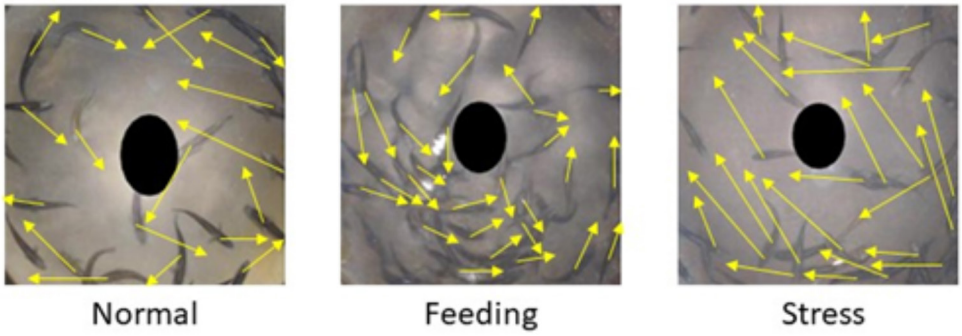


Fig. 1 Example of tree behavior classes: left – normal behavior where the fish swim all directions, middle – feeding behavior where the fish search for the food, right - stress behavior where the fish swim out of the noisy place.

References

Farokhi et al., 2016, S. Farokhi, J. Flusser, U. Ullah Sheikh, Near infrared face recognition: a literature survey, Comp. Sci. Rev., 21 (2016), pp. 1-1

Pautsina et al., 2015, A. Pautsina, P. Císař, D. Štys, B.F. Terjesen, Å.M.O. Espmark, Infrared reflection system for indoor 3D tracking of fish, Aquacult. Eng., 69 (2015), pp. 7-17

Saberioon and Cisar, 2016, M.M. Saberioon, P. Cisar, Automated multiple fish tracking in three-dimension using a structured light sensor, Comp. Electron. Agricult., 121 (2016), pp. 215-221

Nichols, J.A., Herbert Chan, H.W. & Baker, M.A.B. Machine learning: applications of artificial intelligence to imaging and diagnosis. Biophys Rev 11, 111–118 (2019). https://doi.org/10.1007/s12551-018-0449-9

Szegedy et al., 2015, W. Liu, Y. Jia Y., "Going deeper with convolutions." In Proceedings of the IEEE conference on computer vision and pattern recognition, pp. 1-9. 2015.

A METHODS COMPARISON FOR PERACETIC ACID DETERMINATION

Megan Murray[1*], Natalie Redman[1], Christopher Good[1], Anna DiCocco[1], Curtis Crouse[1], Travis May[1]

[1]The Conservation Fund Freshwater Institute, Shepherdstown, WV 25443, USA
Email: mmurray@conservationfund.org

Introduction

Due to its rapid reaction time with organics and potent efficacy as an oxidative disinfectant, the usefulness of peracetic acid (PAA; chemical formula $C_2H_4O_3$) has been demonstrated in a range of industrial applications. Previously, the antimicrobial, bactericidal, virucidal, fungicidal, and sporicidal properties of PAA have been exploited for highly effective wastewater treatment (Kitis, 2004). More recently, PAA has been used to improve water quality in recirculation aquaculture systems (RAS) without disturbing the microbial composition and nitrification function of biofilters (Suurnäkki et al., 2020). While PAA has not yet been approved by the U.S. Food and Drug Administration for use in aquaculture in the United States, PAA has been approved in Europe for use in aquaculture systems and by the U.S. Environmental Protection Agency (EPA) for use as an aquaculture surface disinfectant (e.g. tanks and pipes) when food fish are not present (DiCocco et al., 2021). With increasing use of PAA as an alternative to other therapeutants and disinfectants, having a rapid and accurate method for PAA concentration analysis is very useful. This comparison includes two methods: (i) CHEMetrics Peracetic Acid Test Kit — Vacu-vials Instrumental Kit and (ii) Hach Total Chlorine Method 8167. The CHEMetrics Peracetic Acid Test Kit features self-filling ampoules for the colorimetric analysis of PAA, 0-5.00 ppm. The kit employs DPD (N,N-diethyl-p-phenylenediamine) chemistry. The sample is treated with an excess of potassium iodide, PAA oxidizes the iodide to iodine, then iodine oxidizes DPD to form a pink coloured species in direct proportion to the PAA concentration. Hach Total Chlorine Method 8167 uses the same DPD chemistry process. This method features a powder pillow reagent added to a glass vial along with the option of self-filling ampoules. For this comparison, the power pillow variation was used. Results through this method are given as 0.02-2.00 mg/L Cl_2 and used to calculate mg/L PAA with a multiplier of 1.07. Both methods require use of a colorimeter or spectrophotometer.

Materials and methods

This comparison was conducted by reviewing and analyzing multiple factors of each method. To analyze the accuracy of both methods, four different standard solutions of PAA were created using a commercial product containing 15% PAA and 10% H_2O_2. Each standard was then tested in triplicate using both methods. Results were compared to the target standard solution for accuracy. Standard solutions were neutralized using sodium thiosulfate before disposal. Other factors taken into consideration for comparison include time and effort required, cost, and disposal.

Results & Conclusions

After comparison of accuracy, time and effort required, cost, and disposal, the CHEMetrics vacu-vials were superior only in the time and effort required by the method. The vacu-vials had a reaction time of only one minute, where the Hach Total Chlorine Method 8167 reaction time was three minutes. The steps required for the vacu-vials were also less, making the process more streamlined and easier to accomplish in a shorter time than the Hach Total Chlorine Method 8167. The vacu-vials covered a wider range of PAA concentration, eliminating any need for dilution for a concentration less than 5.0 mg/L of PAA. Otherwise, the two methods were comparable in terms of accuracy and disposal requirements. The average accuracy of the CHEMetrics vacu-vials being 97% and the Hach Total Chlorine Method 8167 being 95%. The Hach Total Chlorine Method 8167 was more economical in the upfront cost with $0.23 per powder pillow, while the CHEMetrics test was $1.06 per vacu-vial.

References

DiCocco, Anna, et al. “Reducing mortality associated with opportunistic infections in Atlantic salmon Salmo salar fry using hydrogen peroxide and peracetic acid.” *Aquaculture Research* (2021).

Kitis, Mehmet. “Disinfection of wastewater with peracetic acid: a review.” *Environment international* 30.1 (2004): 47-55.

Suurnäkki, Suvi, et al. “The effect of peracetic acid on microbial community, water quality, nitrification and rainbow trout (Oncorhynchus mykiss) performance in recirculating aquaculture systems.” *Aquaculture* 516 (2020): 734534.

OPTIMIZATION OF PRODUCTIVITY, FATTY ACID AND FUCOXANTHIN CONTENT OF *Chaetoceros muelleri* AND *Thalassiosira pseudonana* IN THE PROVIAPT FLAT-PANEL PHOTOBIOREACTOR

M. Muys[1,*], L. Roef[1], M. Wille[2], V. Vermeylen[2], M. Michiels[1]

[1] Proviron Holding NV, George Gilliotstraat 60, 2620 Hemiksem, Belgium.
[2] Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Coupure Links 653, 9000, Gent, Belgium
Mailing address: Maarten.Muys@Proviron.com

Introduction

The production of microalgae with a stable and high nutritional quality within hatcheries and nurseries of crustaceans and bivalves is still a major bottleneck. Culture conditions are often suboptimal, reducing culture density, productivity and resulting in lower and unstable nutritional microalgae quality. Furthermore, contamination often spoils the product and culture crashes reduce hatchery yields, all increasing the operational costs. Finally, regular photobioreactors are often unsuitable for the cultivation of sensitive microalgae species such as *Chaetoceros* sp., because of induced shear stress, again resulting in low productivities and a reduced nutritional quality.

Proviron (Hemiksem, Belgium) has developed, engineered and patented a closed photobioreactor system for indoor cultivation of microalgae, named ProviAPT (Proviron Advanced Photobioreactor Technology), which is particularly suited for the mass production of high quality algae biomass for aquaculture purposes. One ProviAPT production reactor features a total of 35 vertical flat panels and a volume of 220 Liters, made out of a feed-grade plastic film material, illuminated artificially by means of LED lights. The vertical stacked reactor configuration allows high areal productivities and the specific reactor geometry allows operation both at low pressure gradients and at reduced airflows. Other than resulting in major cuts in energy cost for aeration, this geometry provides gentle cultivation conditions allowing the system to host sensitive, recalcitrant species. Rigorous control and standardization of the operational parameters results in a product of constant and high quality.

The presented work includes: (1) the optimization of productivity, lipid content, fatty acid profile and fucoxanthin (bioactive carotenoid) content in a lab-scale proviAPT reactor by means of light conditions (wavelength mixing ratios) and dilution rate; and (2) the validation of these laboratory scale observations at full production scale.

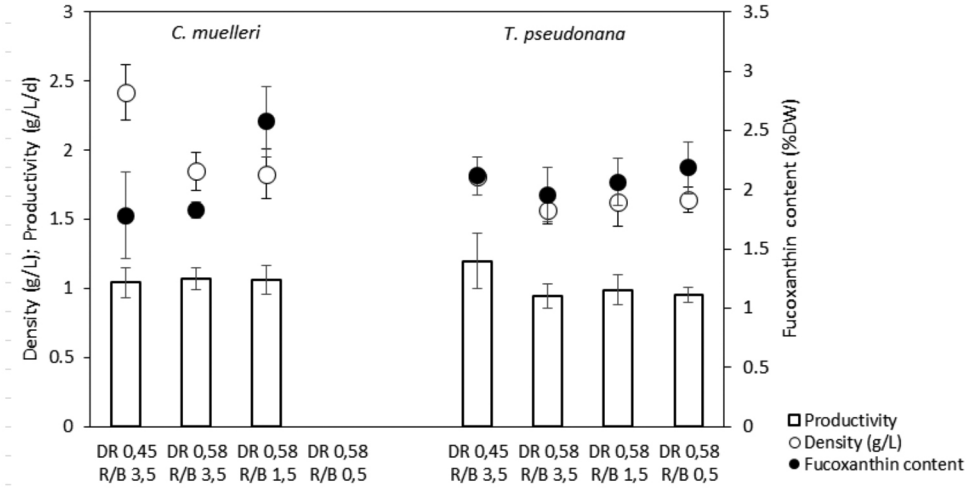


Figure 1. Influence of light conditions (R/B: wavelength ratio red-to-blue) and dilution rate (DR: dilution rate) on the productivity and fucoxanthin content (%DW) of *C. muelleri* and *T. pseudonana* (data at DR 0.58 R/B 0.5 for *C. muelleri* to be determined).

(Continued on next page)

Materials and methods

Chaetoceros muelleri (CCAP 1010/3) and *Thalassiosira pseudonana* (CCAP 1085/12), two diatoms with importance in larval feeds, were cultivated in 6.4 Liter lab-scale ProviAPT photobioreactors in semi-continuous operation mode, with a feed cycle of 12 hours. A temperature of 20.5 ± 1°C, CO_2 concentration of 0.5 %(v/v) and a salinity of 25 ppm was applied, while light intensity was kept at 473 μmol photons/m^2/s in a 20:4h day-night regime. Tested operational conditions are presented in figure 1. Temperature, pH, salinity and cell density were logged daily. After a minimum of 7 days in equilibrium conditions, microalgae biomass was harvested and stored at -20°C upon freeze-drying and analysis for ash fraction, total lipids, fatty acid profile and fucoxanthin content. Subsequently, lab-scale observations were validated at industrial scale in one reactor battery counting 6 production units, each composed out of 16 proviAPT reactors of 220 Liter, containing 21 m^3 of microalgae culture. Hereby, the laboratory scale

ProviAPT reactor of 6.4 Liters is an exact copy of 1 of the 35 flat panels of a full-scale reactor with a volume of 220 Liters.

Results and discussion

For both, *C. muelleri* (CM) and *T. pseudonana* (TP), enriching the light in the blue spectrum over a red-to-blue ratio (R/B) from 3.5 to 0.5 or increasing the dilution rate (DR) from 0.45 to 0.58, did not influence the average growth rate significantly with a value of 1.06 ± 0.02 g/L/d for CM, compared to 1.02 ± 0.12 g/L/d for TP (Figure 1). In contrast, for CM, fucoxanthin content increased significantly with 44% (from 1.8 ± 0.4 to 2.6 ± 0.3 %DW) when the light was enriched with blue wavelengths (R/B from 3.5 to 1.5). Fucoxanthin content of TP was not influenced by the color of light, presenting a stable value of 2.1 ± 0.1 %DW. An increase in dilution rate, resulting in a decrease in biomass density, was not observed to influence fucoxanthin content.

Enriching the light in the blue spectrum (from a red-to-blue ratio of 3.5 to 1.5) resulted in an increase in total fatty acid content from 55 to 68 mg FAME/g AFDW for TP, with a significant increase in eicosapentaenoic acid (EPA; C20:5n-3) from 1.3 to 2.0%AFDW. Similarly, docosahexaenoic acid (DHA; C22:6n-3) increased from 0.19 to 0.27%AFDW.

The full fatty acid profile and total lipid content for both microalgae species will also be presented, as well as the validation of laboratory scale observations in production scale proviAPT photobioreactors.

Conclusion

The presented findings indicate that light conditions (e.g. decreasing the mixing ratio red-to-blue) can be tuned at industrial scale microalgae production facilities to increase the fatty acid, EPA and DHA content of the diatom *T. pseudonana* and fucoxanthin content, a promising bioactive compound in larval feeds, of *C. muelleri*. Furthermore, the suitability of the proviAPT laboratory scale photobioreactor was verified to maintain a constant microalgae productivity with a stable nutritional value over time under fixed operational conditions, while it was found that optimized growth conditions at laboratory scale can be applied at industrial scale.

Acknowledgements

This study is part of the BlueMarine^3.Com project funded by the Flemish government through Flanders Innovation and Entrepreneurship (VLAIO) and is facilitated by the Blue Cluster program.

THE APPLICATION OF NACL IN PIKE-PERCH AQUACULTURE TAKING INTO ACCOUNT ONTOGENY

Christopher Naas * [ab], Werner Kloas [b], Andreas Müller-Belecke [a]

a Institute of Inland Fisheries in Potsdam-Sacrow, Im Königswald 2, 14469 Potsdam
b Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin
E-Mail: christopher.naas@ifb-potsdam.de

The optimal salinity for rearing juvenile pike-perch (*Sander lucioperca*) in recirculating aquaculture systems (RAS) is 3g NaCl L^{-1}. Hereby, an increased biomass gain and feed utilization can be expected. However, throughout pike-perch's development from egg to yearling, fundamental morphological and physiological but also major nutritional changes take place. These alterations are likely to affect the optimal salinity of this species when reared in RAS.

In a set of different experiments, we investigated the performance of five different life stages of pike-perch exposed to different NaCl concentrations - and where possible - under RAS or RAS-like conditions, respectively. First, a standardized fish egg test was conducted in order to investigate the embryonic development of pike-perch considering NaCl (0 to 12g NaCl L^{-1}). Free-swimming and feeding larvae (0.8mg body weight (bw)) and dry-feed adapted pike-perch (0.4 g bw) were exposed to NaCl (0 to 9g NaCl L^{-1}) in static exposures. Furthermore, pike-perch fingerlings (30g bw) and yearlings (100g bw) were reared in RAS under different saline conditions (0 to 12g NaCl L^{-1}). Based on aquaculture performance parameters (growth, feed efficiency), as well as further welfare indicators an optimal NaCl concentration for every life stage was determined.

Pike-perch's embryonic development was not affected at concentrations up to 6g NaCl L^{-1}. Beneficially, the exposure to NaCl significantly reduced fungal infections during the incubation. Free-swimming and artemia-feeding larvae grew best under 6g NaCl L^{-1}, but did not tolerate 9g NaCl L^{-1}. Dry-feed adapted pike-perch showed best growth at 3g NaCl L^{-1} with a clear decrease in performance when NaCl concentrations increased. Also, pike-perch fingerlings showed best performance under 3g NaCl L^{-1}, but displayed growth depression at 6 and 9g NaCl L^{-1} and did not tolerate 12g NaCl L^{-1} at all. Pike-perch yearlings can be reared up to 6g NaCl L^{-1} without negative effects on their growth performance.

Consequently, the optimal NaCl concentration varies with the ontogenetic development of pike-perch. Depending on the life stage, 3 to 6g NaCl L^{-1} is optimal for pike-perch in RAS and can thus safely be applied by fish farmers. In the context of available literature, an increase of tolerance towards salts with an increase in biomass can be derived.

THE COMPARISON OF SPERM MOTILITY AND DENSITY IN FOUR DIFFERENT GOLDFISH (*Carassius auratus*) TYPES

Borbála Nagy*[1], Gergely Bernáth[1], Levente Várkonyi[1], József Molnár[1], Levente Zete Láng[1], Tibor Izsák[1], Tamás Bartucz[1], István Ittzés[2], Áron Ittzés[2], Béla Urbányi[1], Bokor Zoltán[1]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1., H-2100 Gödöllő, Hungary
[2]Self- entrepreneur („Dunamenti Aranyhalak"), H-2440 Százhalombatta, Hungary
E-mail: nagy.borbala@uni-mate.hu

Introduction

Different goldfish types play an important role both in ornamental fish farming and science (Ahmadmoradi et al. 2012). Considering its historical background the goldfish is a suitable model animal for the study of artificial selection as well as for developmental biological studies (Li et al. 2019). Chilled storage of sperm can contribute to the genetic research, artificial reproduction, and shipping or trade of valuable samples (Glenn et al. 2011). Sperm motility and cell density is an important parameter in determining sperm quality. Both parameters can directly affect the fertilization rate in fish (Fauvel et al. 2010). Difference was observed in case of the chilled sperm storage of three goldfish variants in the previous study of Bernáth et al. 2017.

Materials and methods

The aim of our study was to examine the effects of different goldfish types on the sperm quality. The males were kept in a recirculating system at 22 °C. The spermiation was hormonally induced using 2 mg bodyweight kg^{-1} of carp pituitary 24 hours prior to sampling. Sperm motility of four different goldfish types (Common goldfish-"wild type" *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5) was compared during 60 hours (at 12-hour intervals) of refrigerated storage (4 °C). In our experiments, progressive motility (pMOT, %), curvilinear velocity (VCL, μm/s), linearity (LIN, %), amplitude lateral Head Displacement (ALH, μm), Beat Cross Frequency (BCF, Hz), straight line velocity (VSL, %) in various types were analysed using a CASA (Computer-assisted Sperm Analysis) system. Sperm cells were activated in an activating solution for cyprinids by adding 0.01 g ml^{-1} bovine serum albumin (BSA). In addition, sperm density in the four variants was also investigated. Sperm samples of the goldfish types were diluted in a sugar-based extender at a ratio of 1:999 and were loaded into a Bürker-type haemocytometer.

Results

Based on the examined CASA parameters, significant difference was not observed between the variants, as well as significant decrease was not recorded during 60 hours of storage in neither of them. Furthermore, a similar cell concentration was determined among the variants (Common goldfish $2{,}01x10^{10} \pm 3{,}46x10^{9}$; Shubunkin $1{,}71x10^{10} \pm 3{,}25x10^{9}$; Black Moor $1{,}66x10^{10} \pm 3{,}02x10^{9}$; Oranda $1{,}56x10^{10} \pm 5{,}83x10^{9}$).

Discussion and conclusion

According to our results the chilled storage for few days is applicable in the case of the four investigated goldfish types. Although statistically significant difference in cell density was not observed, a variability among the types was observable. Our results can contribute to the improvement of the common hatchery propagation and the investigation of the possible effects of the artificial selection on the reproductive biology in different goldfish types.

Acknowledgements

This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12). The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

References

Ahmadmoradi, E., Rezaie, A., & Mousavi, S. M. (2012). Histopathological study of the kidney, liver and intestine tissues in goldfish (*Carassius auratus*) and angelfish (*Pterophyllum sp*.). Aquaculture, Aquarium, Conservation & Legislation, 5(4), 282-288.

Bernáth, G., Ittzés, I., Szabó, Z., Horváth, Á., Krejszeff, S., Lujić, J., Várkonyi, L., Urbányi, B., & Bokor, Z. (2017). Chilled and post-thaw storage of sperm in different goldfish types. Reproduction in Domestic Animals, 52(4), 680-686.

Fauvel, C., Suquet, M., & Cosson, J. (2010). Evaluation of fish sperm quality. Journal of Applied Ichthyology, 26(5), 636-643.

Glenn III, D. W., Lang, R. P., & Tiersch, T. R. (2011). Evaluation of extenders for refrigerated storage of koi carp and goldfish sperm. Cryopreservation in aquatic species, 2, 107-124.

Li, I. J., Lee, S. H., Abe, G., & Ota, K. G. (2019). Embryonic and postembryonic development of the ornamental twin-tail goldfish. Developmental Dynamics, 248(4), 251-283.

DEVELOPMENT OF A MEDIUM DENSITY SNP-ARRAY FOR THE BLUE MUSSEL SPECIES-COMPLEX

Jennifer C. Nascimento-Schulze* [1,2], Tim P Bean[3], Bonnie A Fraser[2], Ross D Houston[3], Josephine R Paris[2], Carolina Penaloza[3], Matthew B Sanders[1] James R Whiting[2] and Robert P Ellis[2]

[1]University of Exeter, EX4 4PS, Exeter (United Kingdom)
[2]Centre for Environment Fisheries and Aquaculture Sciences, DT4 8UB, Weymouth (United Kingdom)
[3]The Roslin Institute, University of Edinburgh, EH25 9RG Midlothian (United Kingdom)
E-mail: jn378@exeter.ac.uk

Background

Blue-mussels from the Mytilus species-complex (*M. edulis* x *M. trossulus* x *M. galloprovincialis*) are an abundant component of the benthos community, found in the high latitude habitats. Aside from their ecological value (Norling & Kautsky, 2007), these foundation species are of extreme relevance to the aquaculture industry, with 2 149 534 tonnes produced globally in 2019 (FAO, 2021).

Blue mussels are able to withstand a wide range of environmental conditions and significant effort has been made to understand the physiological consequences of environmental stress in this species (e.g Lesser, 2016; Melzner, 2011; Riisgard, 2013). Our understanding on the genomic mechanisms underlying species distribution, hybridisation and local adaptation remains incomplete, as the development of genetic resources began to be emphasized in recent years. To help overcome this, we developed a novel genomic tool, a medium-density SNP array, for rapid, high-throughput genotyping of individuals in the *Mytilus* species complex. The array contains globally polymorphic SNPs, which capture the genetic diversity present in mussel populations thriving across a gradient of diverse environmental conditions (e.g. temperature, salinity and CO_2), and showing high population divergence in response to habitat, facilitating the investigation of genomic structure in this species complex. Whilst genetics and breeding technologies have yet to be widely applied to improve blue mussel aquaculture production. This multipurpose tool can also benefit shellfish aquaculture, facilitating genome wide association studies (GWAS) for key production traits, and testing genomic selection approaches.

Material and Methods

For the creation of the array, 23 *Mytilus* spp populations were sampled from across their global distribution, incorporating 4 species in this complex (*M. edulis*, *M trossulus M. galloprovincialis* and *M. chilensis*), as well as hybrids. Populations were selected based on prevailing environmental conditions to capture habitat diversity (e.g. upwelling regions, low salinity regions; Figure 1), and subsequently genetic diversity associated with local adaptation. Samples were sequenced using a low coverage (4-6x) whole-genome resequencing approach, with sequences aligned to the *Mytilus galloprovincialis* genome prior to filtering and calling of SNPs. In addition, existing sets of published and validated SNPs were included on the array as informative for speciation, as well as diagnosis of transmissible cancer. The final SNP set was selected and incorporated on the in medium-density SNP-array, developed in collaboration with Affymetrix.

Results and future perspectives

In this study, we have identified SNPs representative of global and within species diversity and explored levels of introgression in *Mytilus* spp populations distributed worldwide. A subset of 60K SNPs was used to develop a SNP array the *Mytilus* species complex. This tool will allow the consistent, fast and affordable genotyping of individuals, facilitating the investigation of ecological and evolutionary processes in these taxa. Unravelling global genetic diversity in *Mytilus* spp populations will contribute to our understanding on ecological and biological processes ongoing in these taxa. The applications of this array extends to shellfish aquaculture, contributing to the optimisation of this industry via (a) genomic selection of blue mussels,; (b) parentage assignment; (c) inbreeding level assessment and (d) species/product identification and provenance.

Subsequently, we will undertake analyses to assess to the heritability and mechanisms of stress tolerant phenotypes, as well as conserved mechanisms of adaptation/divergence among populations inhabiting challenging environments. By combining ‘omic approaches with physiology, we can investigate the genetic basis of phenotype and characterise animals resilient to environmental stress. Such research is especially relevant to safeguard aquaculture production under climate change.

(Continued on next page)

References

FAO (2021). Global production statistics (http://www.fao.org/figis/servlet/TabSelector). Visited on 12 May 2021.

Lesser, MP (2016), Climate change stressors cause metabolic depression in the blue mussel, Mytilus edulis, from the Gulf of Maine. Limnol. Oceanogr., 61: 1705-1717. https://doi.org/10.1002/lno.10326

Melzner F, Stange P, Trübenbach K, Thomsen J, Casties I, et al. (2011) Food Supply and Seawater pCO2 Impact Calcification and Internal Shell Dissolution in the Blue Mussel Mytilus edulis. PLOS ONE 6(9): e24223. https://doi.org/10.1371/journal.pone.0024223

Norling P & N Kautsky N (2007). Structural and functional effects of Mytilus edulis on diversity of associated species and ecosystem functioning. Marine Ecology-progress Series - MAR ECOL-PROGR SER. 351. 163-175. 10.3354/meps07033.

Riisgård, H.U., Lüskow, F., Pleissner, D. et al. Effect of salinity on filtration rates of mussels Mytilus edulis with special emphasis on dwarfed mussels from the low-saline Central Baltic Sea. Helgol Mar Res 67, 591–598 (2013). https://doi.org/10.1007/s10152-013-0347-2

IMMUNE TRANSCRIPTOME RESPONSES TO BACTERIAL AND VIRAL STIMULATION IN ATLANTIC SALMON (*Salmo salar*)

S. Naseer*, D.J. Macqueen and S.A.M. Martin

Fish Immunology Research Centre, University of Aberdeen, UK
Roslin Institute, University of Edinburgh, UK
E-mail: shahmir.naseer.19@abdn.ac.uk

Introduction

Infectious disease represents the most pressing biological threat to modern aquaculture at a global scale. Control of disease outbreaks is essential for maintaining fish health and welfare, which is crucial for sustainable and profitable aquaculture production. The work presented here is part of the EU Horizon 2020 project, AQUA-FAANG, which aims to improve understanding of genome functional regions and genotype-to-phenotype pathways in the six most commercially important European aquaculture fish species, with a major focus on dissecting immune responses and the basis for disease resistance. The outcomes of this project will contribute towards sustainability and competitiveness in European aquaculture in addition to cross-national food security.

Our focus within AQUA-FAANG is Atlantic salmon, which represents a major food production species globally and an excellent source of protein, omega 3 oils and micronutrients. One of the goals of our research is to examine immune transcriptomic responses using both *in vivo* and *in vitro* models that mimic viral (poly I:C) and bacterial (formalin killed *Vibrio)* infections. RNA-Seq was thus performed on head kidney tissue (*in vivo*) as the primary lymphoid organ and from primary cell cultures of head kidney leucocytes.

Material and Methods

Juvenile freshwater Atlantic salmon with an average weight of 50g were used. For *in vivo* stimulation, fish were stimulated intraperitoneally with *Vibrio* extract (n=6), poly I:C (n=6), or PBS (n=6, control) and sampled 24hr later. For the *in vitro* experiment, head kidney leukocytes were extracted by percoll gradient separation, then incubated for 24 hr with the same three immune treatments (n=6 each).

For transcriptomic analysis paired end 150bp sequencing was performed using an Illumina NovaSeq 6000. The Nf-core (Ewels, *et al*., 2020) RNA-seq pipeline was used for analysis. Following trimming and removal of low quality reads the sequences were mapped to the new Atlantic salmon reference genome (Ssal_v3.1 (GCA_905237065.2)) using STAR. FeatureCounts was used to generate read count for the annotated genes before DESeq2 (Love *et al*., 2014) was used to identify differentially expressed transcripts.

Results

36 RNA-seq libraries were sequenced (~30M paired end 150bp reads per sample) and the resulting transcriptomes showed a strong clustering of samples by treatment (Figure 1). The number of differentially expressed genes (adjusted $P<0.05$) per treatment is shown in Table 1. We are currently comparing the responses of the leukocytes to the *in vivo* head kidney responses, where other cell types are present. When both *in vivo* and *in vitro* responses to the stimulants are examined we find many common responses for each stimulant, along with some unique responses (Table 1). Further downstream analysis of the data will be presented in this poster.

(Continued on next page)

Table 1: Differential gene analysis between different immune stimulants in Atlantic salmon. Genes differentially expressed in common between in vivo and in vitro for each stimulant Poly I:C and vibrio extract) are shown. Adjusted p-value cut-off was set at 0.05.

	Up regulated	**Down regulated**
Poly I:C vs control	1302	233
Vibrio vs control	4459	4392

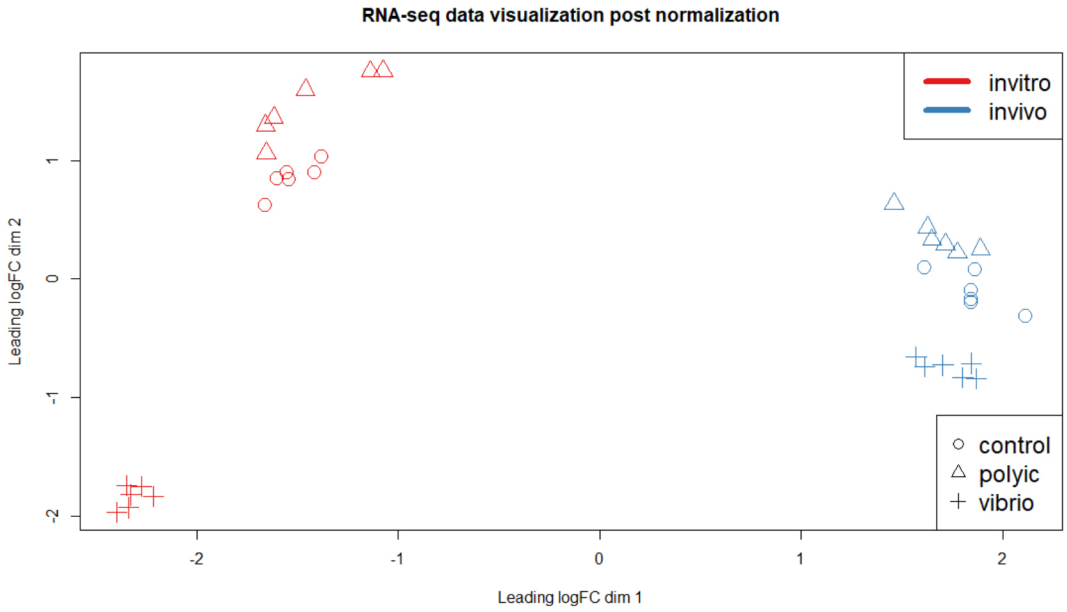


*Figure 1: **MDS plot of RNA-seq counts generated using featureCount and normalised using DESeq2**. Different clustering patterns can be seen between two conditions (in vitro vs in vivo) and different treatment groups (control, poly I:C and Vibrio).*

Acknowledgements: The AQUA-FAANG project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement 817923.

References

Ewels, P.A., Peltzer, A., Fillinger, S., Patel, H., Alneberg, J., Wilm, A., Garcia, M.U., Di Tommaso, P. and Nahnsen, S., 2020. The nf-core framework for community-curated bioinformatics pipelines. Nature biotechnology, 38(3), pp.276-278.

Love, M.I., Huber, W. and Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*(12), pp.1-21.

SUSTAINABLE MICROALGAE PROTEIN FOR AQUAFEEDS OF THE FUTURE

João Navalho[1*], Patrícia Diogo[1], Benjamin Schmid[1], Ana Coelho[1], Hugo Pereira[2], Alexandre Rodrigues[1], Victória del Pino[1]

[1] Necton SA, Olhão, Portugal
[2] GreenCoLab, University of Algarve, Faro, Portugal
* Correspondence: jnavalho@necton.pt

Aquaculture is one of the most relevant sources of protein to fulfil a constant growing human population. However, it is urgent to overcome the lack of sustainability of protein production sources, since the demand for high-quality protein, and the depletion of wild fishstocks are forecast to increase aquacultural fish demand by 37% between 2016-2030[1]. One of the most promising protein biomass sources for aquafeeds is microalgae, due to its high nutritional quality and high production sustainability[1]. Nevertheless, microalgae industrial production still requires optimizations to improve the sustainability of its production. For that purpose, an integrated strategic plan is essential, considering the most relevant bottlenecks of this area such as the energetic use, water supply and nutrient sources. PROFUTURE is a H2020 research project focused on improving productivity, sustainability and marketability of microalgae protein for innovative foods and feeds. It counts with 31 partners, 21 of which industrial, from 13 different European countries. Currently under the scope of PROFUTURE project, several biotechnological optimization steps in the protein production pipeline are being performed, including microalgae cultivation, drying, and processing. The ultimate objective is to develop novel foods and feeds from different protein sources. Necton's tasks are focused in microalgae production and reduction of its costs, testing a novel drying method, and leading industrial-level protein production pipeline. Microalgae production costs is being tackled by the installation of a highly efficient and low energy consuming, off-the-grid, tubular photobioreactor. Solar panels were installed in the facilities to reduce grid energy consumption for microalgae production. The drying behaviour of several microalgae species are being evaluated in a hybrid solar drier assisted by an algorithm that controls internal heat and humidity for improved drying. This work shows the recent advances in sustainable solar drying of *Nannochloropsis oceanica* and *Tetraselmis chui*, two widely used species in aquaculture, being the latter recently approved as novel food. Drying results will be compared with other innovative dryers from partners to select the one that provides best biomass quality, sustainability and cost-effectiveness. Finally, with all the improvements chosen and in place, industrial partners led by Necton will produce sustainable microalgae protein and innovative foods and feeds at industrial scale, including feeds for shrimp, catfish and carp.

References

1. Yarnold, J., Karan, H., Oey, M. & Hankamer, B. Microalgal Aquafeeds As Part of a Circular Bioeconomy. *Trends Plant Sci*. **24**, 959–970 (2019).

Acknowledgements

Work funded by PROFUTURE- H2020 862980.

CHARACTERIZATION OF DIGESTIVE ENZYMES IN THE GREATER AMBERJACK (*Seriola dumerili*) REARED AT DIFFERENT TEMPERATURES

C. Navarro-Guillén*, E. Perera, M. Yúfera

Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro, 11519 Puerto Real, Spain
Email: carmen.navarro@csic.es

Introduction

Temperature is one of the most important factors regulating the rate of many biological processes, especially in poikilotherms. In fish, water temperature has a significant effect on digestion efficiency through the modulation of feed intake, gut transit time and enzymatic activity. The study of fish digestive biochemistry is essential to obtain valuable information on factors that affect the net efficiency of food transformation and growth, and therefore aquaculture profitability. In this context, the evaluation of types and functional features of the digestive enzymes involved in the hydrolysis of the main nutrients is one of the most important aspects to understand digestive capacities of species of interest.

The greater amberjack, *Seriola dumerili*, is a fast-growing pelagic teleost with great interest for the diversification of marine fish aquaculture in the Mediterranean region. The seeding of fry in cages for on-growing will face seawater temperatures which, in the case of the Mediterranean Sea, range between 15-28 ºC, approximately. The aim of the present study was to assess the activity and functional characteristics of key digestive enzymes in juveniles of greater amberjack, as well as of the possible modulation of their relative importance by water temperature.

Materials and methods

Juveniles of greater amberjack (initial body weight 56.2 ± 19.6 g) were randomly distributed in three independent RAS systems with water temperature set to 18, 22 and 26ºC, respectively. Fish were fed *ad libitum* five times daily for 17 days. At sampling, fish were dissected (n = 8, per temperature) and each digestive tract was separated into stomach and pyloric ceca for preparation of enzyme extracts. Extracts were used for the assay of pepsin in the stomach, and trypsin, chymotrypsin, lipase and leucine aminopeptidase activities in the pyloric ceca following the protocols described by Perera et al. (2017) and Yúfera et al. (2019). Optimal pH for enzymes were determined by measuring the activities at different pHs. Optimal temperature was determined in a range from 10 to 90°C. Classes of proteases in the pyloric ceca were determined by the use of specific protease inhibitors according to Perera et al. (2008).

Results

Fish body weight at the end of the experiment was 61.4 ± 21.5, 101.5 ± 27.5 and 145.9 ± 41.5 for fish reared at 18, 22 and 26ºC, respectively. Optimal pH and temperature for the different enzymes are resumed in Table 1. The use of the specific inhibitor for serine proteases SBTI resulted in inhibition ranging from 37 to 47%, for fish reared at 18 and 26ºC, respectively. In fish reared at 18ºC, higher inhibition was obtained with the specific inhibitor for chymotrypsin TPCK (27.83%) while higher inhibition was recorded with the specific inhibitor for trypsin TLCK in fish reared at 26ºC (38.15%).

Discussion

The present study presents for the first time a partial characterization of the main digestive enzymes from *S. dumerili*, being the optimal pH obtained for the different enzymes similar to those reported by others authors for closely related species (Kishimura et al., 2006). By contrast, optimal temperature for trypsin and chymotrypsin was higher to those usually reported for fish, ranging from 50 to 70ºC, suggesting the presence of thermally robust proteases. Lipase activity was more sensitive to heat and alkaline pH than proteases, suggesting a different and maybe more complex three-dimensional structure of the active enzyme.

Enzyme activity	Optimal pH	Optimal T (ºC)
Pepsin	2.5	60 – 70
Trypsin	10	90
Chymotrypsin	9	90
Lipase	7.5 – 8	50 – 60
Leucine aminopeptidase	11	65 – 75

Table 1. Optimal pH and temperature (T) for digestive enzymes in the greater amberjack *Seriola dumerili*.

(Continued on next page)

The inhibition assay showed the importance of the activity of serine proteases in the digestive tract of the greater amberjack, as expected for a carnivorous species. The incubation of intestinal extracts with the specific inhibitor SBTI caused, on average, a reduction of 42% in overall casein digestion, being this inhibition 10% higher in fish reared at 26 than fish reared at 18ºC, probably due to changes in the relative contribution of different serine proteases and their different susceptibility to SBTI. Indeed, the use of the specific inhibitors TLPC and TPCK suggested inverse trypsin and chymotrypsin relative activity levels with changes in temperature, with higher trypsin relative contribution in 26ºC-fish while higher chymotrypsin contribution in 18ºC-fish. In Atlantic salmon, an increasement in the relative activity of chymotrypsin related to trypsin in the pyloric ceca was observed when growth was limited (Rungruangsak-Torrisen, 2007). However, in the present study, although the use of specific inhibitors suggested different relative contribution of trypsin and chymotrypsin depending on water temperature in *S. dumerili*, activity levels of both enzymes in the pyloric ceca were similar for the three treatments. These results might be explained by other factors, likely the presence of isoforms.

In conclusion, the results of the present study increased the knowledge on the digest biochemistry of the greater amberjack, and the effects of rearing temperature on its digestive enzymes functional flexibility, aiding in improving management practices for this species industrialization.

References

Kishimura, H. et al. 2006. Journal of Food Biochemistry, 30: 521-534.
Rungruangsak-Torrissen, K. 2007. Journal of Food Biochemistry, 31: 509-540
Perera, E. et al. 2008. Comparative Biochemistry and Physiology Part B, 150: 247-254.
Perera, E. & Yúfera, M. 2017. Fish Physiology and Biochemistry, 43: 563-578.
Yúfera, M. et al. 2019. Comparative Biochemistry and Physiology Part A, 230: 71-80.

Acknowledgements

This research was funded by the project RTI2018-096134-B-I00 from the Spanish Ministry of Science granted to MY. CN-G was supported by the researcher contract DOC_01203 funded by the Andalusian Plan for Research Development and Innovation (PAIDI 2020).

WATER TEMPERATURE AFFECTS FEEDING BEHAVIOR AND GUT TRANSIT TIME IN GREATER AMBERJACK JUVENILES (*Seriola dumerili*) FED MULTIPLE DAILY MEALS

C. Navarro-Guillén*, N. Gilannejad†, G. Martínez-Rodríguez, M. Yúfera

Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro, 11519 Puerto Real, Spain
† Current affiliation: Norwegian Research Centre (NORCE), 5008 Bergen, Norway
Email: manuel.yufera@icman.csic.es

Introduction

Water temperature is a primary factor in fish production systems, directly affecting the production efficiency. Specifically for open sea cages, water temperature is poorly controlled and determined by environmental factors, having a special impact the current scenario of global warming.

The efficiency with which the on-growing fish feeds are digested is a key factor determining the feed conversion ratio and, therefore, the profitability and environmental cost of the production process. The efficiency of digestion depends on the time the food is available for hydrolysis within the gut, being food residence time dependent on the feeding frequency and the environmental conditions. In particular, water temperature affects food intake, food transit time and, consequently, growth. However, the estimation of food transit times in fish has usually been performed under unrealistic conditions, from gut filling or evacuation after a single meal.

The greater amberjack (*Seriola dumerili*) stands out as an interesting species to support Mediterranean aquaculture diversification due to its high added commercial value and good adaptation to captivity. Nevertheless, as emerging species, the knowledge on its digestive function is in its infancy. The objective of the present work was to examine the effects of temperature on the feeding behavior and gut transit rate over a daily cycle in greater amberjack juveniles under standard feeding conditions.

Materials and methods

Greater amberjack juveniles with 34.2 ± 15.7 g of body weight (BW) were randomly distributed in three independent RAS systems set to 18, 22 and 26 ºC. Juveniles were reared for 37 days and fed *ad libitum* three times daily with a commercial diet (SPAROS Lda., Portugal). To properly evaluate the transit of each meal, feeds from the different meals were labelled with different inert markers, lanthanum, ytterbium or yttrium. After the experimental period, three fish per temperature were sampled every 4 h during 24 h for feed content and transit determinations. The gastrointestinal tracts (GIT) were dissected in stomach, pyloric ceca, anterior intestine, middle intestine, and distal intestine. Inert markers content was analyzed following the protocol described in Yúfera et al. (1). Transit time for each meal was calculated as the time comprised between feeding and 95% of the ingested meal been evacuated.

Results

Average BW at the end of the experiment was 82.9 ± 35.7, with no statistical differences due to rearing temperature. Voluntary feed intake (VFI) for each meal is summarized in table 1. Globally, 26ºC-fish consumed more feed over the day, mainly supported by a trend of higher feed intake in the first meal of the day compared to 18 and 22ºC-fish.

Overall, food transit time along the GIT was faster in fish reared at 26ºC, with an average of 9h 41min ± 45min for each meal ($P = 0.001$). The specific transit time for each meal is shown in table 2. Transit time of the second meal tended to be slower at 18 and 22ºC, by contrast, at 26ºC the second meal tended to be faster. Moreover, transit time in each segment differed between meals and water temperature (results not shown).

Discussion

The present work evaluated for the first time the transit time throughout the digestive tract of three consecutive daily meals in greater amberjack juveniles reared at three different temperatures. Fish reared at 26 ºC showed both, higher daily feed intake and faster gut transit time compared to 18 and 22 ºC-fish, however, a higher feed consumption was not reflected in higher growth. Gut transit rate defines the duration and physiochemical conditions in which the ingesta is processed by digestive enzymes in the GIT, and consequently influences the utilization of the nutrients (2). Thus, the present results suggested that, in greater amberjack juveniles, slower transit time is correlated with a more favorable nutrients utilization as described for other fish species, such as cobia juveniles (1).

(Continued on next page)

Water temp	VFI for each meal			Daily VFI
	1st meal	2nd meal	3rd meal	
18ºC	0.87±0.14	0.62±0.62	2.12±1.07	3.61±0.33[b]
22ºC	0.99±0.45	0.90±0.58	1.80±0.44	3.69±0.27[b]
26ºC	2.36±0.25	0.83±0.76	2.09±0.87	5.28±0.43[a]

Table 1. VFI (% BW) for each meal and total per day in *S. dumerili* reared at 18, 22, and 26ºC. Results are presented as mean ± SEM. Different letters indicate statistical differences in daily VFI between treatments ($P < 0.05$).

Table 2. Transit time for each meal at the three rearing temperatures. Results are shown as mean ± SEM. Different letters indicate statistical differences in transit time between treatments ($P < 0.05$).

Water temp	Transit time for each meal		
	1st meal	2nd meal	3rd meal
18ºC	12h 56min±1h 36 min	15h 41min±2h 21min[b]	14h 28min±1h 20min
22ºC	13h 11min±1h 22min	17h 7min±1h 21min[b]	13h 8min±1h 20min
26ºC	9h 50min±2h 1min	8h 26min±1h 18min[a]	10h 48min±55min

Transit rate assessment is a challenging task when more than one meal is offered because the ingested feed is mixed up with the feed of the previous and the next meal(s). The present work demonstrated that each meal tended to progress in a different rate along the GIT and not all meals were affected in the same way by the temperature increase. However, meals transit rate between temperatures was only statistically different for the second one. In addition, the progression of the inert markers along the different GIT segments revealed that at 26 °C the meals transited fast enough to progress as clearly three separate batches, while at 18 °C consecutive meals became mixed in the GIT as a consequence of a higher residence time.

In conclusion, the present study analyzed for the first time how successive meals are processed within the GIT of on-growing greater amberjack juveniles. As expected, results confirmed that the passage time of the ingesta throughout the GIT varies among the different daily meals and it is modulated by water temperature, suggesting differences in the digestion efficiency of the meals.

References

1. Yúfera, M. et al. 2019. Comp Biochem Physiol A, 230: 71-80.
2. Gilannejad N. et al. 2019. Aquaculture 513: 734438.

Acknowledgements

This research was funded by the project RTI2018-096134-B-I00 from the Spanish Ministry of Science granted to MY. CN-G was supported by the researcher contract DOC_01203 funded by PAIDI 2020.

UNRAVELLING THE IMPACT OF INTESTINAL MICROBIOTA ON HOST TRANSCRIPTOMICS IN GILTHEAD SEABREAM JUVENILES FED NON-CONVENTIONAL FEED INGREDIENTS

F. Naya-Català[1], G.V. Pereira[2], M.C. Piazzon[3], A.M. Fernandes[2], J.A Calduch-Giner[1], A. Sitjà-Bobadilla[3], L.E.C. Conceição[2], J. Pérez-Sánchez[1]

[1]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Spain. [2]SPAROS Lda, Olhão, Portugal. [3]Fish Pathology Group, IATS-CSIC, Spain
E-mail: fernando.naya@iats.csic.es

Introduction

In a sustainable aquafeed scenario, processed animal proteins (PAP), insect, microbial and algae-products appear as suitable replacers of fish meal (FM) in practical fish diets (Glencross et al., 2020; Basto et al., 2021). However, such new and emerging ingredients have been tested one-by-one rather than using different formulation combinations. In this regard, a previous study in gilthead sea bream (*Sparus aurata*) pointed out that graded levels of sustainable PAP- and NoPAP-based feed formulations are able to support optimal growth performance when nutrient requirements are met (Fernandes et al., 2021). However, PAP-based feed formulations caused a slight impairment of feed conversion ratio (FCR) associated with a down-regulation of the hepatic insulin-growth factor-I and the up-regulation in head-kidney of pro-inflammatory cytokines, chemokines and T-cell markers. Here, we focused on gut health indicators to assess the main effects of PAP and NoPAP- based diets on the mucosal adherent microbiota of the anterior intestine (AI) and its impact on changes in the host transcriptomic profile of selected markers of intestinal function and integrity.

Methods

Quadruplicate groups of gilthead seabream were fed *ad libitum* daily with three different diets in a 77 days feeding trial. The control diet (CRTL) followed a commercial type formulation. In both NoPAP and PAP diets, insect meal, fish by-products, microbial and yeast biomasses were used as FM and vegetable protein replacers. The PAP diet also comprised several PAP ingredients such as poultry meal, feather meal hydrolysate, and porcine blood meal. The NoPAP diet included *Spirullina* and *Chlorella* meal as additional protein sources. At the end of the feeding trial, RNA from the AI was collected and run through a PCR-array to profile the expression of a panel of 44 genes, including markers of epithelial integrity, interleukins and immunoglobulins, among others. DNA from the adherent bacteria of the AI was collected and the V3-V4 region of the 16S rRNA of each sample was amplified and sequenced by Illumina MiSeq. Taxonomic assignment was performed against the RDP database. Alpha diversity was calculated using Phyloseq, and beta diversity using partial least-squares discriminant analysis (PLS-DA) models. Metagenome prediction and pathway analysis were performed using Piphillin. Differentially expressed (DE) genes and discriminant OTUs were correlated using the *corrplot* R package.

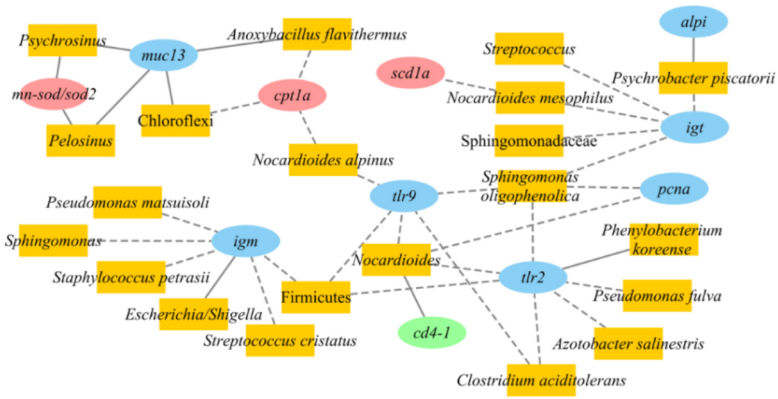


Figure 1: Integrative analysis of discriminant gut intestinal OTUs (orange rectangles) and intestinal (blue ellipses), hepatic (red ellipses) and head-kidney (green ellipses) genes. Edges show positive (solid grey lines) or negative (dashed grey lines) OTU-gene correlations (Spearman correlation test; $P < 0.01$).

(Continued on next page)

Results

The gene expression analysis revealed 13 DE genes (out of 44) ($P < 0.1$) in response to the experimental diets. Markers of epithelial integrity (*pcna*) and pro-inflammatory genes (*alpi*, *il8* and *igm*) were significantly up-regulated in fish fed PAP diet. Fish fed NoPAP diet presented an up-regulation of the anti-inflammatory cytokine *il10*. In the microbiota analysis, Illumina sequencing reads were assigned to 2,180 OTUs and a significantly lower richness and alpha diversity were found in the NoPAP group in comparison to CTRL fish. Detailed differences in microbiota composition were analysed with a statistically validated PLS-DA which clearly separated CTRL fish from fish fed PAP and NoPAP diets, with 135 OTUs mainly driving this separation (VIP ≥ 1). Inferred metagenome results showed a differential regulation of 34 pathways. Both NoPAP and PAP groups showed, among others, the up-regulation of routes tailoring immune response and inflammation (C-type lectin receptor, VEGF, TNF and NFκ-β signalling pathways), with a lower degree of activation in fish fed NoPAP diet. Fish fed PAP and NoPAP diets also displayed an exclusive type of response at this level, with the differential regulation of 14 and 10 inferred pathways, respectively. Correlation tests disclosed a remarkable amount of DE intestinal genes (12) involved in 38 significant ($P < 0.01$) correlations with 29 of the discriminant OTUs (Fig. 1). This highlights the relation between host gene expression and changes in the gut microbiota. In addition, 11 out of the 29 correlated OTUs were also associated with changes in the relative expression of liver and head-kidney genes, retrieved from previous studies performed in the same fish.

Conclusions

Even though growth performance was not affected by the experimental diets, these results disclosed a pro-inflammatory response to PAP-based formulation in terms of gene expression and intestinal microbiota. This inflammatory condition seems to be ameliorating using NoPAP-based feed formulation, which becomes especially interesting as an alternative eco-efficient feed for seabream. At the same time, a remarkable correlation between changes in gut microbial population and gene expression was unravelled at local and systemic levels. This highlights the potential action of the gut microbiome as a "second genome", probably being involved in the regulation of the transcriptomic response of this marine farmed fish when fed innovative diet formulations based on increased circularity and resource utilization principles.

References

Basto et al., (2021). *Frontiers in Physiology*, 12, 659567. doi: 10.3389/fphys.2021.659567
Fernandes et al., (2021). Presented at: *EAS Aquaculture Europe 2020 Virtual*, April 2021.
Glencross et al., (2020). *Fishes* 5, 1–39. doi: 10.3390/fishes5030022

Funding: GAIN (EU-H2020 #773330); AQUAEXCEL2020 (EU-H2020 #652831, TNA project AE150004); Bream-AquaINTECH (RTI2018–094128-B-I00); RYC2018-024049-I/AEI/10.13039/501100011033.

EFFECT OF DIFFERENT TYPES OF SINGLE CELL PROTEIN ON FEED INTAKE, GROWTH, BODY COMPOSITION AND DIGESTIBILITY OF WHITELEG SHRIMP (*Litopenaeus vannamei*).

Marit A.J. Nederlof*[1], Fariz K. Harwinda[1], Remon van Ginkel[1], Johan W. Schrama[1]

[1]Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands.
*marit.nederlof@wur.nl

Introduction

The aquaculture industry is reliant on land and marine protein sources, and fishmeal is one of the major sources of protein used in the aquaculture sector. Limited supply, resource-use conflicts and environmental concerns force the aquaculture sector to look at alternative sources of protein that can improve the sustainability of aquaculture. To keep up with the increasing demand for high quality shrimp feeds in a growing industry, there is an interest in alternative protein sources that can (partly) replace fishmeal in shrimp diets (e.g. Amaya et al. 2007, Panini et al. 2017, Shao et al. 2019).

Single cell proteins (SCP) have been used as an alternative protein source for aquafeeds (Glencross et al. 2020). SCPs are (dried) cells of micro-organisms and can be of algal, fungal or bacterial origin. Of the three different SCPs sources, bacterial SCPs have in general the highest protein levels (50 – 80% on a dry basis). Nevertheless, compared to micro-algae, SCPs of bacterial origin have been studied less frequently for their potential as fishmeal alternative (Glencross et al. 2020).

The success of alternative protein sources depend among others on their impact on attractability and palatability (i.e. feed intake) and their bioavailability (i.e. digestibility and growth). The way SCPs are processed may affect attractability, palatability or bioavailability (Teuling et al. 2019), which in turn may lead to differences in fish or shrimp performance.

This study aims to assess the potential of four bacterial SCP products (provided by String Bio), which differ in the way they are processed, as a substitute for fishmeal in shrimp diets, by studying their effect on feed intake, growth, body composition and digestibility of whiteleg shrimp (*Litopenaeus vannamei*). This was tested against a basal diet, a diet containing a high-quality fishmeal and a diet containing soy protein concentrate as protein source.

Materials and Methods

The SCP products for this study were provided by String Bio (www.stringbio.com). The products were made using their proprietary fermentation process from Gammaproteobacteria *Methylococcus capsulatus*. The bacteria were grown on methane, either derived from biogas or natural gas. SCP1 was produced by aerobic fermentation of *Methylococcus capsulatus*, SCP2 and SCP3 were produced by optimization of the downstream processing to have varying levels of peptides, SCP4 was formulated to have an additional immunomodulatory function by the inclusion of metabolites naturally expressed by the bacteria.

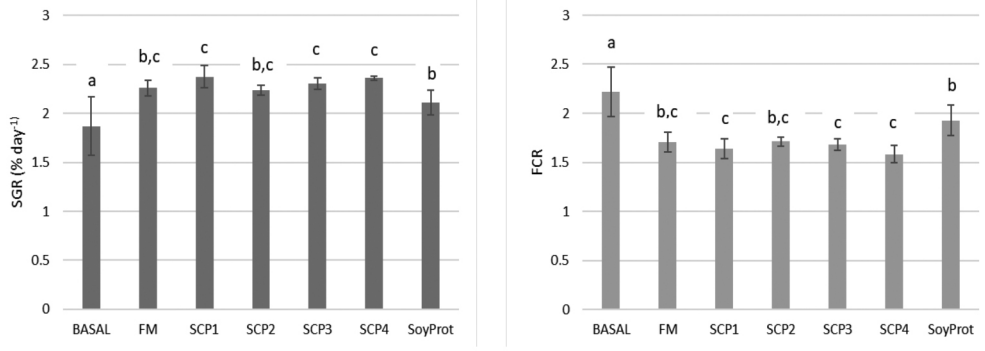


Figure 1. Growth (SGR, % day^{-1}) and FCR of *Litopenaeus vannamei* fed the experimental diets for a period of 10 weeks.
Shrimp were fed to apparent satiation, with one of the seven experimental diets: a basal diet (BASAL), four diets each with one of the SCP products at an inclusion level of 15% (SCP1, SCP2, SCP3 and SCP4), a fishmeal diet (FM, inclusion level of 15%) and a soy protein concentrate diet (SoyProt, inclusion level of 15%). Bars are mean values (n = 4); error bars are standard deviations. Different superscripts indicate significant differences between the diets.

(Continued on next page)

To study the effect of the four bacterial SCP products on feed intake, growth and body composition of *L. vannamei* a growth trial of 10 weeks was performed. During the growth trial, whiteleg shrimp (average initial weight 2.7 ± 0.2 gram) were fed to apparent satiation with one of seven experimental diets; a basal diet (BASAL), four diets each with one of the SCP products at an inclusion level of 15% (SCP1, SCP2, SCP3 and SCP4), a fishmeal diet (FM, inclusion level of 15%) and a soy protein concentrate diet (SoyProt, inclusion level of 15%). Each diet was tested in 4 replicates.

The growth trial was followed by a digestibility trial, during which the same shrimp were fed with the same experimental diets. Shrimp faeces was collected for a period of 7 weeks, to study the effect of the four bacterial SCP products on total N, amino acid and P digestibility.

Results

Growth was affected by diet ($P<0.05$; Figure 1). Post hoc analysis showed that shrimp fed the basal and SoyProt diet had the lowest SGR. Shrimp growth on the diets with SCP did not statistically differ from the diet with fishmeal ($P>0.05$). Numerically, shrimp fed the SCP1 diet had the highest SGR (Figure 1). Patterns in feed intake were comparable to SGR and affected by diet ($P<0.05$), but differences were less pronounced. Feed intake at the basal and SoyProt diets (0.25 g/d) was lowest, while feed intake was higher at the other diets. Feed intake was equal for shrimp fed the SCP diets and the FM diet, ranging between 0.26 and 0.27 g/d. Feed intake was highest for SCP1 and SCP3 diets. FCR was also affected by diet ($P<0.05$). FCR was highest for the basal diet. FCR was lowest for FM and all SCP diets. SCP diets did not differ from the FM diet, but the FCR tended to be lower for SCP1, SCP3 and SCP4 compared to FM (Figure 1). The current results show that bacterial SCP at 15% inclusion give similar results as fishmeal, thus being a potential alternative for fishmeal in shrimp diets. Analysis on nutrient digestibility are in progress and are included in the presentation.

References

Amaya, E.A., Davis, D.A. and Rouse, D.B. (2007) Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. Aquaculture 262 (2-4): 393-401.

Glencross, B.D., Huyben, D. and Schrama, J.W. (2020) The application of single-cell ingredients in aquaculture feeds—a review. Fishes 5 (3): 22.

Panini, R.L., Pinto, S.S., Nóbrega, R.O., Vieira, F.N., Fracalossi, D.M., Samuels, R.I., Prudêncio, E.S., Silva, C.P. and Amboni, R.D.M.C. (2017) Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp *Litopenaeus vannamei*. Food Research International 102: 445-450.

Teuling, E., Wierenga, P.A., Agboola, J.O., Gruppen, H., and Schrama, J.W. (2019) Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture 499: 269-282.

Shao, J., Zhao, W., Han, S., Chen, Y., Wang, B. and Wang, L. (2019) Partial replacement of fishmeal by fermented soybean meal in diets for juvenile white shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition 25 (1): 145-153.

DEVELOPMENT OF AN *IN SILICO* METHOD TO DESIGN NEW SPECIES POLYCULTURE IN AQUACULTURE

Nellya Amoussou*, Thomas Lecocq, Alain Pasquet and Marielle Thomas

University of Lorraine, INRA, UR Animal and Functionality of Animal Products, Team Domestication in Inland Aquaculture, Nancy, France

*Corresponding author: +33372745200, nellya-lydie-yabo.amoussou@univ-lorraine.fr

Among the range of practices that promote aquaculture efficiency and sustainability, polyculture (i.e. production of two or more fish species in the same physical space at the same time, adapted from [1]), is one of the most ancient fish farming practice in the world. In Western Europe, intensive monoculture has been favored during the last decades instead of the traditional extensive pond polyculture production. Nevertheless, the recent theorization of long-standing productions as well as developments of new concepts (agroecology) and practices (integrated multi-trophic aquaculture, aquaponics, and integrated agriculture-aquaculture) reflect a renewed interest in polyculture for future aquaculture developments [2]. Indeed, polyculture is seen as an opportunity to promote aquaculture by mitigating some of the negative impacts and unsustainable development of current aquaculture [3]Sander lucioperca (L.. In order to be efficient, polyculture requires that the involved species are compatible (i.e. species which can live in the same production system without detrimental interactions or competition for resources) and moreover complementary (i.e. species which can use different parts of the available resources or develop commensal/mutualist interactions) [4]. Ensuring compatibility and complementarity requires to promote an optimal use of all resources; trophic (e.g. by combining species with different diets), spatial (by combining species occupying different areas in the living environment), and temporal (by combining species with different periods of activity; e.g., day and night) [4]. Although long-standing polyculture are available, new species combinations are requested to maximize the benefits of polyculture, to address new socio-economic and environmental challenges (e.g. climate change, new technological developments and new consumer demand). For example, considering the context of climate change, new species combinations are needed since some species that were initially compatible in a given environment may no longer be so due to the resistance of some of them to variations in environmental parameters such as temperature and salinity. Moreover, considering a large number of species could maximize the chances to determine the best combination(s) of species to provide to fish farmers.

Problematic and objectives

Designing new species combinations involves considering a large number of possible species combination, which cannot be assessed by empirical methods. Indeed, such methods are based on trial-and-error essays, and are thus time-consuming and costly. They do not maximize the chances of identifying combinations of polyculture that respond to new socio-economic and environmental issues. Such empirical approaches are reasonably unlikely to be feasible for practical and ethical reasons [4]. Moreover, considering only empirical methods cannot be regarded as the most efficient solution to develop new fish polyculture because the most useful species combination(s) for aquaculture purpose could be missed out. To overcome this problem, here we propose an integrative conceptual method to standardize and rationalize the choice of the best species combinations for future polyculture.

Background and steps of the *in silico* method

In order to reach an efficiency and sustainability aquaculture, a polyculture should (i) use optimally all available resources in the aquaculture system, (ii) to maximize fish welfare while addressing regulation, economic and environmental expectations. This implies to consider interspecific interactions to design new combinations, because the functioning of an agro(eco)system depends mainly on the nature of these interactions [5]. Therefore, considering functional approach through species functional traits is necessary to understand how organisms interact with their environment and with other species [6].

(Continued on next page)

Based on a functional approach, we propose the development of a new prospective and integrative method, divided into four successive assessing steps, requiring the intervention of scientists and stakeholders at several levels. The first step consists in theoretically predicting the compatibility of a wide number of fish species using their relevant functional traits (morphological: e.g., size, mass, physiological: e.g., growth rate, phenological: life span, and behavioral: e.g. swimming activity, predation, intra and interspecific relationships), available in different databases. The fish species compatibility is based on the calculation of an index, considering their stage of development, their abiotic requirements, the degree of competition for space/time/trophic resources and the risk of predation. This index is based on a contrasting gradient of compatibility ranging from 0 for incompatible species to 1 for highly compatible species. The second step aims to select one or more (new) species combination considering the stakeholder's advices. The third step assesses species combinations performances from bioassays in Recirculated Aquaculture systems or in ponds. A fourth step corresponds to the transfer of technologies in real aquaculture exploitation conditions. During the conference, the presentation of the *in silico* method will be widely illustrated with examples of species combinations which have been tested in Recirculated Aquaculture systems.

Acknowledgements
This research program was funded by FEAMP SEPURE, INTERREG V A Perciponie and the 'Zone Atelier Moselle'.

References

[1] Stickney RR (2013) Polyculture in Aquaculture. In: Christou P, Savin R, Costa-Pierce BA, et al. (eds) Sustainable Food Production. Springer New York, New York, NY, pp 1366–1368
[2] Chopin T, Buschmann AH, Halling C, et al (2001) Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J Phycol 37:. https://doi.org/https://doi.org/10.1046/j.1529-8817.2001.01137.x
[3] Boyd CE, D'Abramo LR, Glencross BD, et al (2020) Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J World Aquac Soc 51:578–633. https://doi.org/10.1111/jwas.12714
[4] Thomas M, Pasquet A, Aubin J, et al (2020) When more is more: taking advantage of species diversity to move towards sustainable aquaculture. Biol Rev 4:. https://doi.org/10.1111/brv.12677
[5] Hooper DU, Chapin FS, Ewel JJ, et al (2005) Effect of biodiversity on ecosystem functioning: a consensus of current knowledge. 75:3–35. https://doi.org/https://doi.org/10.1890/04-0922
[6] Violle C, Navas M-L, Vile D, et al (2007) Let the concept of trait be functional! Oikos 116:882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x

CAPACITY BUILDING IN AQUACULTURE: A TOOLBOX FOR REGIONAL DEVELOPMENT

A. Neyts*[1], K. Thyholt[2], M. Bull[3], L.B. Brubakken[3], B.E. Asbjørnslett[1], A. Misund[4]

[1]Norwegian University of Science and Technology, 7491 Trondheim (Norway).
[2]Fremtidens Industri AS (NCE Aquatech Cluster), Verkstedveien 4, 7125 Vanvikan (Norway)
[3]Blått Kompetansesenter, Nordfrøyveien 413, 7260 Sistranda (Norway)
[4]SINTEF Ocean, Otto Nielsens veg 10, 7052 Trondheim (Norway)
E-mail: alexandra.neyts@ntnu.no

Introduction

The aquaculture sector is in transition. Not only is the sector expected to contribute to global food security, it also needs to comply with increasing societal demands for responsible and sustainable production and adopt the new digitalization opportunities. Enabling technologies provide a new window of opportunities, with the exploration of high-tech solutions as steppingstones towards this paradigm shift. The development of efficient, green and smart production systems requires expertise across a wide range of technology and social science disciplines, whilst setting the farmed species and the production ecosystem in the centre. The Bridgehead Aquaculture 2050 project is developing a toolbox to acquire a strengthening of the innovation capacity across the value chain.

Methodology

The region of Mid-Norway is used as a pilot area for the development of a knowledge exchange and capacity building platform. The county of Trøndelag is ranked high in the top 25 Regional Innovation Leaders in Europe[1]. It also represents about 1/3rd of the total national aquaculture production (amounting to 1,45 million tonnes in 2019[2]), and locates two major aquaculture educational and research centres, i.e. NTNU and SINTEF. Alongside fish, shellfish and algae farming companies, Bridgehead Aquaculture involves mainly businesses supplying technology, services, or feed to the aquaculture sector. Many of them are connected through their membership in a regional cluster[3].

The knowledge and innovation capacity in the aquaculture sector can be considerably strengthened through a more efficient utilisation of existing scientific and practice-based competences.

Applying the toolbox across the aquaculture value chain has an expected impact on three different levels:
1. Education: improved aquaculture relevance of studies, especially in enabling technological education areas and for those with limited knowledge about the sector
2. Research: stronger engagement of industry in the creation of research projects
3. Industry: increased competence level of company employees

Results

The toolbox of Bridgehead Aquaculture 2050 has developed and implemented routines for more and stronger cross-stakeholder interactions at the education and skill development level. More specifically, the presentation wants to highlight four pilot instruments that were developed in 2020/21.

Minor in aquaculture[4] is a set of aquaculture courses and seminars directed to civil engineering students across a variety of specialities, i.e. marine technology, renewable energy, environmental engineering, ICT, cybernetics and robotics, mechanical engineering, electronics systems design. The minor enabled the students to recognise how their specific expertise can be applied to problem-solving in aquaculture planning, operations, and development across the value chain. In addition, they developed biological and cross-disciplinary communication skills and increased their knowledge about the sector's structure and its stakeholders.

At a Bachelor level, a study programme in aquaculture engineering[5] was developed. This is a 3-year's professional education programme within operations and maintenance of aquaculture farming systems, both at sea and on land. The study programme was developed and set off in 2020 because of a clear demand by the sector, emphasising a lack of candidates with this specific set of skills.

(Continued on next page)

Students represent a powerful instrument for change, of which the sector is not adequately aware. In order to lower the threshold for student-industry interactions, an online portal[6] for the aquaculture sector was established at the university. It provides, amongst others, guidelines, agreement templates and examples of past student assignments in aquaculture, an annual wheel for collaborating opportunities and deadlines, and open summer job assignments or internships. Overall, it helps both students and companies with little experience or network to interact.

Additional instruments in the toolbox are existing collaborative mechanisms adapted to the particularities of the aquaculture sector: the industry PhD scheme, continued education courses at bachelor and master level, trainee programmes, and lifelong learning.

Discussion and conclusion

As a response to the need for a more efficient and mutual exchange of theoretical and practical knowledge, the Bridgehead Aquaculture 2050 project developed a regional knowledge platform with a set of new and improved instruments.

It was shown that the instruments stimulated aquaculture stakeholders in the target region to build up trust, competence and experience in collaborating with groups they previously had little knowledge about. Participating students and researchers opened a wide range of expertise within the fields of technology, natural sciences and social sciences. The sharing of knowledge, creation of engagement and general networking across the stakeholder groups using one or several Bridgehead instruments, contributes to the realisation of the paradigm towards a sector-wide acknowledgement of the importance of research-based innovation in the green and digital transformation.

The Bridgehead Aquaculture toolbox is based on strengthening existing assets that have so far been underexploited by the aquaculture sector. Being supported and implemented by strong institutions and networks, the legacy of the instruments is secured. As a next step, the toolbox can possibly be expanded to other regions in Europe with similar needs and area of opportunities.

References

1. European Commission (2019). Regional Innovation Scoreboard. Report, pp.97.
2. Statistics Norway (2019). Aquaculture, final figures. Statistics. https://www.ssb.no/en/jord-skog-jakt-og-fiskeri/statistikker/fiskeoppdrett/aar/2020-10-29?fane=tabell&sort=nummer&tabell=433976
3. NCE Aquatech Cluster https://aquatechcluster.no/?lang=en
4. Minor i havbruk - NTNU
5. Bachelor in Engineering, Aquaculture (BIHAV) - NTNU
6. Aquaculture | Bridge NTNU

DEVELOPMENT OF AN EASY TO OPERATE HATCHERY PROTOCOL FOR BLUE MUSSEL (*Mytilus edulis)* SEED PRODUCTION

Pernille Nielsen*[1], Clarie Ng[1], Pascal Barreau[1], Camille Saurel[1], Iarfhlaith Connellan[2], Colin Hannon[3]

[1] Technical University of Denmark, DTU Aqua, Section for Coastal Ecology, Øroddevej 80, 7900 Nykøbing Mors, Denmark
[2] Cartron Point Shellfish Ltd, New Quay, Burrin, Clare, Ireland
[3] Galway-Mayo Institute of Technology, Marine & Freshwater Research Centre, Dublin Road, Galway, Ireland

E-mail: peniel@aqua.dtu.dk

Introduction
Blue mussel (*Mytilus edulis*) cultivation largely depends both on seed availability and food from the natural environment and is therefore highly impacted by natural variations in seed and feed supply. Currently, the blue mussel production industry has an over reliance on seed provided by wild harvest or use of suspended collectors for natural settlement of spat. Hatchery produced blue mussel seed has been proposed as a solution to ensure sufficient seed supply and provides the opportunities for selective breeding and possibilities of triploid production giving the product increased added value. However, production of hatchery produced blue mussel seed is challenged by the fact that hatchery produced seed can be prohibitively expensive compared to the actual sale value of harvested mussel seed from the wild. Consequently, further development and optimisation of hatchery produced blue mussel seed is required for the further advancement and implementation at an industry scale in an economic timeframe.

Results and discussion
A protocol for blue mussel hatchery reared seed has been developed in Ireland. The protocol has shown to be successful in a traditional shellfish hatchery setting but is not operational for mussel farmers without access to a hatchery facility. The goal of our research is to implement, adapt and optimise this robust and repeatable protocol to other hatchery conditions and to develop in parallel a low-technological protocol that will be implemented at an industry level, operational by mussel farmers and thereby reduce the overall cost of hatchery produced mussel seed. The presentations will focus on the simplified production, the feeding setup for the larvae rearing as well as the settlement success comparison for commonly used spat collectors by farmers in Denmark vs settling material commonly used in hatchery. The results will be discussed in terms of trade-offs of the two different hatchery protocols and if the low-tech method would be transferable to the farmers as an affordable source of mussel seeds and if it can improve the economic feasibility of hatchery produced blue mussel seed.

EFFECTS OF POST OVULATORY AGING ON FERTILISATION, DEVELOPMENT, SURVIVAL AND HATCHING IN ATLANTIC HALIBUT *(Hippoglossus hippoglossus)* EGGS

N. Niepagen*, L. Berg, E. Kjørsvik

Norwegian University of Science and Technology, Brattørkaia, 7010 Trondheim
E-mail: nils.niepagen@ntnu.no

Introduction

While established for decades, aquaculture of Atlantic halibut is challenged by many obstacles one of which is insufficient production of juveniles (Engelsen et al., 2004). One of the problems encountered is a highly variable egg quality, possibly caused by overripening or post ovulatory ageing. Post ovulatory ageing is a process of degeneration on eggs which begins right after ovulation. In rainbow trout, incorrect stripping time can result in very low egg quality, changes in morphology, composition and maternal gene expression due to post ovulatory ageing (Aegerter et al., 2005). Mating of Atlantic halibut in captivity is rarely observed and eggs and milt need to be stripped by hand (Holmefjord, Lein, 1990). Decades ago, wild fish were used for broodstock, but some hatcheries have since bred up to F3 generation fish which are in production today. Wild females were typically closely monitored by hatchery personnel to estimate the optimal stripping time shortly before they would spontaneously release their eggs in the water (Norberg et al., 1991). Farmed broodstock typically obtain lower fertilisation rates than wild fish, higher variability and in many individuals the lack of spontaneous egg release. Today, in three of the four Atlantic halibut hatcheries in Norway, technicians are stripping females based on how much their ovaries are visually built up and on experience. If no eggs are obtained, the fish are checked again after 12-80 hours. Fish that gave eggs are stripped again after a protocol, such as e.g. in 81-84 hour intervals. This study is aimed at understanding the implications of post ovulatory ageing on egg quality of Atlantic halibut during fertilisation, development and hatching.

Materials and methods

Experiments were conducted at Nordic Halibut AS, Midsund, Norway during summer 2020 and spring 2021 spawning seasons. The natural spawning season for Atlantic halibut is spring but spawning in summer is achieved by photomanipulation. All broodstock were stripped by hatchery personnel, and a flexible stripping protocol was implemented to obtain egg batches as close to ovulation as possible. Briefly, based on the fertilisation percentage of a given batch, the fish was stripped again after 60-72 hours. The lower the percentage of the previous batch, the shorter the time for the next stripping. Only gentle pressure was applied to strip the eggs from the females, if no eggs were obtained,the fish were checked later. Six egg batches were obtained this way as shortly after ovulation as possible. Each egg batch was divided into six fractions (50-150 mL each), which were stored in a concealed container filled with eggs to the top at 6°C. To simulate post ovulatory ageing, one egg fraction at a time was fertilised using cryopreserved milt (Cryogenetics AS) at 0, 1, 2, 4, 6 and 12 hours after stripping. For eggs from each fertilization time, between 200-400 eggs were distributed to each of three petri dishes and to nine 800 mL cell culture flasks (three per sampling stage) containing 1 L of seawater with 0.25mL/L solution of terramycin (100mg/mL) and incubated at 6°C. Eggs were sampled for evaluation of fertilisation rates (from petri dishes) and of embryonic development at 14h post fertilisation (hpf) for 8 cell stage, , at 48-50 hpf for blastula stage, at 300 hpf (after blastopore closure) and at 540 hpf for hatching rate (from cell culture flasks).

Results

Data evaluation for spring 2021 is still in progress. However, in summer 2020, fertilisation rates were slightly but not significantly decreasing up to 12 hours past stripping. Survival was constant for four hours after stripping and at 6 hours past stripping it decreased dramatically from 70 % to 30 % in one of the batches. Other observed trends were higher mortality during incubation with longer post ovulatory ageing, a higher percentage of eggs lacking activation during the fertilisation process and higher occurrence of failures in body axis formation after gastrulation. The flexible stripping protocol led to an average increase in fertilisation rate and batches followed according to this protocol were either stripped too early (no eggs obtained) or increased in fertilisation rate compared to the previous batch of the same fish.

(Continued on next page)

Discussion and conclusion

Survival and fertilisation rate in artificially aged Atlantic halibut eggs are correlated over a longer period. We showed however, that post ovulatory ageing had a higher influence on survival than on fertilisation rate. This indicates that previous findings based only on fertilisation rate may have underestimated the problem of overripening in Atlantic halibut broodstock (for example Norberg et al., 1991). Thus, our data is highlighting the importance of a flexible stripping protocol that is suitable to avoid overripening of the eggs as much as possible. In turbot, low egg qualiry leads to reduced ability to cope with environmental stress, abnormal development, deformities and incomplete eye migration (Kjørsvik et al., 2003). It is likely that similar to turbot, fry from low quality batches in Atlantic halibut will suffer higher mortalities and possibly more deformations during development and metamorphosis. Low fertilisation rates and high variability in egg quality in Atlantic halibut can at least to some extents be explained by post ovulatory ageing due to inadequate stripping protocols for farmed broodstock. This study shed light on how to improve halibut egg quality by modifying the established stripping protocols based on the fertilisation rates of previous batches. This not only dramatically increased the quality of successive batches, but it also enables researchers to obtain batches that are less affected by post ovulatory ageing when exploring differences in egg quality between individual broodstock fish and their different egg batches.

References

Aegerter, Sandrine, Bernard Jalabert, and Julien Bobe. “Large scale real-time PCR analysis of mRNA abundance in rainbow trout eggs in relationship with egg quality and post-ovulatory ageing.” Molecular Reproduction and Development: Incorporating Gamete Research 72.3 (2005): 377-385.

Engelsen, R., Asche, F., Skjennum, F., & Adoff, G. (2004). New species in aquaculture: some basic economic aspects. Culture of Cold-Water Marine Fish, 487-515.

Holmefjord, Ivar, and Ingrid Lein. “Natural spawning of Atlantic halibut (*Hippoglossus hippoglossus)* in captivity.” International Council for the Exploration of the Seas, CM (1990).

Kjørsvik, E., K. Hoehne-Reitan, and K. I. Reitan. “Egg and larval quality criteria as predictive measures for juvenile production in turbot (*Scophthalmus maximus* L.).” Aquaculture 227.1-4 (2003): 9-20.

Norberg, Birgitta, et al. “Ovulatory rhythms and egg viability in the Atlantic halibut (*Hippoglossus hippoglossus*).” Aquaculture 97.4 (1991): 365-371.

PREDICTION OF AQUAFEED PERFORMANCE OVER A FULL PRODUCTION CYCLE

A. Nobre[1]*, S. Cabaleiro[2], F. Soares[1], T. Silva[1], L. Conceição[1]

[1]SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[2]CETGA, Cluster de la Acuicultura. 15965.Punta Couso-Aguiño (Riveira) Apdo 126. 15960 Riveira. A Coruña, SPAIN

Introduction

At the fish farm, with feed costs accounting for 50% to 70% of total production costs, feeding management is a critical activity. An evaluation of the suitability of the commercially available aquafeeds for the particular farming conditions of each farm/site is of paramount importance to address the sustainability challenges raised by fast growth of the aquaculture industry and the increasing expected demand for farmed fish. In this work, we illustrate the application of a nutrient-based model (FEEDNETICS™) to evaluate seabass aquafeeds used by the Mediterranean industry, considering two different temperature profiles. In the FEEDNETICS™ virtual environment, users can compare different production settings, feeds or feeding regimes and analyse which ones have the potential to improve their FCR, reduce the time to harvest, enhance growth, improve their economic conversion, or reduce their waste of N and P, all of this within hours of field implementation for the entire production cycle. This work was carried out in the context of the EU's H2020 PerformFISH project (No. 727610) which aims to contribute to sustainable growth of the Mediterranean Marine Fish Farming industry.

Methods

FEEDNETICS™ is a web-application developed by SPAROS Lda (Olhão, Portugal) that includes a mechanistic nutrient-based model to predict fish growth and composition along time, using information on temperature, feed intake and feed properties. The model has been calibrated with a wide range of datasets and is currently available for gilthead seabream, European seabass, rainbow trout and Nile tilapia, with several applications done at fish farms and for generic conditions (Soares et al., 2020, Nobre et al., 2021).

Long-term term simulations of European seabass growth were carried out with FEEDNETICS™ considering three aquafeeds applied in the Mediterranean farms (Table 1). The feeding rates were defined using a feeding table per feed generated by a protein and energy flux model similar to the FeedEST engine (available at http://performfish.eu/industry-corner). The farming conditions considered a cage with 300,000 fish (initial weight: 50 g), mortality rate of 1% per month, a harvest weight of 450 g and two temperature profiles (Besson et al. 2016):Western Greece (average 19.3 ºC, 14.3 ºC to 24.2 ºC) and Eastern Coast of Spain (average 18 ºC, 11.9 ºC to 24.1 ºC).The simulated conditions included two different scenarios of feed waste, both within the range considered for the industry (Ballester-Molto´ et al., 2016): i) 8% to 10% of feed waste, and ii) 10% to 15% feed waste.

The prediction results were used to evaluate the feed performance concerning FCR, growth rate, time to reach harvestable weight, total feed quantity, N and P waste, and savings on feed.

Results

According to the model results (Table 2) for the Eastern Spain temperature profile, Aquafeed 1 leads to a better fish performance with a shorter production cycle (435 days), lower FCR (2.0), and less N and P waste. However, and considering the simulated conditions, Aquafeed 2 leads to a better economic conversion with savings estimated around less 111 € of feed per ton of fish produced compared with the Aquafeed 1. For this temperature profile, and regarding the economic conversion, Aquafeed 1 is also outcompeted by Aquafeed 3, which is the feed that leads to the worst fish performance.

Table 1 . Feed specifications.

	Aquafeed 1	Aquafeed 2	Aquafeed 3
Proximal composition (as feed basis)			
Digestible Protein (%)	42.3	40.5	38.3
Digestible Lipids (%)	18.4	17.1	16.7
Digestible energy (MJ/kg)	18.7	18.5	18.2
DP/DE (g/MJ)	22.6	21.9	21.0
Amino acid (AA) profile	Assumed default values in accordance with the requirements of the species		
Fatty acid (FA) profile			
Feeding quantity	Feeding table per feed		
Cost of feed (relative to Aquafeed 2)	+10%	ref	-8%

(Continued on next page)

As illustrated in Table 2, the precise evaluation of aquafeeds will depend, among other factors, on the particular temperature profile under which fish are reared. According to the FEEDNETICS™ simulations (Table 2), the more favourable Western Greece temperature profile leads to substantial improvements of the production performance compared with the Eastern Spain and for the same scenario of feed waste (8% to 10% of feed waste). As expected, also in the Western Greece scenario, Aquafeed 1 leads to the best fish performance comparing with both Aquafeed 2 and 3. However, the savings on feed (Table 2) indicate a different trend between the two temperature profiles: under the Western Greece temperature profile, Aquafeed 1 leads to a better economic conversion despite its higher unit cost, with savings estimated around 41 € and 538 € of feed per ton of fish produced, compared with Aquafeed 2 and 3, respectively.

Despite the impact on the production profitability, the feed losses are difficult to estimate with precision over the production cycle. Herein, we illustrate how we can use this tool to build a what-if scenario where, instead of 8%-10% of feed losses, we assume losses in the range of 10%-15%. In Table 2, the impacts related to the increase in feed losses are quantified considering the Western Greece temperature profile, which leads to a substantial increase of days in production, overall, more expenditure of feed, with decreasing growth rates and worst FCRs. It is interesting to highlight that the impacts are non-linear between the 3 aquafeeds. In this case, it was proportionally higher for Aquafeed 2, leading to an increase of the savings on feed for Aquafeed 1 compared to Aquafeed 2 (less 303 € of feed per ton of fish produced).

Final remarks

Nutrient-based models allow farmers to select feeds and plan feeding, towards optimized feeding. As illustrated in this PerformFISH use case, this type of tools can assist farmers in predicting the impact of different feeding strategies on their farm's performance. Mechanistic nutrient-based models include the fish physiological and metabolic processes that are required to predict the effects of feed composition and temperature on fish growth, feed conversion and wastes, among other variables. Farmers can quantify the impact of their actions or build what-if scenarios, such as quantifying the feed cost per ton of fish produced, and estimate time to market size of a given stock based on a certain cage stocking date. Such nutrient-based models will most likely be part of a good precision farming toolset.

References

Ballester-Moltó M, García-García B, Aguado-Giménez F, et al. 2016. Controlling feed losses by chewing in gilthead sea bream (*Sparus aurata*) on growing may improve the fish farming environmental sustainability. Aquaculture, 464:111-116.

Besson M, Vandeputte M, van Arendonk, J.A.M., et al. 2016. Influence of water temperature on the economic value of growth rate in fish farming: The case of sea bass (*Dicentrarchus labrax*) cage farming in the Mediterranean. Aquaculture, 462, 47-55.

Nobre A, Soares F, Silva T, et al. 2021. Prediction tools for trout aquafeed evaluation and on-farm support. International Aquafeed, March 2021: 22-24.

Soares F, Silva T, Conceição L, et al. 2020. Optimisation of feeding strategies at a fish farm through mathematical modelling. FISH FARMING TECHNOLOGY, International Aquafeed, September 2020: 42-45.

JELLYFISH. A USEFUL RESOURCE FOR AQUACULTURE

Natacha Nogueira[1,2,3] *, Sonia Gueroun[1,4], Paula Canada[1,2], Ricardo José[1,3], Paolo Guttuso[1,3], João Canning-Clode[1,4], Carlos Andrade [1,2,3]

[1] OOM – Oceanic Observatory of Madeira, ARDITI – Regional Agency for the Development of Research Technology and Innovation, Ed. Madeira Tecnopolo, 9020-105, Funchal, PT
[2] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, 4450-208, Matosinhos, PT
[3] Mariculture Center of Calheta, Directorate for the Sea, Av. D Manuel I, Nº7, 9370-133, Calheta, PT
[4] MARE – Marine and Environmental Sciences Centre, ARDITI- - Regional Agency for the Development of Research Technology and Innovation, Ed. Madeira Tecnopolo, 9020-105, Funchal, PT

* Email : natacha.nogueira@madeira.gov.pt

Introduction

Historically, jellyfish (JF) products are not new. These gelatinous organisms have been used for centuries as human food in Asian countries, while modern exploitations are applied in medicine and cosmetics. Nevertheless, due to their high water content, JF are often presumed to be a poor food source and a trophic dead end[1]. However, in addition to the vertebrate predators that extensively consume gelatinous species, at least 124 fish species, some of them with economic value like the seabream *Sparus aurata*, the piked dogfish *Squalus acanthias* and the Atlantic saury *Scomberesox saurus*, are reported to prey on JF in natural waters[2].

On a dry-mass basis, JF contain a higher proportion of polar lipids than what is found in standard aquafeed pellets, including n–3 highly unsaturated fatty acids, especially eicosapentaenoic acid, and n–6 HUFAs, such as arachidonic acid[3]. In addition, some jellyfish species are important amino acids, such as glycine and taurine, that dominates the free amino acids (FAAs)[4]. Therefore, several studies have investigated the potential of using JF as feed for aquaculture fish[5,6].

In the framework of the Horizon 2020 'Gojelly' project, three JF species - *A. solida*, *Cotylorhiza tuberculata* and *Catostylus tagi* - were selected for product evaluation as aquafeed, based upon the following criteria: 1) species need to have been recorded in the geographic area to avoid the incidental introduction of non-indigenous species in the ecosystem, in the case of trials with live JF; 2) species need to present biochemical composition potential to be used as feed; 3) biomass availability.

Materials and methods

The first trial, conducted at the facilities of the Mariculture Centre of Calheta, Madeira, evaluated the potential of using live jellyfish to feed juvenile's seabream, using all the life stages benthic polyps (< 1 cm), and the free-swimming ephyrae (≈ 2 m) and medusa (≈ 1cm) of reared *A. solida* (one of the most worldwide distributed jellyfish genera that fulfil the first criteria) *versus* standard seabream aquafeeds. For two days, seabream were fed with the different items for two hours before removal of the remaining food to estimate the ingestion rate.

The second trial aimed to determine the potential of incorporating jellyfish biomass into a compound diet for seabream at the juvenile stage. *C. tuberculata* was collected in Mar Menor, Spain, immediately frozen (-20ºC) and transported to "Sparos Lda." facilities (Portugal). Based on the composition of the jellyfish biomass (dry and fresh forms) determined upon arrival and taking into account the feasibility of its use in industrial feed extrusion processes, the trial comprised six dietary treatments: the control diet (CTRL) that mimicked a commercial feed for gilthead seabream juveniles and four additional diets incorporating at mixing (before extrusion) either dry or fresh jellyfish biomass at 2.5 and 5.0%. A sixth diet contained 2.5% of the fresh JF biomass but incorporated post-extrusion by vacuum coating to potentiate the jellyfish biomass bioactivity role. Seabream juveniles (initial weight of approximately 24g) were fed with the six diets (three replicates per treatment) for two months in a flow-through system. Survival, growth, and biochemical composition were determined at the end of the feeding trial that lasted two months.

Finally, a third trial was conducted to determine the acceptability of freeze-dried JF (*C. tagi*) by the goldfish *Carassius auratus,* one of the most common freshwater fish found in pet shops, *versus* a diet based on commercial fish flakes. Fish were fed four dietary treatments: one exclusively commercial feed and three others with different percentages of JF (25, 50, 75%) plus commercial feed, once a day, for 45 days. The remaining food was weighted, and feeding was video recorded (30 s before feeding, 60 s during feeding and 60 s after feeding). At the end of the trial, biochemical composition and food consumption were analyzed.

(Continued on next page)

Results and Discussion

The first trial showed that seabream displayed predatory activity over benthic (polyps) and pelagic stages (ephyra and medusa). Although standard dry pellets were the most preyed item, within JF, the highest ingestion rates were observed for polyps, followed by ephyrae, and last by medusa. Results of the ingestion rate on polyps and ephyrae highly exceed the values found by Marques et al. (2016), which might be explained by the fact that seabream used in that study were larger (min 70g) and likely less interested in small prey.

Results of the second trial showed no differences in growth, biochemical composition, nor in most immunological parameters, except for peroxidase (p 0.025), showing that inclusion of JF, tended to increase peroxidase levels. Further studies need to investigate the potential of jellyfeeds to increase this enzyme activity, as peroxidase activities in fish erythrocytes suggest the predominant role of this enzyme in the protection of polyunsaturated acids against uncontrolled oxidative processes possibly indicating an important positive role of including jellyfish biomass in aquafeeds.

Results of the goldfish trial indicated that jellyflakes were accepted. As soon as jellyflakes were delivered into the aquaria, fish exhibited a "chasing behavior" (defined as increased swimming speed towards the flakes) and increased biting frequency (refers to the number of times that each fish was seen biting on the flakes deposited at the bottom of the aquarium per meal). Biochemical results indicated that the proximate composition of goldfish fed different percentages of JF had significantly higher protein content and lower lipid content.

Fish generally accepted all JF aquafeeds products in all trials, and no adverse effects were found. Both freeze-dried JF and compound diets with JF as an ingredient present the advantage of availability throughout the year, easier to store and manipulated by users.

References

[1]Sommer U et al. (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production: primary production. Hydrobiologia 484: 11–20.

[2] Pauly D, Graham W, Libralato S, et al (2009) Jellyfish in ecosystems, online databases, and ecosystem models. In: Hydrobiologia. pp 67–85.

[3]Stenvers V., et al. (2020). Seasonal Variability of the Fatty Acid Composition in Aurelia aurita (Cnidaria: Scyphozoa): Implications for Gelativorous Food Web Studies. J Plankton Res 13;42(4):440-452.

[4]Miyajima-Taga Y, et al. (2018) Efficacy of feeding tiger puffer Takifugu rubripes on moon jellyfish with respect to nutritional composition and behavioural traits. Aquac Nutr 24:504–514.

[5]Marques R, et al. (2016) Jellyfish as an alternative source of food for opportunistic fishes. J Exp Mar Bio Ecol 485:1–7.

[6]Miyajima-Taga Y, et al. (2015) Effect of giant jellyfish Nemopilema nomurai as supplemental feed on threadsail filefish Stephanolepis cirrhifer . Nippon Suisan Gakkaishi (Japanese Ed 81:701–714.

MOLECULAR MECHANISMS INVOLVED IN ATLANTIC HALIBUT (*Hippoglossus hippoglossus*) EGG QUALITY

O. Yilmaz[1*], A. Mangor Jensen[1], T. Harboe[1], M. Møgster[1], R. Mangor Jensen[1], O. Mjaavatten[2], E. Birkeland[2], E. Spriet[3], L. Sandven[3], T. Furmanek[4], F.S. Berven[2] and B. Norberg[1]

[1]Institute of Marine Research, Austevoll Research Station, 5392, Storebo Norway
[2]The Proteomics Facility of the University of Bergen (PROBE), 5009 Bergen, Norway
[3]The Molecular Imaging Center (MIC), University of Bergen, 5009, Bergen, Norway
[4]Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817, Bergen, Norway
email: ozlem.yilmaz@hi.no

Introduction

Egg quality has a powerful influence on reproductive success. It remains a serious problem of largely variable cause(s) in human reproductive medicine (Keefe et al., 2015) and livestock production (Bobe and Labbé, 2010). Recent research in teleosts has focused on differential abundance of maternal mRNA and protein stockpiles deposited in the egg for clues to the origin of the problem (Cheung et al., 2019; Ma et al., 2019; Sullivan et al., 2015). Earlier studies revealed several impaired mechanisms involved in early development related cellular processes in zebrafish (Yilmaz et al., 2021, 2017). The evolutionary conserved stereotypical procedure of cellular events led us to investigate whether these findings are common in marine species of aquaculture interest. An unsteady supply of high quality eggs and fry in Atlantic halibut (Hippoglossus hippoglossus), makes this species a perfect candidate to study egg quality related mechanisms. The objective of our study was to analyze proteomic profiles in good (GQ) versus poor quality (BQ) eggs, to identify proteins that can serve as egg quality markers, and to discover molecular mechanisms determining egg quality.

Material and methods

Eggs from a total of 25 spawns were collected in 2019-2021 reproductive seasons. For each egg batch, aliquots were snap frozen at 1 hour post fertilization (1hpf) for subsequent analyses, while 100 ml was incubated for egg quality assessment, based on survival rates prior to hatching at ≃ 12 dpf. Proteomic profiling between GQ and BQ eggs was performed via tandem mass tags labeling (TMT) based liquid chromatography tandem mass spectrometry (LC-MS/MS). Obtained spectra was searched against an in-house built proteome database originated from Atlantic halibut egg transcriptome. Differentially abundant proteins (DAPs) were determined based on p values resolved from Student's t-test ($p < 0.05$) followed by Benjamini Hochberg correction for multiple testing ($p < 0.05$). Functional annotation of DAPs was performed using the GO, KEGG, and UNIPROT databases. Overrepresentation analyses were done via GESTALT using human proteome as reference database. DAPs were additionally tested for protein-protein interaction networks using the STRING Network search tool. Eight DAPs were selected as candidate markers based on fold differences in abundance and were subjected to parallel reaction monitoring (PRM) based targeted LC-MS/MS. Relative abundance of gene expression for 21 DAPs were also determined via TaqMan based quantitative PCR. Eggs from GQ and BQ spawners were also processed for transmission electron microscopic (TEM) evaluations at 1hpf for analysis of differences in mitochondrial number and structure.

Results

A total of 115 proteins were differentially abundant between GQ and BQ eggs with 64 being down-regulated and 51 being up-regulated in BQ eggs compared to GQ eggs. Frequency distribution of DAPs showed clear differences in proteomic profiles between GQ and BQ eggs ($p < 0.05$). Accordingly, GQ eggs seem to contain significantly higher number of proteins related to protein folding, while BQ eggs seem to be enriched with proteins related to transcription, protein degradation and synthesis inhibition, and mitochondrial biogenesis. Overrepresentation-test-based enrichment analysis ($p < 0.05$) revealed significant biological processes, molecular functions and cellular components which are in close agreement with frequency distribution analysis findings. STRING network analysis of proteins down-regulated in BQ eggs revealed a network of three subclusters of proteins related to cytoskeletal regulation and energy homeostasis, protein homeostasis, and fatty acid degradation. Proteins which were up-regulated in BQ eggs formed a network made of two major subclusters of proteins mainly related to mitochondrial biogenesis, energy and protein homeostasis.

(Continued on next page)

PRM based LC-MS/MS results revealed five out of the eight candidate marker proteins (MT-ND5, DHRS9, GATD3a, FBP1, UQCRFS1) to be significantly different in abundance between GQ and BQ eggs. Accordingly, MT-ND5, DHRS9 and GATD3a are down-regulated while FBP1 and UQCRFS1 are up-regulated in BQ eggs. UQCRFS1 and FBP1 were additionally found to be part of the STRING network cluster which was formed by BQ up-regulated proteins. Relative abundance of expressions of 7 out of the 21 tested genes exhibited an increase with the same tendency pattern ($p > 0.05$) and 6 genes exhibited a converse tendency pattern ($p > 0.05$) to protein abundance, while 8 genes exhibit a similar increasing tendency pattern to protein abundance and significant differences between GQ and BQ eggs ($p < 0.05$). These genes (*cyc1*, *fh*, *gcn1*, *ghitm*, *uqcrb*, *uqcrfs1*, *fbp1a*, and *atp5f1a*) were also found within the STRING network cluster revealed to be significant in BQ up-regulated proteins. The overall findings were additionally supported by TEM observations which revealed clear signs of differences in mitochondrial number and structure between GQ and BQ eggs.

Conclusions

Frequency distribution, enrichment and protein-protein interaction analyses of DAPs between GQ and BQ A. halibut eggs revealed matching results pointing to impairments in mitochondrial structure and functions and protein folding related activities. Additional findings based on TEM imaging fortifies the hypothesis concerning the impairments in mitochondrial structure and functions. The overall findings were found to be corresponding to previous findings in zebrafish and thus there seem to be high similarities in the pattern of impaired molecular mechanisms in BQ eggs of zebrafish, a well-established biomedical model, and Atlantic halibut, a representative of marine flatfishes with aquaculture interest. We expect discoveries of such mechanisms in poor quality eggs to spur development of practical strategies to determine and eliminate the potential causes leading to egg quality problems in Atlantic halibut and other farmed fishes, thereby contributing significantly to development of effective strategies for improving breeding practices and sustainable growth of Norwegian and global aquaculture.

References

Bobe, J., Labbé, C., 2010. Egg and sperm quality in fish. Gen. Comp. Endocrinol. 165, 535–548. https://doi.org/10.1016/j.ygcen.2009.02.011

Cheung, C.T., Nguyen, T., Le Cam, A., Patinote, A., Journot, L., Reynes, C., Bobe, J., 2019. What makes a bad egg? Egg transcriptome reveals dysregulation of translational machinery and novel fertility genes important for fertilization. BMC Genomics 20, 584. https://doi.org/10.1186/s12864-019-5930-8

Keefe, D., Kumar, M., Kalmbach, K., 2015. Oocyte competency is the key to embryo potential. Fertil. Steril. 103, 317–322. https://doi.org/10.1016/j.fertnstert.2014.12.115

Ma, H., Martin, K., Dixon, D., Hernandez, A.G., Weber, G.M., 2019. Transcriptome analysis of egg viability in rainbow trout, Oncorhynchus mykiss. BMC Genomics 20, 319. https://doi.org/10.1186/s12864-019-5690-5

Sullivan, C.V., Chapman, R.W., Reading, B.J., Anderson, P.E., 2015. Transcriptomics of mRNA and egg quality in farmed fish: Some recent developments and future directions. Gen. Comp. Endocrinol. 221, 23–30. https://doi.org/10.1016/j.ygcen.2015.02.012

Yilmaz, O., Patinote, A., Com, E., Pineau, C., Bobe, J., 2021. Knock out of specific maternal vitellogenins in zebrafish (Danio rerio) evokes vital changes in egg proteomic profiles that resemble the phenotype of poor quality eggs. BMC Genomics 22, 308. https://doi.org/10.1186/s12864-021-07606-1

Yilmaz, O., Patinote, A., Nguyen, T.V., Com, E., Lavigne, R., Pineau, C., Sullivan, C.V., Bobe, J., 2017. Scrambled eggs: Proteomic portraits and novel biomarkers of egg quality in zebrafish (Danio rerio). PLOS ONE 12, e0188084. https://doi.org/10.1371/journal.pone.0188084

PIGMENT QTL GENOTYPE BY DIET INTERACTION ON GROWTH IN ATLANTIC SALMON (*Salmo salar*)

P. Sae-Lim*, S. Boison, S. Gonen, M. Baranski , J. Å. Stene, G. Rosenlund, B. Hatlen, B. Ruyter, R. Fontanillas, L. Martinez Rubio and A. Norris

Mowi Genetics AS, Sandviksbodene 77AB, 5035 Bergen, Norway
Email: Panya.Sae-Lim@mowi.com

Introduction

Growth and fillet color are two important traits in farmed Atlantic salmon. The color of the fillet results from an uptake of supplementary astaxanthin - an antioxidant which has a positive effect on growth in early life (Bazyar Lakeh et al., 2010). However, the ability of fish to utilize and retain astaxanthin is limited while astaxanthin in the diet accounts for approximately 2–4% of feed cost. This trait is highly heritable, and in recent years there has been progress in understanding the underlying biological pathways, including identification of a number of QTLs and candidate genes that explain a large portion of the genetic variance (Helgeland et al., 2014). The relationship between astaxanthin deposition and growth is not fully understood but are likely to be connected because of the antioxidant effect of the pigment. Furthermore, such association or interaction may depend on the amount of marine omega-3 in the diet which has a positive effect on fillet color and robustness (Bou et al., 2021). The aim of this study was to examine the pigment QTL genotype by diet interaction in Atlantic salmon in terms of its effect on growth.

Materials and Methods

Data in this study was from AquaIMPACT project (H2020 BG2018-818367). Previously, Mowi Genetics had performed genome-wide association studies for pigment levels in several year classes of fish from the breeding nucleus using a ThermoFisher Axiom genotyping array containing approximately 55,000 single nucleotide polymorphism (**SNP**) markers distributed over the salmon genome. From this data, two major QTLs were mapped, with the locus having the largest effect being subsequently used in this study. In June 2020, 959 Atlantic salmon with known pedigree and pigment QTL genotype from the Mowi breeding nucleus in Norway were distributed across six 5x5m^2 sea pens located in Averøy, Norway. The sea pens were evenly split between two different diets (3 replicates each) from Skretting ARC: a common extruded feed kernel coated with a standard oil mix rich in vegetable oil (standard diet or **K**) or coated with an oil mix (Veramaris®) enriched with omega-3-PUFA from microalgal oil (diet **H**). A bivariate animal mixed model, where body weights from different diets (measured in March 2021) were treated as different traits, was fitted using **REML** in WOMBAT with genomic relationship matrix (**GRM**) generated from 53,186 SNP markers. The genomic based heritability ($\boldsymbol{h^2}$) for growth, genetic correlation ($\boldsymbol{r_g}$) – as a measure of genotype re-ranking and their SE were subsequently estimated.

Results

The genomic based h^2 for growth with diet H (0.40±0.01) was slightly higher than for growth with diet K (0.35±0.01). The r_g was close to unity (0.94±0.09), indicating marginal or no genotype re-ranking of growth between H and K diets. The plot of phenotype-genotype association shows no re-ranking of pigment QTL genotypes across different diets (**Fig 1**). For the K diet, high pigment deposition genotype (**PP**) had higher and significant ($P<0.05$) mean weight (2.80 kg) than low pigment deposition genotype (**pp**; 2.67 kg). The use of H diet resulted in considerably positive effect on growth in both genotypes but the difference in mean weight between PP and pp genotypes became less and statistically non-significant ($P>0.05$). Mean genomic EBVs shows the same pattern.

Discussion and Conclusions

To the best of our knowledge, this is the first report on the association between pigment QTL genotype and growth in Atlantic salmon. The genomic based h^2 for growth is in the upper range with the previous estimates reported in literature. Higher growth in PP than in pp indicates that pigment genotypes may be associated with growth but it may be diet-dependent trait as the difference in growth is less in H than in K. Selection for PP genotype not only increases astaxanthin deposition, leading to less pale fillet and higher level of antioxidant for health benefits, but also higher growth. Further study to confirm this finding should be conducted.

(Continued on next page)

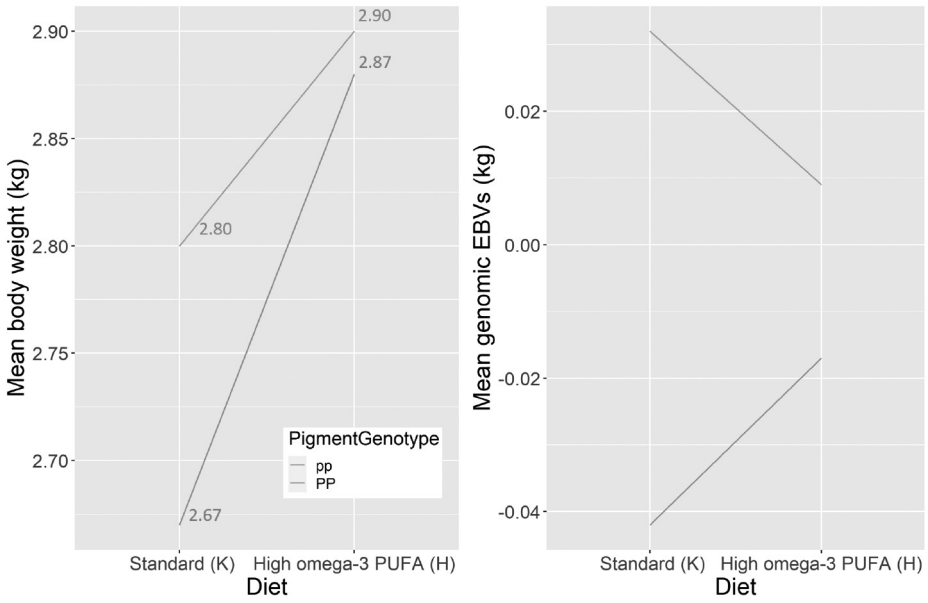


Fig 1. The observed phenotype-genotype association (*left*) when farmed Atlantic salmon fed by two different diets. The plot of genomic EBVs of growth for different diets (*right*) show no genotype re-ranking.

References

Bazyar Lakeh, A.A., Ahmadi, M.R., Safi, S., Ytrestøyl, T., Bjerkeng, B., 2010. Growth performance, mortality and carotenoid pigmentation of fry offspring as affected by dietary supplementation of astaxanthin to female rainbow trout (*Oncorhynchus mykiss*) broodstock. Journal of Applied Ichthyology 26, 35–39.

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Østbye, T. K., Ruyter, B., 2021. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages. Journal of Nutritional Science 6, 1–14.

Helgeland, H., Sandve, S.R., Torgersen, J.S., Halle, M.K., Sundvold, H., Omholt, S., Våge, D.I., 2014. The evolution and functional divergence of the beta-carotene oxygenase gene family in teleost fish—Exemplified by Atlantic salmon. Gene 543, 268–274.

INTERMITTENT FEEDING AND GROWTH OF EUROPEAN SEABASS: INSIGHTS FROM GENE EXPRESSION AND BLOOD BIOCHEMICAL PARAMETERS

Ntantali O.[1]*, Malandrakis E.E.[2], Abbink W.[3], Golomazou E.[1], Bastiaansen J.W.M.[3], Chatzoglou E[2]., Miliou E.[2] and Panagiotaki P.[1]

[1]Laboratory of Aquaculture, Department of Ichthyology and Aquatic Environment, University of Thessaly, Fitokou str., Volos, Greece
[2]Laboratory of Applied Hydrobiology, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, Athens, Greece
[3]Animal Breeding and Genomics, Wageningen University and Research, Droevendaalsesteeg 1, Wageningen, The Netherlands.
*E-mail: ntolga@uth.gr

Introduction

In nature, fish regularly have to deal with a lack of food, and subsequently have evolved mechanisms of coping with limited food availability. Compensatory growth, during a specific period of time, is considered significantly faster than the growth rate of fish that have not experienced feed deprivation (Nikki *et al*., 2004). European seabass, *Dicentrarchus labrax*, a widely distributed marine teleost, is considered of great commercial importance, as it exhibits high growth rates, high market demand, and good feed-conversion ratio values. Many interacting environmental and genetic factors play a role in the various physiological pathways of fish growth. Expression levels of specific mRNAs can explain variation in growth rates, and therefore can be used as valid biomarkers for fish selection. The study will present information on identification of target-genes related to the nutritional status during short-term intermittent feeding in European seabass, that can be used as potential biomarkers for breeding programs.

Materials and Methods

After a 4-week acclimatization period to laboratory conditions, 540 graded fish weighing 87.5±16.2 g were randomly distributed into two treatments with three replicate tanks per treatment in a recirculating aquaculture system (RAS). Fish of the first treatment (control) were fed continuously to apparent satiation for 40 days, whereas the second treatment was fed in a feeding-fasting scheme of 80-20% (2 days of fasting followed by 8 days of refeeding to apparent satiation) for 40 days. Water quality parameters were monitored daily.

At the end of the experiment (D40), all fish were graded in two groups according to their final weight (FG-fast growers and SG-slow growers from the fasted treatment and from the control treatment; four treatment-group combinations) and sacrificed with phenoxyethanol overdose (1 ppt). Growth parameters, serum glucose, cholesterol, triglycerides, non-esterified fatty acids (NEFA) and lactate dehydrogenase (LDH) activity were measured according to standard protocols.

Total RNA was extracted from liver tissue and white muscle and isolated RNA quantity and quality were determined via spectrophotometry. For cDNA synthesis, 500 ng of RNA were reverse transcribed with PrimeScript RT Reagent Kit. The qPCR analysis was carried out with RT^2 SYBR Green qPCR Mastermix (Qiagen) in a Rotor-Gene Q 5plex. The qPCR assay was designed for the profiling of six glycolytic genes *(hk, gpi, eno1, g3pdh, alda, pk*), two genes related to fatty acid metabolism (*lpl* and *acc*) and two genes involved in cholesterol biosynthesis (*sqle, 7dhcr*). The geometric mean of β-actin (*actb*) and elongation factor 1 (*ef1*) expression was used as normalization factor.

Results

Within the FG and SG groups, the experimental feeding treatment did not significantly affect final body weight. Among slow growers (SG) the intestine somatic index (ISI) was smaller in fasted fish ($P < 0.05$). However, dietary treatments did not significantly affect condition factor (CF), hepatosomatic index (HIS), viscerosomatic index (VSI), spleen somatic index (SSI) and intraperitoneal fat ratio (IFR).

Glucose and NEFA levels were different between fed SG and FG ($P < 0.05$), and such difference was not observed for their fasted counterparts. Glucose of fed FG fish was significantly higher (27.6 ± 1.1 mg/dL) than in fed SG fish (16.9 ± 1.7 mg/dL) and NEFA of fed FG fish was significantly lower (5.6 ± 0.3 mg/dL), than in fed SG fish (10.7 ± 1.4 mg/dL).

(Continued on next page)

In the muscle tissue, all genes demonstrated significant differential expression in at least one -treatment-group, while in liver 5 genes (*g3pdh, alda, gpi, hk, 7dhcr*) out of 11 exhibited no significant difference ($P > 0.05$). Muscle transcription levels of *g3pdh, alda, gpi, ldha* exhibited upregulation in fed FG compared to fed SG, although such differences were not observed between fasted fish groups. Furthermore, mRNA levels of *g3pdh, hk, pk, lpl, acc, sqle, 7dhcr* were significantly different ($P < 0.05$) between fish in different dietary treatments (fed versus fasted), regardless of the growth group (SG or FG). In the liver tissue, only *pk* was significantly up-regulated ($P < 0.05$) in fed fish compared with the fasted ones, for both growth groups. *ldha, lpl* and *acc* mRNA levels were differentially expressed between fed and fasted fish, only for the SG group.

Discussion

In aquaculture, feeding strategies are critical because feeds typically represent more than 40-50% of the production cost and both underfeeding and overfeeding can have negative consequences for the production. As fish are relatively high-value products, conditions for successful aquaculture farming strongly relies on feed management (Chatzifotis *et al*., 2011). A key factor in view of obtaining the desired growth compensation after feed deprivation for a specific species, is to manage the appropriate duration and intensity of feed deprivation.

In the present study, the final weight between the fast growing (FG) and slow growing (SG) groups were not significantly affected by the short-term food deprivation periods. Any potential loss of weight during the 2-day (out of 10 days) fasting period was compensated during the refeeding to satiation periods, at least for a 40 days experimental period. The short-term intermittent fasting did not have substantial effect either on condition factor or other zootechnical parameters. No differences in blood biochemical parameters were observed between fed and fasted fish, supporting the hypothesis that short-term intermittent fasting (2 days out of 10) does not affect fish physiological status in the long term. On the contrary, expression of certain genes differentiated between groups. In conclusion, the study provides new insights on the impact of intermittent feeding of European seabass on gene expression levels.

Acknowledgements

This research was financed by the AQUAEXCEL2020 project (grant agreement no. 652831), under the TNA programme GeneComp: "Compensatory growth and single nucleotide variation in European sea bass (*Dicentrarchus labrax*)" (Project ID AE120002).

References

Chatzifotis, S. et al. (2011) 'Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (*Dicentrarchus labrax*)', Aquaculture, 316(1–4), pp. 53–59. doi: 10.1016/j.aquaculture.2011.02.044.

Nikki, J. *et al*. (2004) 'Compensatory growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), held individually', *Aquaculture*, 235(1–4), pp. 285–296. doi: 10.1016/j.aquaculture.2003.10.017

PRELIMINARY COMPARISON OF THREE DIFFERENT INDUCTION METHODS FOR THE SPAWNING OF EUROPEAN ABALONE (*Haliotis tuberculata coccinea)*

B. Costa[ab], C. Nunes[a*], M. De Girolamo[a], E. Isidro[a]

[a]Okeanos – Institute of Marine Sciences, Rua Prof. Doutor Frederico Machado, 4 – 9901- 862 Horta, Faial, Açores, Portugal. [b]Aquaculture and Fisheries Group, Wageningen University Research, the Netherlands
Corresponding author: E-mail: mirko.d.girolamo@uac.pt

Introduction

Abalone spawning induction is considered to be well described, with three main reported methods: thermal shock, hydrogen peroxide and UV irradiation (Leighton, 1972; Moss et al., 1995; Leighton, 2008). Although all methods have been described to induce spawning in *H. tuberculata coccinea,* no comparative study has been conducted on the performance of each method. The aim of this study is to understand which method among thermal shock (Temp), hydrogen peroxide (H2O2), and UV irradiation (UV) is the most adequate for spawning induction of *H. tuberculata coccinea*, in order to optimize the reproductive performance of the species.

Materials and Methods

F2 broodstock of European abalone cultured at 20°C were selected according to weight (13.4 ± 4.5g) and gonad development (Stage 3). Animals were divided in groups consisting of 14 animals, 7 males and 7 females per treatment. Each specimen was kept in separate container, filled with 800ml of 1μm filtered sea water (FSW). Control (Ctrl) animals were placed in the spawning containers and only disturbed for spawning checks. Temp treatment was performed introducing specimens in preheated FSW at 23°C. The temperature was maintained for 1.5h. If no gametes were released, the temperature was increased to 25°C for 0.5h. H2O2 spawning induction method was carried out according to the description of Courtois de Viçose (2011). UV treatment was conducted using a flow through system with UV treated sea water with a calculated irradiation of 600m.W.h.l^{-1} applied for 2h. All treated animals were checked for spawning at every 30min for the 5h extent of the trail. When a spawning event occurred a 30min interval was given before sample collection. Eggs and sperm were counted for density (cell/ml). 50 eggs/ind were photographed and analyzed for diameter (mm) and area (mm^2).

Results and Discussion

H2O2 and Temp were the only treatments that induced spawning in the tested animals, in contrast with the initial expectation that all treatment, besides control, would be able to promote these events. According to Kikuchi and Uki (1974) time in between the initiation of the UV stimulation and spawning is inversely related to the amount of UV irradiation. According to Courtois de Viçose (2011) *H. tuberculata coccinea* starts spawning after 2 – 3 hours with a irradiance of 800m.W.h.L^{-1}. This way we might assume that our irradiance and stimulation time was not adequate for spawning. From the two treatments which induced spawning events, H2O2 had the highest percentage of spawning for both males and females. H2O2 induced spawning in 71% of the males and in 86% of the females compared to 29% and 43% for males and females respectively in the temperature treatment. No significant differences were observed for the frequency of spawning in males and females between the two treatments (χ^2 test, $p_{Treatment*female} = 0.094$; $p_{Treatment*male} = 0.109$). When looking at the number of gametes emitted by males, H2O2 presented a higher cell concentration (1.7E+07 ± 9.0E+06 cell/ml) compared to the Temp treatment (9.8E+06 ± 8.9E+06 cells /ml). The inverse was observed for the female gametes which revealed a higher concentration emitted in Temp (≈200 ± 96 cell/ml), compared with H2O2 (≈159 ± 52 cell/ml). Female gametes also showed significant differences in area (mm^2) and diameter (mm) between treatments, having higher values for Temp treatment (Fig. 1).

Conclusion

These results even though preliminary in variables and analysis, reveal that there might be differences in gamete quantity and quality when using different spawning induction protocols, suggesting further research into the influence of these protocols on the reproductive performance of *H. tuberculata coccinea*.

(Continued on next page)

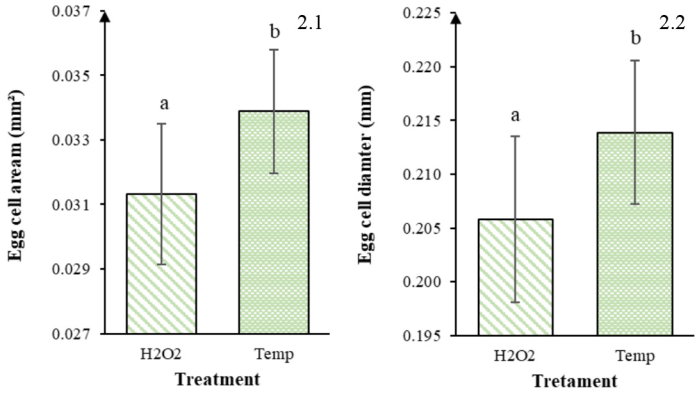


Figure 1 – Mean values of female egg cell area (mm²) (2.1) and egg cell diameter (mm) (2.2) of H2O2 and Temp treatments. Error bars represent standard deviation. Different letters represent significantly different results ($p<0.05$) of means by a T-test with equal assumed variances.

Acknowledgments

This work was carried in the framework of project AQUAINVERT (ref. INTERREG MAC 2/1.1a/282.

References

Leighton, D. L. (1972). Laboratory observations on the early growth of the abalone, Haliotis sorenseni, and the effect of temperature on larval development and settling success. Fish. Bull, 70(2), 373.

Kikuchi, S. and Uki, N., 1974. Technical study of artificial spawning of abalone, genus Haliotis III. Reasonable sperm density for fertilization. Bull. Tohoku Reg. Fish. Res. Lab. 24, 67-71

Leighton, P. (2008). Abalone Hatchery Manual - Aquaculture Explained No. 25. Irish Sea Fisheries Board, 25, 1–89.

Courtois de Viçose, G. C. (2011). *Early life of the abalone Haliotis tuberculata coccínea: development, settlement and growth* (Doctoral dissertation, Universidad de Las Palmas de Gran Canaria).

INVASIVE VS. NON-INVASIVE METHODS OF REPRODUCTION IN *Patella candei* D'ORBIGNY, 1840: FROM FERTILIZATION TO SETTLEMENT

[a]C. Nunes*, [b]A. Ramirez, [b]J. Rodeia, [a]M. De Girolamo and [a,b]E. Isidro

[a]Okeanos – Institute of Marine Sciences, Rua Prof. Doutor Frederico Machado, 4 – 9901- 862 Horta, Faial, Açores, Portugal
[b]IMAR – Marine Institute, Rua Prof. Dr. Frederico Machado, n° 4, 9901-862 Horta, Açores, Portugal
*E-mail: carla.pr.nunes@uac.pt

Introduction

P. candei has great economic value in the Macaronesia region. The interest on developing an aquaculture production of this resource has grown in recent years, due to high demand and sensitivity to exploitation (Hawkins *et al*., 2000). Therefore, this study is focused on the performance of two different methods of obtaining *P. candei* larvae: one by spawning induction, a non-invasive method, and another one using dissected gonads. Differences between methods were evaluated on the base of survival, settlement and growth of the larvae. As the suitability of a substrate with adequate biofilm coverage is a key factor for the induction of metamorphosis to the juvenile stage (Ferranti et al. 2018), the effect on the settlement and growth in two types of biofilms, *Ulvella lens* and *Ulvella leptochaete,* was also analysed. The results presented here represent the first steps on the definition of a reproduction protocol for *P. candei* and the optimization of larval and post-larval rearing.

Materials and methods

Wild *P. candei* specimens were collected from intertidal areas in Faial Island (Azores). Reproduction trials were performed in an acclimatized room, with filtered (1μm) and UV-sterilized seawater (FSW) at 17.3±0.4ºC (±SD).

Spawning induction (SI): The protocol, based upon the work of Ferranti *et al*. (2018), tested five treatments: intense bubbling, UV-light, hydrogen peroxide (H_2O_2) at 6%, thermal shock and a control group (FSW). All treatments had 8 independent replicates (1 *P. candei* per 500ml experimental container; sexes were not identified). Treatments lasted until the first individuals started to spawn (4h30min). Oocytes obtained were then fertilized with a mixed sperm sample obtained from the male spawners (table 1). Sperm acquired with the H_2O_2 (6%) treatment was not used.

Artificial reproduction (AR): At least 2 individuals from each of the treatments tested in the SI were sacrificed for the extraction of mature gonads. The protocol used for AR was based upon Peréz *et al*. (2016). Oocytes from 3 females (262.7oocyte.ml^{-1}) were matured in NH_4OH for 3 hours, resulting in 90.5±8.2% of active oocytes. A mixed sample of sperm obtained from 5 males was activated in FSW for 4h before fertilization (table 1).

Larval rearing and settlement: Swimming trocophora were reared at room temperature until they reached the competent pediveliger stage (table 1). They were then transferred to a 30l settlement tank, containing 6 corrugated fiberglass plates (172 800mm^2 total area per plate). Three plates had *U. leptochaete* as biofilm and three had *U. lens*. Progeny obtained from SI and AR were always kept separate.

Table 1 Fertilization conditions and the product of each type of reproduction at various stages of *P. candei* life cycle.

Stage	Age (days)	Spawning induction	Artificial reproduction
Fertilization		28.8±5.7oocyte.ml^{-1} 4.66x10^{5}sperm.ml^{-1}	53.3±0.6oocyte.ml^{-1} 4.66x10^{5}sperm.ml^{-1}
Gamete contact time		3h	3h
Fertilization rate		98.3 ± 3.0%	83.7 ± 4.0%
Density of trocophora	1	10.0 ± 2.8 ind.ml^{-1}	8.7 ± 3.9 ind.ml^{-1}
Density of pediveliger	3	10.0 ± 4.9 ind.ml^{-1}	4.8 ± 0.8 ind.ml^{-1}
Larval survival (72h)	3	100%	55.7%
Density settlement tanks	3	1.0 ind.ml^{-1}	0.5 ind.ml^{-1}
Settlement *U. lens*	30	0.48 ± 0.39%	0.07 ± 0.05%
Settlement *U. leptochaete*	30	0.04 ± 0.02%	0.02 ± 0.02%
Total survival	30	1.6%	0.3%

(Continued on next page)

Results and discussion

This study provides the first record of a successful spawning induction in *P. candei*. The most effective treatment was intense bubbling in which 37.5% of individuals spawned. Thermal shock induced 25% and exposure to H_2O_2 induced spawning in 12.5% of the individuals. There were no significant differences between treatments where gamete emission occurred (Fisher's exact test of independence, $p_{\text{Bubbling vs. H2O2}} = 0.2846$; $p_{\text{Bubbling vs. Thermal shock}} = 0.50$; $p_{\text{Thermal shock vs. H2O2}} = 0.50$).

Regarding fertilization, it was successful both with gametes obtained through SI and AR. The amount of pediveligers obtained (table 1) was significantly higher (repeated measures ANOVA, followed by a Tukey post hoc test, $p<0.05$) in the SI larvae. Overall survival was also higher in SI (table 1). Deformed larvae were only found in the AR larval culture. This could be consequence of over exposure of mature oocytes to NH_4OH, during the egg maturation process (Castejón *et al*., 2020). Post-larvae obtained from SI also performed better in terms of growth and overall survival after 30 days post-fertilization. Post-larvae from the SI had 0.456 ± 0.014mm (±SE) in length, which was significantly higher (Mann-Whitney U test, $p < 0.05$) than the ones originated by the AR (0.360 ± 0.033mm). They were also larger in width but not significantly different (Mann-Whitney U test, $p = 0.06$).

Overall, the settlement percentages on *U. lens* were higher than *U. leptochaete* both for SI and AR (table 1), although the differences between the biofilms were not statistically significant (Mann-Whitney U test, $p_{SI} = 0.100$; $p_{AR} = 0.400$). Additionally, post-larvae obtained by SI and grown on *U. lens* were significantly bigger (Mann-Whitney U test, $p<0.05$) in size (0.456±0.014mm in length and 0.380 ± 0.014mm in width) than larvae grown on *U. leptochaete* (0.309 ± 0.039mm in length and 0.225 ± 0.049mm). This trend was also verified in AR post-larvae (*U. lens*: 0.360 ± 0.033mm in length and 0.295 ± 0.039mm in width; *U. leptochaete*: 0.345 mm in length and 0.275mm in width). Although the highest settlement obtained was only 0.48 ± 0.39% in *U. lens*, these data represent a baseline for future settlement studies with *P. candei*.

Acknowledgments

This work was carried in the framework of project AQUAINVERT (ref. INTERREG MAC 2/1.1a/282).

References

Castejón, D., Cañizares, J.M., Nogueira, N. and Andrade, C. (2020). Artificial maturation and larval production of the limpet *Patella aspera* Röding, 1798 (Patellogastropoda, Mollusca): Enhancing fertilization success of oocytes using NaOH-alkalinized seawater. *Aquaculture Research*. 2020;00:1–11.

Ferranti, M.P., Monteggia, D., Asnaghi, V. and Chiantore, M. (2018). Artificial reproduction protocol, from spawning to metamorphosis, through non invasive methods in *Patella caerulea* Linnaeus, 1758. *Aquaculture Research*, 49, 3386–3391.

Hawkins S.J., Corte-Real H.B., Pannacciulli F.G., Weber L.C. and Bishop J.D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia* 440, 3–17.

Pérez, S., Fernández, N., and Ribeiro, P.A. (2016). Standardization of a *Patella* spp. (Mollusca, Gastropoda) embryo–larval bioassay and advantages of its use in marine ecotoxicology. *Ecotoxicology and Environmental safety*, 127, 175–186.

OWNERSHIP PERCEPTION OF SUSTAINABLE DEVELOPMENT IN AQUACULTURE: AN EVENT STUDY OF SHAREHOLDER'S REACTION TO NEWS CONCERNING SUSTAINABILITY

R. Nygaard, *F. Asche, and B. Misund

University of South Eastern Norway
Raveien 215
3184 Borre, Norway
rune.nygard@usn.no

Producing food in a sustainable way to feed the world's growing population is a global challenge. Many claim aquaculture to be an important part of the solution. However, the industry has been criticized for not solving many sustainability challenges, such as emissions, escapees and disease. These issues are increasingly important for consumers of aquaculture products, and there is evidence that they are willing to pay a premium for sustainable products, potentially adding revenues to producers. Other stakeholders, like local societies, regulators and equity holders of aquaculture companies (green investors), have increasingly been working as pressure groups for the industry to move towards more sustainable production processes. However, this will not come without significant costs: New technology, equipment, on the job training and compliance routines require time and capital investments for successful implementation. New investments may come through either yearly profits, bank loans, issue of new shares, or a combination of these. All of these alternatives will affect return on equity (ROE). If the company is publically listed, the daily stock price is also affected by these decisions. In other words, moving towards more sustainable production processes is not only likely to affect the value of owners' equity, but its implementation may also need further investments from owners. We therefore believe that understanding ownership perception of issues concerning sustainability is critical for the industry going forward.

To address ownership perception of issues concerning sustainability we look at Norwegian salmon aquaculture using event study methodology. Salmon aquaculture production is the second largest aquaculture species in the world in terms of market value, and the major companies are listed on the Oslo Stock Exchange. They also follow IFRS accounting reporting standards. Event dates are determined through media databases and stock exchange announcements. These are categorized as either positive or negative sustainability news. We then combine these with financial data in an event study model and find whether there are abnormal share price movements at the event dates (news announcements). Our major findings is that owners perceive negative sustainability news as significantly negative. However, our findings on positive news are inconclusive. Our interpretation of this is that shareholders also have to take potential costs into consideration in deciding whether these news are positive for their investments.

A CLOUD PLATFORM FOR PRECISION AQUACULTURE

F. O'Donncha*, A. Akhriev, S.R. Planellas, G. Micallef, J. Grant, M. Lopez, J. Icely, E. Royer, J.G. Ferreira, R. Panicz, P. Eljasik, H. Moore, B.H. Buck, R. Pastres

*IBM Research – Ireland, Damastown Ind. Park, Mulhuddart, Dublin 15
Email: feardonn@ie.ibm.com

Introduction
Precision aquaculture is founded on a set of disparate, interconnected sensors deployed in aquatic environments to monitor, analyse, interpret, and provide decision support for farm operations. This trend parallels developments in agriculture where sensors and other observing technologies lead to enhanced insight into crop production as well as animal health and welfare (Precision Livestock Farming or PLF). The precision aquaculture fundamental approach has been summarised as a series of steps: observe, interpret, decide, and act (Føre et al., 2018).

One of the major challenges in Internet of Things (IoT) applications is the effective management of large amounts of time-series data and associated predictive models. Besides the sheer volume of data, substantial complexity arises from the heterogeneity of data sources. In aquaculture farms, these IoT data encompass multiple sensor and data types, with little or no uniformity in terms of communication protocols, or semantic descriptors explaining the data (O'Donncha & Grant, 2020). In this talk we present a service to integrate data from aquaculture sites (with data from pertinent external sources) and interrogate site conditions with a combination of machine learning and mechanistic models.

Materials and Methods
We demonstrate a cloud-based system for contextual IoT time-series data and model management at scale. The framework is designed to provide a bridge between the disparate data collected at aquaculture farms and the data scientists and subject-matter experts who analyse, model, and interpret the data. A contextual layer provides meta-descriptors to the data to aid the development of models which are then seamlessly stored and deployed in a cloud production environment. The tight coupling of time series data and associated models allows for efficient monitoring, visualisation, and retraining of the models at scale (i.e. hundreds-to-thousands of models). The main features of the system are: (1) an efficient pipeline for ingesting IoT time series data in real time; (2) a scalable, hybrid data management service for both time series and contextual data; (3) a versatile semantic model for contextual information which can be easily adapted to different application domains; (4) an abstract framework for interacting with the system in R or Python; (5) deployment services which automatically train and/or score predictive models upon user-defined conditions.

The architecture is composed of cloud-based microservices and provides an efficient pipeline for real-time data ingestion, time series, and model data management based on unified and intuitive application programming interfaces (APIs) to interact with the data and models.

Both the data scientist and the end user interact with the system using IBM Watson® Studio. This provides a unified user interface where the data scientist, the subject-matter expert, and the end user can collaborate and iterate to visualise and analyse (automated) time series forecasts, and augment with bespoke modelling systems that address specific farm requirements (as an example, in a related paper we describe how the system was used to interrogate the relationships between environmental data and hydroacoustic estimates of distribution of caged salmon (O'Donncha et al., 2021)). Figure 1 presents an overview of the different components of the service.

Results and Discussion
We present an overview of the system from the perspective of the data scientist, subject-matter expert, and farmer. Specifically, we describe the process of integrating data from disparate sources (including environmental sensors, hydroacoustic sensors, operational welfare indices, and open-ocean data from the E. U. Copernicus Marine Information Service) and contextualising with a semantic layer. We detail the development of time-series forecasting models of environmental variables in a scalable framework using serverless cloud, and an automated monitoring framework that tightly couples observation and forecast. Finally, we describe the interaction with the system using IBM Watson® Studio and how it enables a unified development (for both the data scientist and the subject matter expert) and dissemination platform to operationalise the heterogenous datasets collected on aquaculture farms. Results are presented for offshore, inshore, and land-based farms.

(Continued on next page)

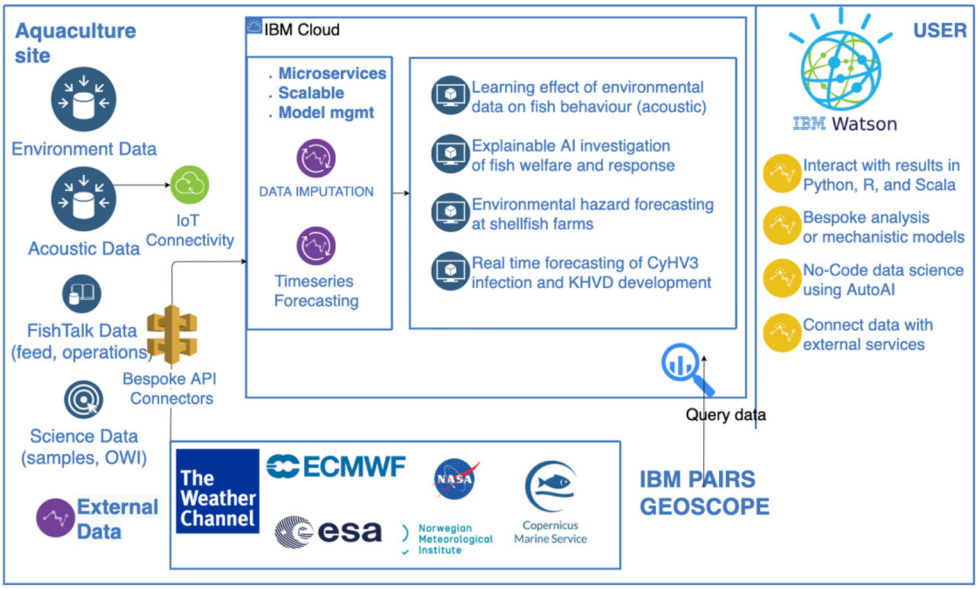


Figure 1: Overview of the system presenting IoT data integration, connectors to external data, microservices and automated model training and scheduling, and user interaction with the system in terms of bespoke (species and farm specific) model development, and dissemination to end user

References

Føre, M., Frank, K., Svendsen, E., Alfredsen, J. A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L. M., Schellewald, C., Skøien, K. R., & Alver, M. O. (2018). Precision fish farming: A new framework to improve production in aquaculture. *Biosystems Engineering*, *173*, 176–193. https://doi.org/10.1016/J.BIOSYSTEMSENG.2017.10.014

O'Donncha, F., & Grant, J. (2020). Precision Aquaculture. *IEEE Internet of Things Magazine*, *2*(4), 26–30. https://doi.org/10.1109/iotm.0001.1900033

O'Donncha, F., Stockwell, C. L., Planellas, S. R., Micallef, G., Palmes, P., Webb, C., Filgueira, R., & Grant, J. (2021). Data Driven Insight into Fish Behaviour and their use for Precision Aquaculture. *Manuscript Submitted for Publication*.

DATA DRIVEN INSIGHT INTO FISH BEHAVIOUR AND THEIR USE FOR PRECISION AQUACULTURE

F. O'Donncha[1], Caitlin L. Stockwell[2], Sonia Rey Planellas[3], Giulia Micallef[4], Paulito Palmes[1], Chris Webb[5], Ramon Filgueira[6], Jon Grant[2]

[1]IBM Research – Ireland, Damastown Ind. Park, Mulhuddart, Dublin 15
Email: feardonn@ie.ibm.com
[2]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4R2 Canada
[3]University of Stirling, Scotland
[4]Gildeskål Research Station, Norway
[5] Cooke Aquaculture, Scotland
[6]Marine Affairs Program, Dalhousie University, Halifax, NS B3H 4R2 Canada

Introduction

Precision aquaculture (Føre et al., 2018) involves a variety of sensors used to gain insight into the farm environment, make decisions which optimize fish health, welfare, growth, and economic return, and reduce risk to the environment. Fundamental to those is monitoring of environmental and animal processes within a cage and processing those data towards farm insight using models and analytics. This paper presents an analysis of environmental and fish behaviour datasets collected at three salmon farms in Norway, Scotland, and Canada. Information on fish behaviour were collected using hydroacoustic sensors that sampled the vertical distribution of fish in a cage at high spatial and temporal resolution, while a network of environmental sensors characterised local site conditions. We present an analysis of the hydroacoustic datasets using AutoML (or automatic machine learning) tools that enables developers with limited machine learning expertise to train high-quality models specific to the data at hand. We demonstrate how AutoML pipelines can be readily applied to aquaculture datasets to interrogate the data and quantify the primary features that explains data variance.

Materials and Methods

Hydroacoustic methods provide a proxy measure for density and distribution of marine animals in form of acoustic backscattering (Foote, 2009). Advantages linked to hydroacoustic sampling techniques include, high spatial and temporal resolution, autonomous long-term sampling duration, range (especially during poor visibility when visual-based methods tend to fail), and a non-invasive surveying approach (Scherelis et al., 2020).

This study considers three salmon cage farms in Norway (NOR), Scotland (SCO), and Canada (CAN). For each site several environmental sensors were deployed monitoring a range of parameters, including temperature, dissolved oxygen (DO), and current speed. These were complemented with weather data from *in-situ* weather stations or model generated reanalysis, and open-ocean model from the E.U. Copernicus Marine Service Information model repository.

Data were processed using an automatic machine learning framework. AutoML systems uses a variety of techniques, such as, differentiable programming, tree search, evolutionary algorithms, and Bayesian optimization, to find the best machine learning pipelines for a given task and dataset (Drori et al., 2018)an automatic machine learning (AutoML.

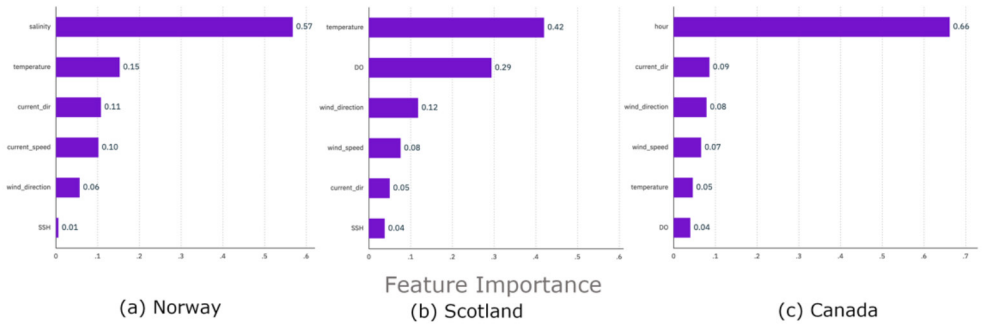


Figure 1: Plot of the relative amount of variance explained by environmental and external drivers of fish behaviour. The x-axis presents the proportion of variance explained for each variable described on the y-axis. Plots are presented for Norway, Scotland, and Canada sites in (a), (b), and (c) respectively.

(Continued on next page)

Results

We interrogated relationships between vertical distribution of fish in a cage and environmental variables at the three geographically disparate sites. Statistical analysis explored the diel patterns, and how data distributions varied over the duration of the study, while IBM AutoAI was used to quantify the effects of environmental variations on the vertical movement of the fish. For the machine learning interrogation, we provided input features that literature suggests influence salmon behaviour (and were available at the study sites). For our study, these were temperature, DO, current speed, wind speed, and salinity, together with hour-of-day (see Fig 1, Y-Axis). The resultant model explained 59%, 64% and 61% of variance for the NOR, SCO, and CAN sites, respectively. Figure 1 presents the variable importance computed for the three locations. While there were similarities in the drivers that influenced fish behaviour at the three sites, pronounced variations existed based on the different geography and characteristics of each site.

Results presented in this paper indicate pronounced differences between sites and the need to consider these variations for farm management. One could readily use this approach to quantify the difference between sites, and further to identify the fundamental drivers to these variations. This could be particularly valuable when comparing different farm systems such as inshore and offshore and the associated operational implications.

References

Drori, I., Krishnamurthy, Y., Rampin, R., De, R., Lourenco, P., Ono, J. P., Cho, K., Silva, C., & Freire, J. (2018). AutoML Workshop. *AutoML Workshop at ICML*. https://www.cs.columbia.edu/~idrori/AlphaD3M.pdf

Foote, K. G. (2009). Acoustic Methods: Brief Review and Prospects for Advancing Fisheries Research. In *The Future of Fisheries Science in North America* (pp. 313–343). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9210-7_18

Føre, M., Frank, K., Svendsen, E., Alfredsen, J. A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L. M., Schellewald, C., Skøien, K. R., & Alver, M. O. (2018). Precision fish farming: A new framework to improve production in aquaculture. *Biosystems Engineering*, *173*, 176–193. https://doi.org/10.1016/J.BIOSYSTEMSENG.2017.10.014

Juell, J., Furevik, D., & Bjordal, A. (1993). Demand feeding in salmon farming by hydroacoustic food detection. *Aquacultural Engineering*, *12*(3), 155–167. https://doi.org/https://doi.org/10.1016/0144-8609(93)90008-Y

Scherelis, C., Penesis, I., Hemer, M. A., Cossu, R., Wright, J. T., & Guihen, D. (2020). Investigating biophysical linkages at tidal energy candidate sites; A case study for combining environmental assessment and resource characterisation. *Renewable Energy*, *159*, 399–413. https://doi.org/10.1016/j.renene.2020.05.109

SIMULATING THE HYDRO-ENVIRONMENTAL EFFECTS OF SUSPENDED AQUACULTURE INSTALLATIONS IN A THREE-DIMENSIONAL NUMERICAL MODEL

Fearghal O'Donncha[1], Michael Hartnett[2]

[1]IBM Research – Ireland, Damastown Ind. Park, Mulhuddart, Dublin 15
Email: feardonn@ie.ibm.com
[2]Department of Engineering, National University of Ireland, Galway

Introduction

Aquaculture structures affect hydrodynamics of their environments by impeding and redirecting flow. Cages, rafts, and lines used in aquaculture systems often form porous suspended canopies extending from the free surface, see Figure 1. Field studies have shown that suspended canopies impact flow profiles in a number of ways, including reduced velocities within a canopy, accelerated flows beneath a canopy, and the development of shear layer processes at canopy interfaces (O'Donncha et al., 2015). Aquaculture canopies may alter vertical velocity profile as shown schematically in Figure 1.

Determining the hydrodynamic properties of these aquaculture systems is important in predicting the transport of nutrients and waste products to and from the system. Simulations can identify ways to improve productivity while minimizing environmental impacts. We applied an amended version of the hydrodynamic model Environmental Fluid Dynamics Code to explore hydro-environmental conditions within Killary Harbour, a fjord-like inlet on the West Coast of Ireland.

Materials and Methods

EFDC (Hamrick, 1996) is a public-domain, open-source, modelling package for simulating three-dimensional (3-D) flow, transport, and biogeochemical processes in surface-water systems. In the modified version of EFDC, we represent physical obstructions to flow by including an additional drag term in the momentum-conservation equation so that momentum was removed from model cells in which the canopy was specified (i.e., the region of the harbour containing aquaculture structures). In this formulation, the model incorporates a momentum sink due to the suspended canopy, as well as modification term terms representing the net changes to turbulent kinetic energy.

An EFDC model for Killary Harbour was developed at a horizontal resolution of 64m with 20 equidistant vertical layers. Open boundary water levels were prescribed on the Western boundary using data from a nearby tide gauge. Freshwater inflows from two rivers (Bundorragha and Erriff) were prescribed, while meteorological data was extracted from a local weather station. Location of aquaculture installations were prescribed from line surveys, and associated model cells were specified with appropriate canopy densities. Model coefficients were fine-tuned based on field data.

Results

We present results on the hydrodynamic calibration of the model against ADCP and water level data. The effects of the aquaculture installations on vertical flow profiles both within and around the canopy were investigated. Model simulations were conducted with three mussel dropper densities (high, medium, and low) and associated hydrodynamics characterised. A significant attenuation of flows through the canopy was observed with flow primarily being diverted under the farms rather than around (farms are land-bounded on one side). Flow dynamics were quantified in terms of velocity differentials of velocity and relative velocity. Figure 2 presents the relative velocity differential computed within the harbour. Differentials are presented relative to unimpeded flows. Hence, a positive value signifies a decrease in flows; while negative values indicate an increase in flows.

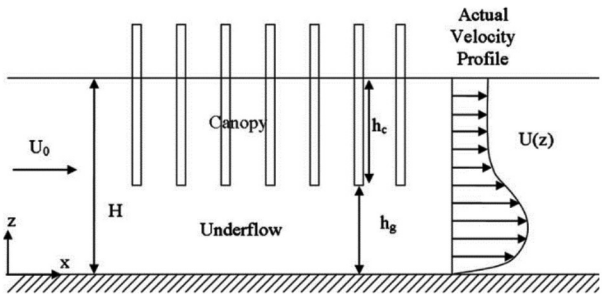


Figure 1: Schematic illustrating the configuration of suspended aquaculture installations representative flow profile that may develop

(Continued on next page)

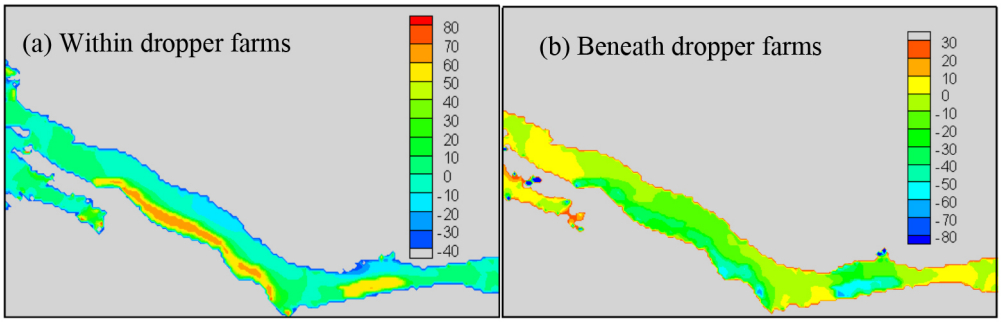


Figure 2: Relative percentage differentials of velocity (a) within mussel dropper farms and (b) beneath mussel dropper farms.

.

Effects of the aquaculture structures on material transport and residence times were also examined. The primary focus was on material transport time scales within the actual aquaculture farms. All cells containing aquaculture installations were coded as having uniform initial concentrations of trace, C_0 with subsequent spatial concentrations, $C_{n,}$ computed after n tidal cycles.

One of the significant conclusions from this research is that the impedance of flows due to the presence of aquaculture structures must be included in models when planning and developing environmental impacts assessments of certain types of aquaculture installations.

References

Hamrick, J. M. (1996). *User's Manual for the Environmental Fluid Dynamics Computer Code Code*. https://doi.org/10.21220/V5M74W

O'Donncha, F., Hartnett, M., & Plew, D. R. (2015). Parameterizing suspended canopy effects in a three-dimensional hydrodynamic model. *Journal of Hydraulic Research*. https://doi.org/10.1080/00221686.2015.1093036

FIVE INTEGRATED MULTITROPHIC AQUACULTURE LABORATORIES, ONE GOAL – RESILIENT AQUACULTURE IN THE FACE OF EMERGING THREATS. ASTRAL PROJECT

Pauline O'Donohoe*[1], Brett Macey[2], Tomás Chalde[3], Luis Poersch[4], Adrian Macleod[5] & Elisa Ravagnan[6]

[1] Marine Institute, Rinville (MI), Oranmore, Co. Galway, Ireland
[2] Department of Forestry, Fisheries & the Environment, Aquaculture Research (DFFE), Sea Point 8001, South Africa & Department of Biological Sciences, University of Cape Town (UCT), Rondebosch 7701, South Africa
[3] Consejo Nacional De Investigaciones Cientificas Y Tecnicas (CONICET), Godoy Cruz 2290, Buenos Aires C1425FQB, Argentina
[4] Universidade Federal Do Rio Grande-Furg (FURG), Avenida Italia Km 8 Campus Carreiros, Rio Grande 96201 900, Brazil
[5] The Scottish Association for Marine Science Lbg (SAMS), Scottish Marine Institute, Dunbeg Oban PA37 1QA, United Kingdom
[6] Norwegian Research Centre As (NORCE), Nygardsgaten 112, Bergen 5008, Norway

*Email: pauline.odonohoe@marine.ie

Introduction

Aquaculture is recognised as being the most promising source of animal proteins (FAO, 2018), however aquaculture faces a challenging future in the face of emerging threats. Increased risk of disease and harmful algae blooms connected with climate change along with production and infrastructure losses from extreme events, fluctuations in water temperature and oxygen levels, ocean acidification, changes in rainfall patterns, create many problems for sustainable aquaculture (Lafferty, 2015). Microplastics are also posing a threat to aquaculture and its products as they are widespread and can be found in aquatic fauna (GESAMP, 2016). ASTRAL will focus on the Integrated Multitrophic Aquaculture (IMTA) production to address these challenges. In IMTA systems, the waste of one crop (fed animals) are converted into fertilizer, food and energy for the other crops (extractive plants and animals). IMTA can reduce the environmental impact, diversify and increase production, lower investment risks, create jobs, increase consumers' trust, as well as supporting sustainable aquaculture and the circular bioeconomy (Chopin, 2015). The aim of ASTRAL is to boost IMTA with the implementation of innovative, and resilient production and the monitoring of environmental hazards with new technology to build long-lasting cooperation across the Atlantic.

Methodology

Five IMTA labs, four case studies and one prospective IMTA lab have been identified along the Atlantic coast. ASTRAL will assess the added value of the species combination throughout the production cycles, through developing health management systems, assessing biosecurity, food safety and profitability.

The IMTA lab in Brazil is an inshore recirculating system using intensive Biofloc technology. Shrimp hyper-intensive, zero water exchange, biofloc systems are a sustainable and biosecure alternative to intensive culture systems. During the shrimp production there is an accumulation of nutrients and organic matter (biofloc) that need to be removed mechanically or biologically. Therefore, the integration of species that consume organic matter (tilapia and oysters) and nutrients (seaweeds and halophytes) are being investigated as an alternative to maintain the water quality in the production system.

IMTA lab South Africa incorporates Buffeljags Abalone, a commercial farm growing abalone in land-based raceway tanks and Ulva in adjacent interconnected paddle raceways using abalone effluent. The Ulva serves as a biofilter allowing 50% of the water from the Ulva systems to be re-circulated back to the abalone tanks with the Ulva used as supplementary feed, contributing to sustainability and the circularity. Concerns regarding biosecurity and a paucity of information on production methods for emerging species, is limiting wider use of this technology. IMTA lab SA aims to develop and validate cost-effective IMTA in land-based pump ashore systems for new high value aquaculture species (sea urchins).

(Continued on next page)

IMTA lab Scotland, an open water system, will optimise cultivation techniques of macroalgal and shellfish to demonstrate an improved economic case for the co-cultivation of kelps spp. and the native oyster, and to achieve production scales necessary to produce widespread positive mitigation effects when collocated together with sources of anthropogenic nitrogen e.g. salmon farms. IMTA lab Scotland will undertake year-round environmental monitoring and sample collection to improve the cultivation system performance aimed at improving yield and composition, stocking density and biosecurity. The development of new cultivation systems will explore options to minimise cultivation waste 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 open water system. This IMTA system will explore the feasibility of the cultivation of Atlantic salmon, lumpfish, European lobster, native oyster, scallop spp., seaweed spp. and spiny sea urchins. Production technologies for these species will be assessed and optimised in the IMTA system. IMTA lab Ireland will operate in line with organic standards to enhance potential profitability and to mitigate environmental impact. IMTA lab Ireland will seek to establish best practice for the cultivation of these species by monitoring and assessing animal welfare, biosecurity and health management.

The prospective IMTA lab in Argentina will gain knowledge from the other IMTA labs. Feasibility studies will be carried out to assess local species and identify appropriate sites within the Beagle Channel to facilitate IMTA. Data will be acquired on water quality parameters as one of the primary inputs for choosing the species assemblages and to simulate a productive cycle. Due to the shortage of high-quality protein sources available locally in Tierra del Fuego for fish feed, it is proposed to assess the viability of earthworm as a dietary protein source for the potential fish species.

Results

ASTRAL will examine the potential of all the IMTA value chains throughout the growing seasons to establish optimal production conditions. Continual monitoring to help establish baseline data to achieve better yield and profitability, less environmental impacts and less waste is being undertaken.

References

Chopin, T., 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28-31

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

GESAMP. 2016. Sources, fate and effects of microplastics in the marine environment: Part 2 of a global assessment, ed. P.J. Kershaw & C.M. Rochman. GESAMP Reports and Studies No. 93. London, IMO

Lafferty KD *et al*.2015 Infectious diseases affect marine fisheries and aquaculture economics. Annu. Rev. Mar. Sci. 7, 471–496. (doi:10.1146/annurev-marine-010814-015646)

Acknowledgments

This work is part of the ASTRAL project, funded by the EU H2020 research and innovation programme under Grant Agreement No 863034.

MODELLING THE RENEWABLE POWER GENERATION OF A NOVEL OFFSHORE AQUACULTURE SYSTEM

M O'Shea[1],

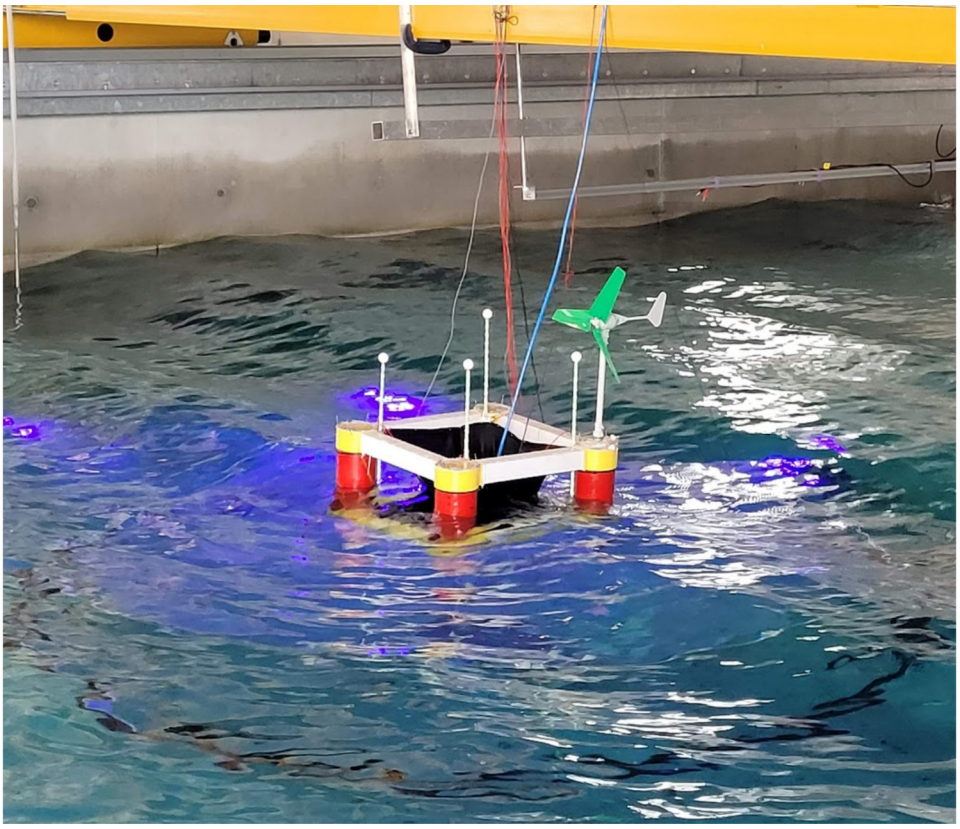
1 University College Cork, 2 Impact 9
Email: Michaeloshea@ucc.ie

Introduction

The potential for offshore renewable energy integration with floating aquaculture becomes operationally and economically very attractive as the industry moves further from the coast. The integration of the two technologies provides multiple benefits including reduced carbon footprint, reduced logistical challenges and crucially cost of production reduction. A renewable powered offshore aquaculture system in deep water however presents some integration challenges that have not yet been addressed.

Study Description

This study selects an Atlantic salmon farm off the west coast of Ireland as a case study for renewable energy technology integration. The power consumption of a novel offshore floating finfish system is simulated and calibrated with existing power consumption data from traditional finfish site. The renewable resources of wind, wave and solar from the site are collated and available renewable power form each is calculated. Renewable power technology solutions based on a range of renewable technologies are compared including the use of hybrid power solutions i.e. battery banks. Wave tank testing of the hybrid concepts is undertasken to assess the structural and hydrodynamic response of converting the floating offshore aquaculture system to renewable power, Figure 1.



TOWARDS AUTONOMOUS ROV OPERATIONS IN FISH FARMS – RECENT DEVELOPMENTS IN NAVIGATION AND CONTROL

Sveinung Johan Ohrem*, Walter Caharija, Herman Biørn Amundsen, Leif Magne Sunde, Eleni Kelasidi, Kevin Frank

SINTEF Ocean, P.O.Box 4762 Torgarden, 7465 Trondheim, Norway
*E-mail: sveinung.ohrem@sintef.no

Introduction

Operations with underwater robots such as remotely operated vehicles (ROVs) have become an important part of the aquaculture industry. Use cases includes net and mooring inspections, net cleaning, and biomass inspection during e.g., crowding operations. Though increasingly common in and around net pens, the potential of ROVs are not fully exploited today and several operations are still conducted using divers. The ROV is mainly used as a mobile camera and is controlled by an ROV pilot. The workload required by an ROV pilot can be demanding: The pilot must control the ROV in a dynamically changing environment and make sure the ROV does not damage the net pen while simultaneously inspecting the video images. These workload challenges, and challenges related to weather conditions and costs can be mitigated through increased use of autonomy. SINTEF Ocean has through research projects such as CageReporter (RCN 269087), Artifex (RCN 256241), Netclean 24/7 (RCN 296392) and SFI Exposed (RCN 237790) developed novel functions for autonomous navigation and robust control of underwater vehicles in net pens. This paper summarises some of the recent developments.

Materials and methods

Navigation under water is a challenging task as signals from global navigation satellite systems, e.g., GPS, does not penetrate the water column. Positioning systems based on acoustics, such as Ultra-short Baseline (USBL), and Doppler Velocity Logs (DVLs) are instead often used. The USBL consists of a transponder that is mounted on the ROV and a transceiver that is placed on the surface support vessel. The transceiver interrogates the transponder to pinpoint its location by determining the bearing and elevation angles as well as the time of flight of the acoustic signal. The DVL is mounted on the underwater vehicle and emits hydro acoustic beams. By calculating the Doppler shift in the returning beams, the speed relative to the seabed or any other fixed structure can be calculated.

One question was, however, how these systems would perform in a fish farm with hundreds of thousands of fish interfering with the signals. This was investigated in [1] and it was found that the performance of the USBL was acceptable, with small levels of noise from acoustic interaction with the biomass when the transponder and transceiver were on different sides of the net pen. Furthermore, it was found that a DVL could be used to calculate the ROVs speed and position relative to the net, instead of the seabed, by facing the DVL in the forward direction. Some interference was experienced if fish passed between the ROV and the net, but this was mostly an issue if the ROV was more than 3 meters from the net. As such, net-relative manoeuvring using a DVL would be possible. A recent study investigated whether a DVL, which is a quite expensive instrument, could be replaced by a combination of less expensive lasers, and cameras [2], see Figure 1. The results are very promising.

The measurements from the sensors are indispensable, but they are also prone to noise, drift, and lag which might render control systems unstable. Therefore, with the purpose of providing both the control systems and the operator with the best possible estimation of the ROV states, i.e., heading and position, model based sensor fusion techniques and filters such as the Kalman filter [3] are employed.

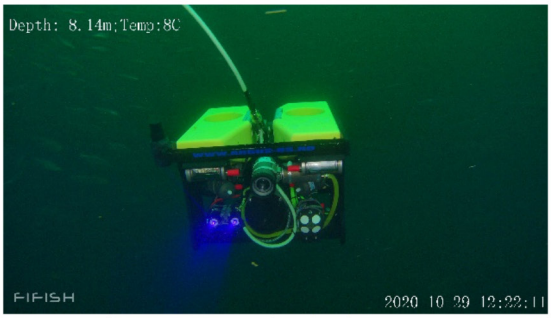


Figure 1: The ROV equipped with a laser.

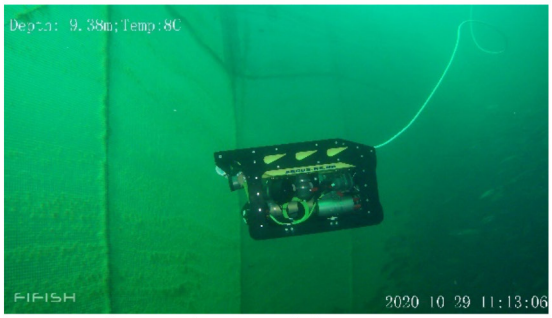


Figure 2: The ROV during net following.

(Continued on next page)

Most industrial ROVs are equipped auto-depth and auto-heading functions that make the ROV hold a given depth and yaw angle, respectively. Such features are very helpful, but more automatic functions are required if the ROV is to perform more advanced autonomous operations. One such example is dynamic positioning (DP) that makes the ROV hold the desired position, and automatically compensates for the disturbances arising from e.g., ocean currents, hence avoiding drift. Another example is path following. Here, the low-level control system in the ROV is provided a desired course angle, depth, and speed from a higher-level path planning algorithm. Combinations of DP and path following algorithms are very useful during operations in fish farms as it allows the ROV to stop and hold its position if it e.g., detects a hole in the net.

Results

SINTEF Ocean has achieved a high level of autonomy within net pen navigation (autonomy level 3, according to [4]). This has been achieved by: (1) estimating the vehicle state with sensor fusion techniques such as the Kalman filter that combines compass measurements, gyro data, DVL measurements and USBL positions; (2) developing a control system for automatic net pen traversing [5], see Figure 2; (3) developing a dynamic positioning control system for station keeping purposes. The systems have been successfully tested at the SINTEF ACE laboratory; a full-scale industrial fish farm laboratory owned by SINTEF Ocean.

Conclusion and future work

New autonomous functions are being implemented on ROVs operating in fish farms. These novel solutions can ensure better working conditions for ROV operators and can, if combined with e.g., machine vision algorithms, become a vital tool for fish farmers worldwide, aiding in the detection of holes and thus reducing risks of fish escaping. The ROVs can also be equipped with manipulator arms and thus aid in underwater maintenance and intervention operations. SINTEF Ocean is also conducting research into the interaction between fish and machines to develop control and navigation solutions that are preserving fish welfare. The overall goal is to achieve autonomy level 4 within the next 3 years [4].

References

[1] P. Rundtop and K. Frank, "Experimental evaluation of hydroacoustic instruments for ROV navigation along aquaculture net pens," *Aquacultural Engineering,* vol. 74, pp. 143-156, 2016.

[2] M. Bjerkeng *et al*., "ROV Navigation in a Fish Cage with Laser-Camera Triangulation," *Journal of Marine Science and Engineering,* vol. 9, no. 1, p. 79, 2021.

[3] M. Candeloro, A. J. Sørensen, S. Longhi, and F. Dukan, "Observers for dynamic positioning of ROVs with experimental results," *IFAC Proceedings Volumes,* vol. 45, no. 27, pp. 85-90, 2012.

[4] *Taxonomy and Definitions for Terms Related to Driving Automation Systems for On-Road Motor Vehicles*, S. International, 2018.

[5] H. B. Amundsen, "Robust Nonlinear ROV Motion Control for Autonomous Inspections of Aquaculture Net Pens," NTNU, 2020.

DIGITAL TWINS OF FISH FARMS – RECENT DEVELOPMENTS IN MODELING AND SENSOR DATA INTEGRATION

Sveinung Johan Ohrem*, Biao Su, Per Christian Endresen, Andrei Tsarau, Jan Tore Fagertun, Pascal Klebert

SINTEF Ocean, P.O.Box 4762 Torgarden, 7465 Trondheim, Norway
*E-mail: sveinung.ohrem@sintef.no

Introduction

Fish farming at exposed locations is demanding with respect to personnel safety, structural integrity and fish welfare during operations. Operations in and around fish farms are often performed with a limited knowledge of how the environmental conditions at the specific site, e.g., waves and ocean currents, affects the structures at the fish farm. This reduced insight into the effect of the environmental conditions and the missing ability to properly assess the situation beforehand and while on site, narrows the operational window of potentially crucial tasks such as well-boat and de-lousing operations. Large volumes of data are gathered from fish farms, but the usage of this data is limited. This abstract presents recent developments in the use of sensor data from fish farms. Measurements from sensors located on a fish cage structure are integrated with a mathematical model of a fish cage, giving a holistic representation of structures and environments during operations. This **digital twin** of the fish cage can provide unique insight into the structural deformations happening below the surface, and as such function as a decision support tool for fish farmers and service providers.

Materials and methods

The digital twin model is developed in the software FhSim, a simulation environment featuring a large collection of mathematical models, including aquaculture net cages, closed containment systems, fish behaviour, underwater vehicles and more [1,2]. The main components of the model consist of a net structure model, a cable and mooring lines model, a floating collar model and a hydrodynamic environment model. The models need to be numerically efficient in order to run in real-time.

An integrated numerical model with adaptive flow field [3] is used to estimate net-cage deformations, where the magnitude and direction of the current flow can be adapted by continuously assessing deviations between the simulated and the measured positions of the net. The integrated modelling and estimation approach is well suited for real-time monitoring of cage deformations by using a significantly reduced number of sensors.

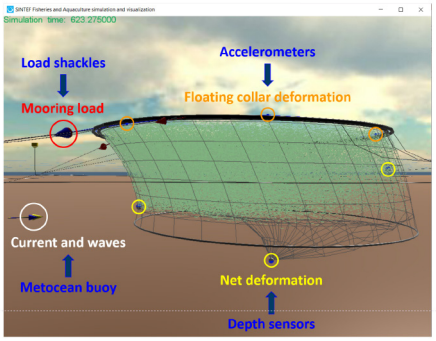
The mooring lines, bridles, chains, ropes and cables of the cage system are modelled as collections of six degree of freedom rigid-bar elements which are connected with axial and angular constraints to provide desired structural properties (i.e., bending, axial stiffness and torsional stiffness). An elastic version of the Baumgarte stabilization method [4,5] is used to avoid numerical instabilities in the cable endpoints when simulating at larger time-steps (which is necessary for real-time implementations).

Similar to the cables and mooring lines, the floating collar is modelled as a collection of rigid-bar elements connected with axial and angular constraints, regulated by the implemented Baumgarte stabilization method. The end points of each element are connected through constraints to create a ring. The vertical displacements of the floating collar, caused by wave excitation forces and the forces applied on the floating collar from attached objects such as the net and bridles, are derived from accelerometer data from the floating collar. This enables the floating collar to exhibit vertical displacements based on measurements while retaining horizontal behaviour based on input forces. To enable real-time implementations of the vertical displacements a cubic spline approximation of the collar's motion is implemented.

FhSim contains implementations of sea environments simulating ocean currents and wave fields, both regular and irregular. The sea environment supports realization of JONSWAP and ISSC wave spectra. An environmental model allowing updates of currents and wave parameters based on sensor inputs was specifically developed for the digital twin model.

(Continued on next page)

Figure 1: Overview of sensor placements and measurements incorporated in a digital model of a cage



Results

Data from load shackles, depth sensors, accelerometers, and a metocean buoy was gathered from a live fish farm in Norway during a 6-month period running from October 2019 to March 2020. This data was integrated in the digital twin model. Prior to integration the data was processed, and a time-period of two days was chosen to be used in the demo case. Data from 6 load shackles were used to estimate the mooring loads, only 3 depth sensor measurements were necessary to accurately estimate the net deformation, while the cubic spline approximation used for the floating collar estimation required measurements from 8 accelerometers to give an accurate result. The metocean buoy data were combined with depth sensor data to estimate the current speed. The variables of the simulated numerical models converged to the measured values, demonstrating the capabilities of the digital twin model.

Conclusion and future work

This work has demonstrated how numerical models can be used to accurately represent structures at fish farms using data collected by sensors. The numerical models are lean and efficient and thus suitable for real-time applications. The real-time capabilities of the floating collar model have been tested in a small-scale experiment using one accelerometer, but a remaining full-scale test is yet to be performed. Combining IMU data of ship motions with the developed models offers a possibility to monitor vessel operations near the cages. A fish behaviour model (as seen in Figure 1) and the corresponding sensor (e.g., echo-sounders) data will also be tested and included in the digital twin for monitoring fish distribution.

References

[1] K.-J. Reite *et al.*, "Fhsim—time domain simulation of marine systems," in *ASME 2014 33rd International Conference on Ocean, Offshore and Arctic Engineering*, 2014: American Society of Mechanical Engineers Digital Collection.

[2] B. Su *et al.*, "A multipurpose framework for modelling and simulation of marine aquaculture systems," in *ASME 2019 38rd International Conference on Ocean, Offshore and Arctic Engineering*, 2019: American Society of Mechanical Engineers Digital Collection.

[3] B. Su et al., "An integrated approach for monitoring structural deformation of aquaculture net cages," Ocean Engineering, vol. 219, pp. 108424[4]

[4] J. Baumgarte, 1972. "Stabilization of constraints and integrals of motion in dynamical systems". Computer Methods in Applied Mechanics and Engineering, 1, pp. 1–16.

[5] V. Johansen, 2007. "Modelling of flexible slender systems for real-time simulations and control applications". PhD Thesis, Norwegian University of Science and Technology, Norway.

A GENOME WIDE ASSOCIATION (GWAS) ANALYSIS FOR PARASITE RESISTANCE IN EUROPEAN SEA BASS *Dicentrarhus labrax*

S. Oikonomou[1,3*], M. Papapetrou[1], Z. Kazlari[1], K. Papanna[2], L. Papaharisis[2], T. Manousaki[3], D. Loukovitis[1,4], L. Kottaras[2], A. Dimitroglou[2], E. Gourzioti[2], C. Pagonis[2], A. Kostandis[2], C. S. Tsigenopoulos[3], D. Chatziplis[1]

[1]Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Department of Agriculture, International Hellenic University, 57400 Sindos, Thessaloniki, Greece
[2]Nireus Aquaculture SA, Greece
[3]Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR) Crete, Greece
[4]Research Institute of Animal Science, ELGO Demeter, 58100 Paralimni, Giannitsa, Greece
Email: valiaekonomou@hotmail.com

Introduction

One of the most important traits in aquaculture, following growth performance, is disease resistance. Diseases can be caused by parasites, viruses, bacteria etc. A common ectoparasite which significantly affects the European seabass aquaculture production is *Lernanthropus kroyeri* (Copepoda: Lernanthropidae) Van Beneden, 1851). Generally, *L. kroyeri* parasitizes the European seabass and affects both production and welfare by increased mortality after the infestation (Sobhana, 2009; Tokşen 2010; Chavanne *et al*., 2016).

Materials and Methods

In total, 2,425 European sea bass (*D. labrax*) juveniles originating from the Nireus breeding program were individually pit-tagged, fin-clipped and challenged to the copepod *L. kroyeri* through natural cohabitation in an environment heavily infested with the parasite (Sagiada site, GR 32 FISH 012). After 4 months in the sea cages, all fish were scarified. The parasite resistance trait was defined according to the counts of parasite found on each individual fish. Experienced personnel counted the number of parasites on each gill arch, on both sides of the fish, with the use of stereoscopes. The heritability of the parasite count was estimated using an animal model (BLUP) and selective genotyping was applied in order to genotype a sample of 1,078 fish with the 30K Affymetrix MedFISH SNP-array. Quality control was performed using plink software (SNP call rate 90%, MAF 0.05, HWE 10^{-6}) (Purcell *et al*., 2007). GWAS analysis was performed using GEMMA (Zhou and Stephens, 2012, 2014), a univariate animal model using as depended variable the parasite count, the genomic relationship matrix among candidates as a random effect while no fixed effect was fitted in the model. Bonferroni correction was also used in the analysis (Bonferroni, 1936). Finally, the linkage disequilibrium was estimated using the pairwise correlation between all pairs of SNPs using plink software.

Results and Discussion

Parasites were counted in all fish and their average number was 25 ±13.26 while the range was from 1 to 84 parasites. Heritability for parasite count (0.29, BLUP) revealed the existence of substantial additive genetic variation. Since both within and between family variation was evident, from the pedigree-based analysis (animal model), selective genotyping was applied based both on the within family variance of the parasite count and the discordant EBVs for parasite count from the animal model. This method of selective genotyping was utilized to capture both within and between family genetic variations in our genotyped sample (1,078 fish). The dataset which passed the quality control successfully was consisted of 1,076 offspring and 26,821 SNPs. SNP distribution is illustrated in Fig 1, while SNPs which were unstructured were collected in chromosome 25.

To our knowledge, this is one of the first applications of the newly designed 30K Medfish SNP-array in a genomic association analysis and our results indicate an average Linkage disequilibrium (LD = 0.07) in all chromosomes. The GWAS analysis performed revealed two SNPs (p-value: 0.0000023034, 0.0000060397, Fig 2) close to the Bonferroni correction limit in chr 8 (a=0.05, 0.0000018642). The phenotypic variation explained by the two SNPs was estimated to be 2% each. Nevertheless, a higher LD would improve the power of the GWAS analysis. Moreover, if a larger sample was available as well as a higher SNP density on chromosome 8 stronger associations could be revealed. Further analysis on genomic heritability and alternative modes of inheritance for the GWAS on parasite count is being conducted.

(Continued on next page)

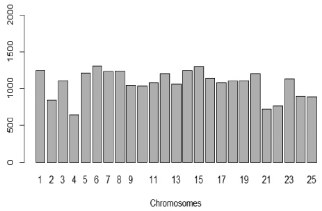


Figure 1. SNPs distribution on chromosomes

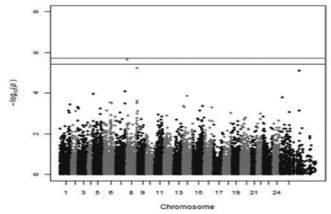


Figure 2. Manhattan plot for *Lernanthropus kroyeri*

Reference

Bonferroni, C. E. (1936) 'Teoriastatisticadelleclassi e calcolodelleprobabilità', *Pubblicazioni del Royal IstitutoSuperiore di ScienzeEconomiche e Commerciali di Firenze*, 8, pp. 3–62.

Chavanne, H. *et al*. (2016) 'A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry', *Aquaculture International*, 24(5), pp. 1287–1307. doi: 10.1007/s10499-016-9985-0.

Purcell, S. *et al*. (2007) 'PLINK: A tool set for whole-genome association and population-based linkage analyses', *American Journal of Human Genetics*, 81(3), pp. 559–575. doi: 10.1086/519795.

R Core Team (2020) 'R: A language and environment for statistical computing. R Core Team (2020).' Vienna, Austria: R Foundation for Statistical Computing, p. 2020. Available at: https://www.r-project.org/.

Sobhana, K.S., 2009. Diseases of seabass in cage culture and control measures, in: National Training on Cage Culture of Seabass. pp. 87–93.

Tokşen, E., 2010. Treatment Trials Of Parasites Of Sea Bass (Dicentrarchus labrax) and Sea Bream (Sparus Aurata) in Turkey. 2nd Int. Symp. Sustain. Dev.

Zhou, X. and Stephens, M. (2012) 'Genome-wide efficient mixed-model analysis for association studies', *Nature Genetics*, 44(7), pp. 821–824. doi: 10.1038/ng.2310.

Zhou, X. and Stephens, M. (2014) 'Efficient multivariate linear mixed model algorithms for genome-wide association studies', *Nature Methods*, 11(4), pp. 407–409. doi: 10.1038/nmeth.2848.

GENOMIC SELECTION FOR STRESS RESPONSE AND BODY WEIGHT IN EUROPEAN SEABASS

S. Oikonomou[1,2], A. Samaras[3], M. Tekeoglou[1], D. Loukovitis[1,4], A. Dimitroglou[5], L. Kottaras[5], K. Papanna[5], L. Papaharisis[5], C. S. Tsigenopoulos[6], M. Pavlidis[3]and D. Chatziplis[1]

[1] Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Dept of Agricultural Technology, School of Geotechnical Sciences, International Hellenic University, Alexander Campus, P.O. Box 141, 57 400 Sindos, Thessaloniki, Greece
[2] Department of Genetics, Development and Molecular Biology, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece
[3] Department of Biology, University of Crete, GR-714 09 Heraklion, Greece
[4] Research Institute of Animal Science, ELGO Demeter, 58100 Paralimni, Giannitsa, Greece
[5] Department of Research & Development, Nireus Aquaculture SA, 341 00 Chalkida, Greece
[6] Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), 71003 Heraklion, Crete, Greece
Email: valiaekonomou@hotmail.com

Introduction

The majority of the aquaculture breeding programs focus on growth performance traits (Chavanne et al., 2016)hindering an overall evaluation of their success. Here, we report on the results of an online survey of the major aquaculture breeding companies operating in Europe. Six main reared fish species were targeted. A total of 31 respondents contributed to the survey, representing 75 % of European breeding organizations. Family-based breeding schemes were predominant, but individual selection was more frequently applied in marine species. Artificial fertilization is the preferred means of reproduction; however, mass spawning is often used as a fallback method. The most frequently selected trait is growth performance, but the number of selected traits has been increasing over the years through the addition of traits such as disease resistance or product quality. The use of molecular tools is now common in all programs, mainly for pedigree traceability. An increasing number of programs use either genomic or marker-assisted selection. Results related to the seed production market confirmed that for Atlantic salmon there are a few dominant players at the European level, with 30–50 % market share. Only part of the European fish aquaculture industry today fully exploits selective breeding to the best advantage. A larger impact assessment still needs to be made by the remainder, particularly on the market share of fish seed (eggs, larvae or juveniles, which are quite inheritable traits. Apart from such traits, stress response could also be included in breeding programs. Literature suggests high stress response negatively affects the fish welfare (Pickering, 1993). Additionally, cortisol levels, which have been used as stress indicators, show negative genetic correlation with body weight. Moreover, a moderate heritability of cortisol levels has been identified (Chatziplis et al., 2020).The aim of this study is to estimate the heritability and genetic parameters in the European seabass for body weight and stress response using as indicators the hormonal (cortisol levels) and biochemical (glucose, lysozyme levels) factors and based on 57k SNP Dlab-chip array (Griot et al., 2021)"ISSN":"00448486","abstract":"Viral Nervous Necrosis (VNN.

Materials and Methods

European seabass offspring coming from two different years batches were used [533 offspring (Batch 10) and 332 offspring (Batch13)]. They were submitted into a stress challenge test as described by Chatziplis et al (2020). After the stress test, blood samples were collected and used to determine the plasma concentration of cortisol, glucose and lysozyme levels (318-334 Days Post Hatching). Offspring and broodstocks were genotyped with the ThermoFisher AxiomTM Sea Bass 57k SNP DlabChip (Griot et al., 2021)"ISSN":"00448486","abstract":"Viral Nervous Necrosis (VNN. Quality control was performed with plink software (Purcell et al., 2007), using the following criteria for exclusion: SNPs with call rate less than 90%, minor allele frequency lower than 0.05, deviation of Hardy –Weinberg equilibrium lower than 0.001. After quality control, 50,136 SNPs were used in the study. Heritability of the stress indicators and body weight as well as the genetic and phenotypic correlations between traits were estimated using Maximum Likelihood methods. The body weight and stress bio-indicators were utilized in a multitrait animal model to estimate heritability and genetic/phenotypic correlations among them. The batch was used as a fixed effect, while the polygenic effect which was fitted in the model, was estimated using a Genomic Relationship Matrix (GRM). All analyses were performed using AIREMLF90 (Aguilar et al., 2014).

$$Y = \mu + X + Zu + e$$

where y corresponds to the matrix of the body weight and stress response, μ is the mean of the body weight, X is the fixed effect, Z is the incidence matrix, is the additive genetic effect, which using GRM, is distributed as ~N (0, $G\sigma_a^2$) (G is the GRM and σ_a^2 is the additive variance). Finally, e is the random residual.

(Continued on next page)

Table 1 Descriptive statistics of the studied traits

Trait	Body weight (g)	Cortisol levels (ng ml^{-1})	Glucose levels (mmol l^{-1})	Lysozyme levels (kU l^{-1})
MEAN	65.26	339.43	6.78	568.2
SDV	20.85	79.77	2.25	287.45

Table 2 Heritability (bold), genetic correlation, and phenotypic correlation (above and below the diagonal, respectively) for body weight and stress response and standard errors are in parenthesis

Ratio	Body weight	Cortisol levels	Glucose levels	Lysozyme levels
Body weight	**0.60 (0.06)**	-0.46 (0.11)	-0.09 (0.12)	0.03 (0.09)
Cortisol levels	-0.11 (0.04)	**0.36 (0.06)**	-0.13 (0.14)	0.00 (0.02)
Glucose levels	-0.02 (0.04)	-0.03 (0.04)	**0.41 (0.06)**	0.06 (0.11)
Lysozyme levels	0.03 (0.05)	0.04 (0.06)	0.08 (0.04)	**0.79 (0.05)**

Results and Discussion

Descriptive statistics of the studied traits are illustrated in Table 1. The heritability of body weight in European seabass was 0.60 and for cortisol, glucose and lysozyme levels were 0.36, 0.41 and 0.79, respectively. Chatzplis et al (2020) estimated heritability of the cortisol, glucose and lysozyme levers to be 0.37, 0.33 and 0.56 using a subset of the present sample, combining three repeated measurements of all the stress indicators and pedigree relationship matrix. In our study, a negative genetic correlation between cortisol and body weight was detected using GRM and confirmed previous results (Vandeputte et al., 2016) and Chatziplis et al. 2020). No correlation between the rest stress indicators and body weight was detected, as well as no correlation between the stress indicators was identified.

References

Aguilar, I., Misztal, I., Tsuruta, S., Legarra, A., Wang., H., 2014. BLUPF90 suite of programs for animal breeding with focus on genomics. No. 11.751. The 11th World Congress of Genetics Applied to Livestock Production, Auckland, New Zealand 203.

Chatziplis, D., Oikonomou, S., Loukovitis, D., Tsiokos, D., Samaras, A., Dimitroglou, A., Kottaras, L., Papanna, K., Papaharisis, L., Tsigenopoulos, C., Pavlidis, M., 2020. QTL for Stress and Disease Resistance in European Sea Bass, Dicentrarhus labrax L. Animals 10, 1668. https://doi.org/doi:10.3390/ani10091668

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Aquatrace Consortium, Komen, H., Nielsen, E.E., Bargelloni, L., 2016. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquac. Int. 24, 1287–1307. https://doi.org/10.1007/s10499-016-9985-0

Griot, R., Allal, F., Phocas, F., Brard-Fudulea, S., Morvezen, R., Bestin, A., Haffray, P., François, Y., Morin, T., Poncet, C., Vergnet, A., Cariou, S., Brunier, J., Bruant, J.S., Peyrou, B., Gagnaire, P.A., Vandeputte, M., 2021. Genome-wide association studies for resistance to viral nervous necrosis in three populations of European sea bass (Dicentrarchus labrax) using a novel 57k SNP array DlabChip. Aquaculture 530, 735930. https://doi.org/10.1016/j.aquaculture.2020.735930

Pickering, A.D., 1993. Growth and stress in fish production, Genetics in Aquaculture. Elsevier Science B.V. https://doi.org/10.1016/b978-0-444-81527-9.50010-5

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795

Vandeputte, M., Porte, J.D., Auperin, B., Dupont-Nivet, M., Vergnet, A., Valotaire, C., Claireaux, G., Prunet, P., Chatain, B., 2016. Quantitative genetic variation for post-stress cortisol and swimming performance in growth-selected and control populations of European sea bass (Dicentrarchus labrax). Aquaculture 455, 1–7. https://doi.org/10.1016/j.aquaculture.2016.01.003

GENOME WIDE ASSOCIATION STUDY (GWAS) FOR GROWTH AND FAT IN GILTHEAD SEABREAM

S. Oikonomou[1], M. Papapetrou[1], Z. Kazlari[1], D. Loukovitis[1,2], A. Dimitroglou[3], L. Kottaras[3], K. A. Moutou, L. Papaharisis[3], D. Chatziplis[1]

[1]Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Dept of Agricultural Technology, School of Geotechnical Sciences, International Hellenic University, Alexander Campus, P.O. Box 141, 57 400 Sindos, Thessaloniki, Greece
[2]Research Institute of Animal Science, ELGO Demeter, 58100 Paralimni, Giannitsa, Greece
[3]Department of Research & Development, Nireus Aquaculture SA, 341 00 Chalkida, Greece
[4]Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece
Email: valiaekonomou@hotmail.com

Introduction

The gilthead seabream is one of the leading species in the Mediterranean aquaculture and many studies have been done to investigate the genetic architecture of growth and body weight. QTL affecting body weight and fat have been reported (Boulton et al. 2011; Loukovitis et al. 2011; Kyriakis et al. 2019)"ISSN":"00448486","abstract":"The gilthead seabream (Sparus aurata. The aim of the study is to identify QTL affecting growth performance and fat content using the newly constructed MedFish Array (Peñaloza et al. 2021).

Materials and Methods

1,428 gilthead seabream individuals, originating from 97 families, were randomly divided into two sea cages, which were fed on different diets; a high plant-protein containing 85% plant proteins and a high animal-protein containing 30% of marine animal protein. The body weight was measured at 11 months (465-481 DPH) and at 16 months (633-650 DPH) after tagging. Fat content was measured during the last body weight measurements. Fin clips were collected from the offspring and genotyped using the MedFish Array. Quality control was performed using Plink software (Purcell et al. 2007), with the following criteria for exclusion: SNPs with call rate less than 90%, minor allele frequency lower than 0.05, deviation of Hardy –Weinberg equilibrium lower than 0.001 were removed. Finally, after the quality control, 24,513 SNP were utilized in the study. A Genome Wide Association Study (GWAS) using growth as the depended variable and a GWAS using fat as the depended variable were performed using GEMMA (Zhou and Stephens 2012; 2014). The additive variance was estimated using a genomic relationship matrix among candidates as a random effect while the diet was used as a fixed effect (2 levels) and fitted in the model. Finally, Bonferroni correction was also used.

Results and Discussion

Table 1. Average and standard deviation of the traits

Population	BW4 (g)	BW6 (g)	GROWTH (g)	FAT (%)
Total	382.13±111.5	820.91±196.0	438.23±107.1	12.05±2.6
Plant-protein	355.73±107.7	754.27±176.1	397.45±90.1	11.56±2.5
Animal-protein	404.50±109.9	877.50±194.4	472.22±108.2	12.47±2.6

One putative QTL, affecting fat independent of the diet, was revealed in chr 13 explaining the 2% of the phenotypic variation. Moreover, SNPs in chr 5, 7 and 10 seem to show increased trailing (Figure 1). Kyriakis et al. (2019) identified SNPs associated with fat content at chr 4, 8, 7, 13 and 21 at 750DPH. Examining the growth in both diets, a QTL affecting growth at high temperatures (19ºC – 26ºC) was found in chr 16, explaining the 2.1% of the phenotypic variation. Kyriakis et al. (2019) identified SNPs associated with body weight in chr 1, 6, 8, 16 and 24 at 750 DPH. Interactions between selected SNPs are under investigation.

(Continued on next page)

Table 2. Significant SNPs for the studied traits

Traits	Chr	SNP	Position	MAF	P-value	PVE
FAT	13	Affx-954375470	16,378,140	0.462	0.00000	0.020
GROWTH	16	Affx-954432035	11,368,731	0.421	0.00000	0.021

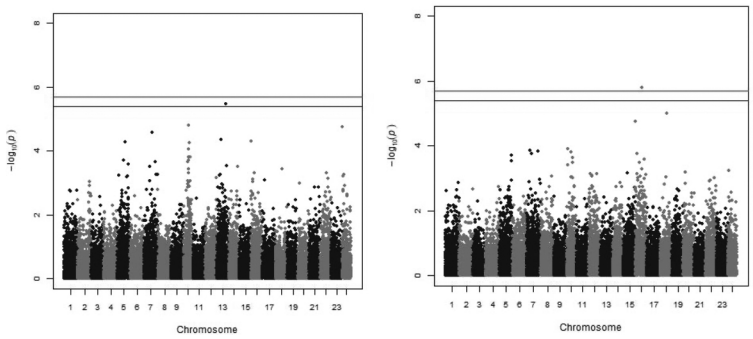


Figure 1. (a) Manhattan plot for fat (b) Manhattan plot for growth

References

Boulton, Kay, Cecile Massault, Ross D. Houston, Dirk Jan de Koning, Chris S. Haley, Henk Bovenhuis, Costas Batargias, Adelino V.M. Canario, Giorgios Kotoulas, and Costas S. Tsigenopoulos. 2011. "QTL Affecting Morphometric Traits and Stress Response in the Gilthead Seabream (Sparus aurata)." *Aquaculture* 319 (1–2): 58–66. https://doi.org/10.1016/j.aquaculture.2011.06.044.

Kyriakis, DImitrios, Alexandros Kanterakis, Tereza Manousaki, Alexandros Tsakogiannis, Michalis Tsagris, Ioannis Tsamardinos, Leonidas Papaharisis, DImitris Chatziplis, George Potamias, and Costas S. Tsigenopoulos. 2019. "Scanning of Genetic Variants and Genetic Mapping of Phenotypic Traits in Gilthead Seabream through DdRAD Sequencing." *Frontiers in Genetics* 10 (JUN): 1–15. https://doi.org/10.3389/fgene.2019.00675.

Loukovitis, Dimitrios, Elena Sarropoulou, Costas S. Tsigenopoulos, Costas Batargias, Antonios Magoulas, Apostolos P. Apostolidis, Dimitrios Chatziplis, and Georgios Kotoulas. 2011. "Quantitative Trait Loci Involved in Sex Determination and Body Growth in the Gilthead Sea Bream (Sparus Aurata L.) through Targeted Genome Scan." *PLoS ONE* 6 (1). https://doi.org/10.1371/journal.pone.0016599.

Peñaloza C, Manousaki T, Franch R, Tsakogiannis A, Sonesson A, Aslam ML, Allal F, Bargelloni L, Houston RD, Tsigenopoulos CS. (2021). Development and testing of a combined species SNP array for the European seabass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata). *Genomics*, 113, 2096-2107.

Purcell, Shaun, Benjamin Neale, Kathe Todd-Brown, Lori Thomas, Manuel A.R. Ferreira, David Bender, Julian Maller, et al. 2007. "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses." *American Journal of Human Genetics* 81 (3): 559–75. https://doi.org/10.1086/519795.

Zhou, Xiang, and Matthew Stephens. 2012. "Genome-Wide Efficient Mixed-Model Analysis for Association Studies." *Nature Genetics* 44 (7): 821–24. https://doi.org/10.1038/ng.2310.

Zhou, Xiang, and Matthew Stephens. 2014. "Efficient Multivariate Linear Mixed Model Algorithms for Genome-Wide Association Studies." *Nature Methods* 11 (4): 407–9. https://doi.org/10.1038/nmeth.2848.

COULD KISSPEPTIN TREATMENT BE USED TO ADVANCE PUBERTY IN GREATER AMBERJACK (*Seriola dumerili*) FEMALES?

C.C.V. Oliveira[1]*, E. Fatsini[1], F. Soares[2], P. Pousão-Ferreira[2], A. Candeias-Mendes[2], P. Gavaia[1], E. Cabrita[1]

[1] CCMAR, University of Algarve, Campus de Gambelas, ed 7, 8005-139 Faro, Portugal
[2] IPMA - EPPO, Av. 5 de Outubro, s/n, 8700-305 Olhão, Portugal
* Corresponding author ccoliveira@ualg.pt

Introduction

The kisspeptin system is now accepted as a key regulator of vertebrate reproductive function, particularly the onset of puberty, acting upstream of the brain–pituitary–gonad (HPG) axis. Recently, this hormone has been used successfully as a hormonal treatment in teleost fish, with proved track records of eliciting gonadotropin release (Felip et al., 2009; Oliveira et al., 2020) and gonadal development (Beck et al., 2012) in different species.

The greater amberjack is a promising candidate for aquaculture diversification, presenting a good adaptability to the captive environment. However, reproductive maturation and spontaneous spawning has proven to be difficult in broodstocks formed of first generation (G1) fish (Mylonas et al., 2004). According to these authors, such reproductive dysfunctions in captivity could be counteract using GnRHa implants. However, for the establishment of greater amberjack as a commercial species, more alternative treatments are needed, namely for puberty development. Thus, the objective of this study was to test an innovative hormonal treatment with kisspeptin, to induce puberty in greater amberjack.

Methodology

To determine the effectiveness of an *in vivo* treatment with kisspeptin as a stimulator of puberty in greater amberjack (*Seriola dumerili*) juveniles, the present trial was performed in summer (July), coinciding with this species reproductive season, using an established stock of greater amberjack (2+ years old, 1171.79 ± 250.54 g, sex ratio 1:1) from IPMA - EPPO (Olhão, Portugal). According to the previously deduced amino acid sequence of the core kisspeptin-10 region in yellowtail kingfish, *Seriola lalandi*, KISS2-10 (NH2-FNFNPFGLRF-CONH2, GenBank Accession No. HQ449730) (Nocillado et al., 2013) amidated decapeptide was synthesized by CPC Scientific Inc. (San José, California, USA). Half of the females were treated with kisspeptin, while the other half were left untreated to serve as a control group. Fish were intramuscularly injected with KISS2 decapeptide at a dose of 250 μg/kg body weight, based on previous studies in fish (Beck et al., 2012; Felip et al., 2009; Oliveira et al., 2020). Both groups of females (Kiss Treated and Control) were sampled 2 days after. Blood samples were extracted, and separated plasma frozen at -80˚C until further analysis. After blood collection fish were sacrificed by anaesthetic overdose (1000 ppm) and immediately dissected. Gonads were extracted both for histological determination of maturation stage, and for gene expression determination through RNA-*seq* and *q*PCR. Gonad samples collected for RNA-*seq* were stored in Trizol (Merk, Lisbon, Portugal) at -80 ºC. Gonad samples collected for histology were fixed in 4 % PFA and later transferred to ethanol. Circulating levels of Testosterone (T) and Estradiol (E_2) were assessed by the respective ELISA kits from Cayman Chemicals (Ann Arbor, Michigan, United States), according to the manufacturer's protocol (Fernández et al., 2019; Oliveira et al., 2020).

Results and Discussion

The results obtained revealed an effect of the kisspeptin treatment on the puberty process of greater amberjack females. The histological analysis showed similar maturation stages in the ovaries of both groups, where oocytes were in previtellogenic stage. This result suggested that the juveniles were probably too immature for the treatment to produce an effect at level of ovary maturation. This information was confirmed in the quality check by nanochip performed in the RNA from ovary samples sent for RNA-*seq*, where an overexpressed peak of 5S rRNA was detected, while the 18S and 28S rRNAs showed low expression in both groups, revealing the ovary immaturity (Rojo-Bartolomé et al., 2016). On the other hand, both estradiol and testosterone levels showed a tendency to be increased in the group of fish treated with kisspeptin, although this difference was not significant. A multiple injection protocol or a slow-release implant would probably be needed to elicit a more pronounced effect. In Senegalese sole, a single injection was enough to activate the whole BPG axis (Oliveira et al., 2020), however the fish were in a more advanced state of maturation. Finally, the RNA-*seq* analysis revealed 99 transcripts differentially expressed in the kiss treated gonad samples (64 up- and 35 down-regulated), in relation to the gonads from the untreated females (FDR<0,05 and |log2FC| >1,5), exposing an effect of the kisspeptin treatment at transcriptional level.

(Continued on next page)

Conclusions

The obtained results reveal that the kisspeptin treatment is a promising therapeutic for advancing puberty in greater amberjack females, opening a window for future research in terms of protocol optimization, namely in terms of the adequate size of the fish and ways of administration.

Acknowledgments

This work was funded by SeriNova Project (Programa Operacional Mar2020, MAR-16-02-01-FMP-0064) and Portuguese national funds (FCT-Foundation for Science and Technology) through project UIDB/04326/2020 and contract DL 57/2016/CP1361/CT0007.

References

Beck, B.H., Fuller, S.A., Peatman, E., McEntire, M.E., Darwish, A., Freeman, D.W., 2012. Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus *Morone*. Comp Biochem Physiol A 162. 10.1016/j.cbpa.2012.03.019

Felip, A., Zanuy, S., Pineda, R., Pinilla, L., Carrillo, M., Tena-Sempere, M., Gómez, A., 2009. Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. Mol Cell Endocrinol 312, 61-71. 10.1016/j.mce.2008.11.017

Fernández, I., Fernandes, J.M.O., Roberto, V.P., Kopp, M., Oliveira, C., Riesco, M.F., Dias, J., Cox, C.J., Leonor Cancela, M., Cabrita, E., Gavaia, P., 2019. Circulating small non-coding RNAs provide new insights into vitamin K nutrition and reproductive physiology in teleost fish. Biochim Biophys Acta 1863, 39-51. https://doi.org/10.1016/j.bbagen.2018.09.017

Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P., 2004. Induction of spawning of cultured greater amberjack (Seriola dumerili) using GnRHa implants. 237, 141-154. https://doi.org/10.1016/j.aquaculture.2004.04.015

Nocillado, J.N., Zohar, Y., Biran, J., Levavi-Sivan, B., Elizur, A., 2013. Chronic kisspeptin administration stimulated gonadal development in pre-pubertal male yellowtail kingfish (*Seriola lalandi*; Perciformes) during the breeding and non-breeding season. Gen Comp Endocrinol 191, 168-176. https://doi.org/10.1016/j.ygcen.2013.06.005

Oliveira, C.C.V., Fatsini, E., Fernández, I., Anjos, C., Chauvigné, F., Cerdà, J., Mjelle, R., Fernandes, J.M.O., Cabrita, E., 2020. Kisspeptin Influences the Reproductive Axis and Circulating Levels of microRNAs in Senegalese Sole. 21, 9051

Rojo-Bartolomé, I., Diaz de Cerio, O., Diez, G., Cancio, I., 2016. Identification of Sex and Female's Reproductive Stage in Commercial Fish Species through the Quantification of Ribosomal Transcripts in Gonads. 11, e0149711. 10.1371/journal.pone.0149711

A SLIPPERY SLOPE FOR AQUACULTURE REGULATION: DEVELOPMENT LICENCES AS A POLICY INSTRUMENT

M. S. Olsen*, T. C. Osmundsen, A. Gauteplass, F. Asche

NTNU Social Research, 7491 Trondheim, Norway
*E-mail: marit.olsen@samforsk.no

Introduction

The Norwegian aquaculture industry is a highly successful one seen in terms of production growth (Garlock et al., 2020), and this rapid production growth is seen as a result of a number of innovations that have improved productivity and competitiveness (Bergesen & Tveterås, 2019). However, the industry is also controversial as it constitutes a new way of utilizing aquatic ecosystems, and there are significant concerns with respect to its environmental sustainability (Belton et al., 2020). The regulation of salmon aquaculture in Norway, is strongly dependent on a licensing system where license gives the right to produce at a given location under a number of conditions that put limitations on how the production can occur (Hersoug et al., 2019). The past decade, increasing concerns with respect to the industry's environmental sustainability has prevented distribution of new commercial licenses for salmon production in Norway. A new regulatory system for growth has been implemented, and growth in terms of increased biomass is awarded through a traffic light evaluation, depending on the environmental performance of all actors within specific geographical areas. The political regulation of the successful, yet controversial, industry must take into account different concerns such as industry growth and development, but also environmental and societal sustainability.

This paper investigates the most recent addition to the licensing system, the development licenses, from the viewpoint of regulation, as a policy instrument and governing tool. The objective of the development licenses is to support the development of new technology that will improve environmental sustainability and benefit the entire industry. These licenses function as both a carrot and a stick, attempting to optimize both legitimacy and effectiveness. With these licenses the Norwegian government introduced a new path towards technology development, spurred by an implicit subsidy as the development license can be converted to standard commercial licenses once the project are completed at the low price of 10 million NOK per license independently of the project's outcome. Importantly, there are no requirements as to further use of the tested technology, after the initial project period is over. The scheme was designed as a competition at a "first-come, first-served" basis, where the degree of innovation in later applications would be compared to already awarded concepts. From the licenses were launched in 2015, 104 applications were filed before the deadline in 2017. Less than 20% of the applied concepts were accepted, however, these sum up to a total of 107 licenses, representing a substantial increase in the national capacity to produce salmon at a time where environmental concerns made it politically impossible to increase production by awarding new ordinary commercial licenses.

Materials and methods

The empirical data for this paper is publicly available material related to Norwegian aquaculture regulation in general, and documents concerning the development licenses in particular, e.g. white papers, green paper, responses to hearing round for the development license scheme, policy papers, regulations, and award and rejection letters from the Directorate of Fisheries and/or Ministry of Trade, Industry and Fisheries. In addition, the material is supplemented with interviews with public authorities.

Results and discussion

This paper draws on interactive governance theory, and our analytical starting point is the relationship between two systems that has earlier been termed the governing system and the system-to-be-governed (Jentoft, 2007). The governing system consists of institutions, and regulatory tools and mechanisms. It is a social system created by politicians and bureucrats, and influences by other stakeholder. The system-to-be-governed, on the other hand, is partly social and partly natural, and consists of the aquaculture industry, other users and stakeholders of the marine environment, as well as nature itself. The governing system aim to impact and steer the behavior of stakeholder in the system-to-be-governed, and though these actors aim to influence the natural system. Although it is too early to evaluate whether the development licenses ultimately will result in major technological advancement for the aquaculture sector, our findings show that the design and implementation of these licenses as a regulatory tool has implications for both the governing system; the overall system of aquaculture regulations, and for the impact that these tools have on the system-to-be-governed; aquaculture production and the aquaculture industry, and for the relationship between the two systems.

(Continued on next page)

We further discuss if this scheme with development licenses is fit for purpose. In a political atmosphere where growth of the Norwegian aquaculture industry has been a contested issue for many years, possible avenues towards growth needs to be coupled with ambitions to improve or solve negative environmental impacts to be politically acceptable. The competition arena, with the first-come, first-serve principle, may not be perceived as fair. The assessment took much time and resources at the Directorate, as the number of applications was much higher than expected and the assessment of the technological advancements was a laborious and difficult task. As the value of commercial licenses rapidly increased, the scheme ended up being much more favorable than planned, giving strong incentives for the industry to suggest more projects as the main motivation for applying may have been (cheap) access to new production capacity. The crucial role of the relationship between significant investments and the estimated value of the awarded licenses in the assessment of the applications, might have led some concepts with high costs to be awarded while others with lower costs to be rejected. The latter group may have had more efficient concepts.

The introduction of development licenses as a tool in 2015 gave environmental issues increased attention since these licenses were to be awarded to companies that would develop technological innovations that ultimately reduce the problem with salmon lice and escapees. However, the development license also provided a venue for obtaining new licenses in a period when no ordinary commercial licenses were awarded because of the environmental concerns.

References

Belton, B., Reardon, T., & Zilberman, D. (2020). Sustainable commoditization of seafood. *Nature Sustainability*, *3*(9), 677–684. https://doi.org/10.1038/s41893-020-0540-7

Bergesen, O., & Tveterås, R. (2019). Innovation in seafood value chains: The case of Norway. *Aquaculture Economics & Management*, *23*(3), 292–320. https://doi.org/10.1080/13657305.2019.1632391

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Ropicki, A., Smith, M. D., & Tveterås, R. (2020). A Global Blue Revolution: Aquaculture Growth Across Regions, Species, and Countries. *Reviews in Fisheries Science & Aquaculture*, *28*(1), 107–116. https://doi.org/10.1080/23308249.2019.1678111

Hersoug, B., Mikkelsen, E., & Karlsen, K. M. (2019). "Great expectations" – Allocating licenses with special requirements in Norwegian salmon farming. *Marine Policy*, *100*, 152–162. https://doi.org/10.1016/j.marpol.2018.11.019

Jentoft, S. (2007). Limits of governability: Institutional implications for fisheries and coastal governance. *Marine Policy*, *31*(4), 360–370. https://doi.org/10.1016/j.marpol.2006.11.003

SHOULD TRACEABILITY SYSTEMS IN THE AQUACULTURE INDUSTRY BE BASED ON BLOCKCHAIN TECHNOLOGY?

P. Olsen[1]*

[1] Nofima, PO Box 6122 Langnes, NO-9291 Tromsø, NORWAY
E-mail: petter.olsen@nofima.no

Introduction

Transparency and traceability is a key issue for food products in general, and for aquaculture products in particular. There are various reasons why the importance of traceability is increasing; partly it relates to the internal need for internal documentation and industrial statistics, and partly it is to meet customer and consumer requirements and preferences both in relation to product characteristics (species, ingredients, origin, processes undergone, etc.) and in relation to so-called secondary characteristics (sustainability, emissions, eco-label status, ethics, fair trade, etc.). Traditionally these characteristics are recorded, stored in relational databases, and transmitted in the chain using some form of Electronic Data Interchange (EDI). In recent years building a traceability system on blockchain technology has become a viable alternative, and this paper attempts to highlight the strengths and weaknesses associated with each option, and in particular to evaluate to what degree and under what circumstances a blockchain based traceability system is suitable for the aquaculture industry.

Blockchain-based traceability in the aquaculture industry

This presentation outlines applications, limitations, costs, and benefits related to the use of blockchain technology in the aquaculture industry, and in particular evaluates the pros and cons of having a blockchain-based traceability system compared to a traditional electronic traceability system. The core principles of blockchain technology are outlined, as well as the fundamental requirements and drivers relating to an electronic traceability system. The presentation compares traditional vs. blockchain-based food traceability systems in terms of database structure, data quality and veracity, immutability, integrity, transparency, confidentiality, trust, robustness, speed, efficiency, and interoperability.

Discussion and conclusion

The overall conclusion is that unless speed of operation or confidentiality are considered to be the most important characteristics of the traceability system, a blockchain-based implementation may be very suitable. The main benefit related to a blockchain-based traceability system is that, at least for now, the blockchain-based systems are more homogenous than traditional electronic traceability systems, so interoperability between different blockchain-based systems is likely to be easier to implement than interoperability between different traditional electronic traceability systems. Lack of interoperability is one of —, or probably the biggest current obstacle preventing system-wide, farm-to-fork aquaculture product traceability, so this advantage associated with blockchain-based implementations is significant.

References

Badia-Melis, R., Mishra, P., & Ruiz-García, L. (2015). Food Traceability: New Trends and Recent Advances. A Review. Food Control, 57, 393–401. http://doi.org/10.1016/j.foodcont.2015.05.005

Bermeo-Almeida, O., M. Cardenas-Rodriguez, T. Samaniego-Cobo, E. Ferruzola-Gómez, R. Cabezas-Cabezas & W. Bazán-Vera (2018). Blockchain in Agriculture: A Systematic Literature Review. Springer, Cham, pp 44–56.

Jansen-Vullers, M. H., van Dorp, C. A., & Beulens, A. J. M. (2003). Managing traceability information in manufacture. International Journal of Information Management, 23(5), 395–413. Retrieved from http://www.sciencedirect.com/science/article/pii/S0268401203000665

Karlsen, K. M., Dreyer, B., Olsen, P., & Elvevoll, E. O. (2012). Granularity and its role in implementation of seafood traceability. Journal of Food Engineering, 112(1-2), 78–85. http://doi.org/10.1016/j.jfoodeng.2012.03.025

Kim, H. M., Fox, M. S., & Grüninger, M. (n.d.). An Ontology for Quality Management — Enabling Quality Problem Identification and Tracing. BT Technology Journal, 17(4), 131–140. http://doi.org/10.1023/A:1009611528866

Nakamoto, S. (2008) Bitcoin: A Peer-to-Peer Electronic Cash System. Available at: https://bitcoin.org/en/bitcoin-paper [Accessed December 30, 2018].

Olsen, P., Borit, M. & Syed, S. (2019). Applications, limitations, costs, and benefits related to the use of blockchain technology in the food industry. Nofima Report 04/2019, available at www.nofima.no

Olsen, P. & Borit, M. (2013). How to define traceability. Trends in Food Science & Technology. Retrieved from http://www.sciencedirect.com/science/article/pii/S0924224412002117

Pizzuti, T., Mirabelli, G., Sanz-Bobi, M. A., & Goméz-Gonzaléz, F. (2014). Food Track & Trace ontology for helping the food traceability control. Journal of Food Engineering, 120, 17–30. http://doi.org/10.1016/j.jfoodeng.2013.07.017

Zheng, Z., S. Xie, H. Dai, X. Chen & H. Wang (2017). An Overview of Blockchain Technology: Architecture, Consensus, and Future Trends. In: 2017 IEEE International Congress on Big Data (BigData Congress). IEEE, pp. 557–564.

SALMON PRODUCED FROM SMOLT TO HARVEST IN SUBMERGED CAGES WITH AIR DOMES REDUCES LICE INFESTATION, BUT CREATES PRODUCTION AND WELFARE CHALLENGES

F. Oppedal*, F. Warren-Myers, T. Vågseth, O. Folkedal, L.H. Stien, and T. Dempster

Institute of Marine Research, 5984 Matredal, Norway
Email: frodeo@hi.no

Introduction

Submerged production of salmon may alleviate surface challenges such as lice infestation (e.g. Sievers et al 2018), extreme environments and open new areas for farming. But to succeed, the physostomous fish must be able to maintain normal buoyancy and swimming behaviour during submergence. Unlike new technologies such as snorkel and skirt cages, use of submerge has halted as salmon require frequent access to the surface to gulp air and fill their swim bladders. Fitting submerged cages with underwater air domes provides an underwater air surface and appears to resolve buoyancy associated issues (Oppedal et al 2020), but they have not been long-term tested. Here, we tested the viability of using submerged cages fitted with air domes at an industry relevant scale over a full sea-cage production cycle.

Methods

Three cages were submerged to 15 m and fitted with air domes, while three standard surface cages acted as controls at Institute of Marine Research sea cage facilities in Smørdal, Masfjorden, Norway. All cages were filled with ~6000 salmon of Aquagen strain per cage at sea transfer (~ 0.2 kg) and grown until harvest size (~5 kg). Parameters compared between control and submerged cages included environmental conditions, growth rates, condition, swimming depth and speed, swim bladder fullness, key welfare parameters and lice infestation levels.

Results

Temperature followed a normal seasonal pattern with warmest waters at the surface in the summer and autumn and at depth in winter months. Oxygen conditions in the upper 5-12 metres were above 80% saturation for the entire trial and below 15 m deep until November, while lower values (70-80% saturation) occurred during winter, decreasing further (consistently < 75% saturation) in spring. The submerged airdomes enabled salmon to refill their swim bladders and maintain normal swimming behaviour for a period of 12 months. Submerged salmon displayed considerably less lice infestation away from the surface layers but slower growth, reduced conditions, higher mortalities and impaired welfare at the lower temperature and poorer oxygen conditions experienced deeper down compared to the surface produced fish.

Discussion

This is the first full-production cycle study to successfully grow Atlantic salmon in submerged cages from sea transfer to harvest with normal behaviours displayed. However, while long-term submergence reduced salmon lice infestation rates, production problems arose, as evidenced by the poorer growth, mortality and welfare experienced by submerged fish. These outcomes were tightly linked to the very different production environment that submerged fish experienced at this site compared to the surface cages, with sub-optimal temperatures and dissolved oxygen conditions for extended periods. Future studies are needed to explore the full potential of submerged salmon farming with airdomes at variable, site-dependent environments in possible combination with flexible, dynamic depth positioning.

A key conclusion of the study is that for submergence to be a successful strategy to alleviate problems such as sea lice infestations, submergence must be matched to farming sites and times when conditions at depth are optimal for production.

References

Sievers M., Korsøen, Ø., Dempster, T., Fjelldal, T., Kristiansen, T., Folkedal, O., Oppedal, F., 2018. Growth and welfare of submerged Atlantic salmon under continuous lighting. Aquacult. Environ. Interact. 10, 501-510.

Oppedal F., Folkedal, O., Stien, L.H., Vågseth, T., Fosse, J.O., Dempster, T., Warren-Myers, F., 2020 Atlantic salmon cope in submerged cages when given access to an air dome that enables fish to maintain neutral buoyancy. Aquaculture, 525, 735286.

SOCIAL LICENSE FOR AQUACULTURE PRODUCTION – PUBLIC SKEPTICISM OR APPRAISAL?

Tonje C. Osmundsen*, Marit Schei Olsen, Vilde Steiro Amundsen, Karen Alexander, Maria Wilke and Ragnheidur Thorarinsdottir

NTNU Social Research, Dragvoll Alle 38b, 7491 Trondheim, Norway
tonjeo@samforsk.no

Sustainable growth in the aquaculture industry presupposes stronger social anchoring in aquaculture producing countries, both locally and nationally. Improving social approval for aquaculture is a two-way process where society's knowledge about and understanding of the aquaculture industry need to be strengthened, while the industry must acknowledge its social responsibility and respond to signals from society. In Norway as well as in other countries, we do know that the industry's social anchorage is surprisingly weak compared to other types of food production. However, we have less knowledge of how social approval and trust towards the industry is distributed in the public, how/whether this is linked to misconceptions, lack of knowledge, poor dialogue between the industry and the public, different values, and/or the influence of economic benefits on different societal levels. Other mechanisms expected to influence a social license include environmental impacts, the character of the relationship between a company and the community (e.g. business strategies for enticing support), and the role of media and public authorities in co-producing/dismantling a social license. Finally, research indicates that lack of a social license may result in public opposition with a high level of conflict. A social license may deteriorate if activities are deemed unacceptable or dubious, and stakeholder groups may protest or engage in other ways against the activities and actors involved (Kelly et al., 2017, 2018; Leith et al., 2014; Syn, 2014). How this plays out related to the aquaculture industry is uncertain, but conflict and opposition may affect perception of the industry and specific companies, their framework conditions, and can even affect stock prices and consumption.

Studies of social license in aquaculture have not been done in a Norwegian or an Icelandic context earlier, but has been more prevalent in other aquaculture regions, such in Tasmania (Alexander & Abernethy, 2019), Scotland (Whitmarsh & Palmieri, 2009; Whitmarsh & Wattage, 2006), Greece (Katranidis et al., 2003), Australia (Leith et al., 2014), Canada (Rayner & Howlett, 2007), and New Zealand (Quigley & Baines, 2014). In Norway, some studies relevant for a social license have been carried out, i.e. surveys from the Norwegian Seafood Council (kyst.no, 2016) portraying the vulnerable reputation of the industry, as opposed to salmon as a product. Other studies (Aanesen et al., 2018; Robertsen et al., 2012) show a greater resistance towards the aquaculture industry in urban vs. rural areas. Historically, the term social license, or social license to operate was used for industrial activities (often mining) in countries with relatively weak regulations, in an attempt to create legitimacy for industry in the absence of well-established formal institutions. In recent years, the concept of social license is increasingly applied to different types of industries. However, literature providing insight into the application of social license within the marine sector is limited. It is still considered an emergent concept and there are considerable research gaps (Kelly et al., 2017).

Data for this paper and presentation is based on a survey conducted in Norway (N=1183), Iceland (N=496) and Tasmania (N=406) comprising 2085 responses. The survey investigates the general perception towards aquaculture in these three countries/regions and compares these to background variables such as age, gender, income, and education level. A comparison to respondents' general trust in the national governance system, concern with environmental issues, knowledge/familiarity with aquaculture production, and with aquaculture companies (i.e. living in close proximity) is conducted.

Findings show in general a positive perception towards aquaculture. We also find that trust in public regulation of the industry corresponds with belief in the accountability of the industry. Our assumption that perception towards the industry is conditioned by how the public perceive that the industry is regulated and controlled is confirmed. In terms of negative perceptions, these are linked to environmental sustainability. Findings also point to comparative differences between Norway, Tasmania and Iceland, with regard to knowledge and familiarity of the industry, and acceptance.

Drawing on these findings, we discuss the determinants and mechanisms of a social license in aquaculture comparing data between Norway, Tasmania and Iceland. The influence of media and public authorities' role in co-producing/dismantling a social license is emphasized.

(Continued on next page)

Aanesen, M., Falk-Andersson, J., Vondolia, G. K., Borch, T., Navrud, S., & Tinch, D. (2018). Valuing coastal recreation and the visual intrusion from commercial activities in Arctic Norway. *Ocean & Coastal Management*, *153*, 157–167. https://doi.org/10.1016/j.ocecoaman.2017.12.017

Alexander, K. A., & Abernethy, K. E. (2019). *Determinants of socially-supported wild-catch fisheries and aquaculture in Australia*. Hobart, Australia.

Katranidis, S., Nitsi, E., & Vakrou, A. (2003). Social Acceptability of Aquaculture Development in Coastal Areas: The Case of Two Greek Islands. *Coastal Management*, *31*(1), 37–53. https://doi.org/10.1080/08920750390168291

Kelly, R., Fleming, A., Pecl, G., Richter, A., & Bonn, A. (2018). *Social licence through citizen science: A tool for marine conservation*. https://doi.org/10.1101/266692

Kelly, R., Pecl, G. T., & Fleming, A. (2017). Social licence in the marine sector: A review of understanding and application. *Marine Policy*, *81*, 21–28. https://doi.org/10.1016/j.marpol.2017.03.005

kyst.no. (2016, April). *The industry have improved their reputation the last year [in Norwegian "Næringen har faktisk bedret omdømmet det siste året»]*. https://www.kyst.no/article/naeringen-har-faktisk-bedret-omdoemmet-det-siste-aaret/

Leith, P., Ogier, E., & Haward, M. (2014). Science and Social License: Defining Environmental Sustainability of Atlantic Salmon Aquaculture in South-Eastern Tasmania, Australia. *Social Epistemology*, *28*(3–4), 277–296. https://doi.org/10.1080/02691728.2014.922641

Quigley, R. J., & Baines, J. T. (2014). *How to improve your social licence to operate: A New Zealand industry perspective*. Ministry for Primary Industries, New Zealand Government.

Rayner, J., & Howlett, M. (2007). Caught in a Staples Vise: The Political Economy of Canadian Aquaculture. *Policy and Society*, *26*(1), 49–69. https://doi.org/10.1016/S1449-4035(07)70100-5

Robertsen, R., Andreassen, O., & Iversen, A. (2012). *Havbruksnæringens ringvirkninger i Troms* (No. 28). Nofima.

Syn, J. (2014). The Social License: Empowering Communities and a Better Way Forward. *Social Epistemology*, *28*(3–4), 318–339. https://doi.org/10.1080/02691728.2014.922640

Whitmarsh, D., & Palmieri, M. G. (2009). Social acceptability of marine aquaculture: The use of survey-based methods for eliciting public and stakeholder preferences. *Marine Policy*, *33*(3), 452–457. https://doi.org/10.1016/j.marpol.2008.10.003

Whitmarsh, D., & Wattage, P. (2006). Public attitudes towards the environmental impact of salmon aquaculture in Scotland. *European Environment*, *16*(2), 108–121. https://doi.org/10.1002/eet.406

PROMOTING BLUE ECONOMY: TOWARDS A SUSTAINABLE AQUACULTURE PRODUCTION AND CONSUMPTION IN THE ATLANTIC

N. Ospina-Alvarez*, T. Li Chen and P. Silva

AIR Centre - Atlantic International Research Centre. 9700-702 Terceira Island, Azores (Portugal)

E-mail: nospina.alvarez@aircentre.org

The AIR Centre

The Atlantic International Research Centre (AIR Centre) is an international collaborative organisation that promotes an integrative approach to space, climate, ocean, and energy in the Atlantic, supported by emerging technological innovations and advances in data science, and through South-North and North-South cooperation.

The AIR Centre is based in Portugal, with headquarters in Terceira Island, Azores, and facilities in Lisbon, and is the outcome of a long process of scientific diplomacy called Atlantic Interactions, which is an ongoing intergovernmental initiative to unleash the full potential of the Atlantic Ocean for society. These diplomatic discussions resulted in an international collaborative scientific agenda for space, climate, energy, and ocean sciences in the Atlantic that started in 2012.

Promoting Blue Economy: The AIR Centre contribution to sustainable aquaculture for food security and ocean conservation

The AIR Centre thematic missions provide a clear orientation to foster knowledge-driven economic development in the Atlantic region and among them, the AIR Centre identifies, provides, and promotes activities, projects, and programs to look beyond their internal resources to develop new products, services, and financial frameworks in alignment with the Sustainable Development Goals (SDGs).

These thematic missions are:

- Clean and productive bays and estuaries.
- Resilience to coastal natural hazards.
- Sustainable food production.
- Improved management of marine and coastal resources.
- Improved environmental and maritime monitoring.

Within this framework, the AIR Centre, fosters through different projects the Blue Farming concept, as a low carbon source of food and feed and a sustainable food system.

Leading, co-leading and being part of those projects, the AIR Centre promotes the '*Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021-2030'* of the European Commission and contributes specifically to the SDGs 1 (No poverty), 2 (Zero Hunger), 3 (Good health and wellbeing), 12 (Responsible consumption and production), 13 (Climate action) and 14 (Life below water).

(Continued on next page)

ASTRAL Project
All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture

ASTRAL focuses 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.

ASTRAL goals include the increase of circularity and the achievement of zero-waste aquaculture systems, as well as the creation of appropriate business models to increase profitability. Potential climate risks and emerging pollutant (microplastics, harmful algae blooms, pathogens) will be assessed, together with the development of innovative technology (specific sensors and biosensors, IoT and AI data analytics), with the final aim to provide monitoring recommendations to policy makers. Sharing knowledge and capacity development are among ASTRAL priorities, to build a collaborative ecosystem along the Atlantic Ocean with industrial partners, SMEs, scientists, policy makers, social representatives, and other relevant stakeholders.

ASTRAL is a HORIZON 2020 project (GA 863034) financed under the Blue Growth programme. Further information: www.astral-project.eu/

CE2COAST Project
Downscaling Climate and Ocean Change: Thresholds and Opportunities

CE2COAST aims to provide 'Climate Services Innovation' in the form of a new way of exploiting observations and modelling tools to deliver climate information (*e.g.* temporal changes, seasonal differences, time of emergence of the ocean change signals). One of the objectives of the project is to investigate the added value of improved downscaled projections for determining future pressure (stressor) changes relevant to key ocean services, such as aquaculture.

Aquaculture is vulnerable to climate change including warming, acidification, changes in rainfall patterns, sea level rise, and deoxygenation. Changes in these ocean pressures can result in serious reduction in productivity. The climatic information provided by CE2COAST will give maximal support to decision-making towards creating adaptation and mitigation plans to aid fish producers and aquaculture farmers to adapt and / or reduce the impact of climate change and extreme climatic events in their production.

CE2COAST is a JPI Climate and JPI Oceans project supported under the 2019 'Joint Transnational Call on Next Generation Climate Science in Europe for Oceans'. Further information: www.ce2coast.com/

Açores IntAIRsect Project
Internationalization of the AIR Centre within the scope of the Azores sectorial challenges
Açores IntAIRsect intends to promote all the scientific potential of the Azores within the international scientific community. The main goal is to strengthen relations between entities and countries and leverage scientific projects of international collaboration, with European, African and American partners.

Açores IntAIRsect will focus on 6 thematic areas, including Marine Resources and Biodiversity. The aim of this thematic area, is to promote the development of projects related to the sustainability of marine resources, including the development of sustainable fisheries and fishing communities, increase the value of fisheries products, and sustainable aquaculture. These projects will focus on addressing and finding solutions for challenges that affect the Azores and/or the Atlantic region.

Açores IntAIRsect is a Açores 2020 project (ACORES-01-0145-FEDER-000138) is co-financed by European Regional Development Fund (FEDER) under Operational Program Azores 2020. Further information: https://www.aircentre.org/projects/acores-intairsect/

(Continued on next page)

NEXTOCEAN
Next Generation of Fishing and Aquaculture Services

NextOcean aims to develop a set of operational Earth Observation based services in Sustainable Fishing and Aquaculture under a common service delivery platform, leveraging on Copernicus data and products and complemented by the assimilation of other sources of data including in-situ.

The project includes use cases that shall consider monitoring aquaculture sites and their impacts, allowing to understand their environmental impact and for risk assessments. Examples of this are detection of ocean color around productions sites, analyses currents before settling sites, and model disease risk. Support to decision making such as need to apply pharmaceutics, delay restocking, anticipate harvesting, or physically move cages are all dependent on the spatial dispersion patterns of pathogens that often follow currents.

NEXTOCEAN is an Innovation Action from H2020 under the frame of H2020-SPACE-2018-2020 topic DT-SPACE-01-EO-2018-2020 with the Grant Agreement nº 101004362.

Enteromyxum leei IN MEDITERRANEAN GILTHEAD SEA BREAM FARMS: HOW MUCH IS OUT THERE?

O. Palenzuela[1*], A. Cook[2], R. del Pozo[1], I. Mladineo[3, 4], J. Hrabar[3,] M. Caffara[5], L. Fioravanti[5], P. Beraldo[6], P. Christofilogiannis[7], D. Gijón[8], I. Petropoulos[9], A. Sitjà-Bobadilla[1]

[1]Fish Pathology Group, Institute of Aquaculture Torre de la Sal, CSIC, Spain
E-mail: oswaldo.palenzuela@csic.es

[2] CEFAS, Weymouth, UK; [3] IZOR, Split, Croatia
[4] BCCAS, Ceske Budejovice, Czech Republic
[5] UNIBO, Bologna, Italy
[6] UNIUD, Udine, Italy
[7] AQUARK, Athens, Greece
[8] Skretting Spain, Burgos, Spain
[9] Andromeda Group, Vonitsa, Greece

Introduction

Enteromyxum leei (Myxozoa) is an important pathogen of gilthead seabream (GSB), *Sparus aurata* and other Sparidae fish cultured in the Mediterranean. In GSB, *E. leei* induces chronic enteritis followed by anorexia, cachexia, and even death. It can cause significant growth delay and mortality in marine cage farms as well as land-based mariculture systems. Water temperature and water recirculation are critical risk factors in the contagion and onset of enteromyxosis (Palenzuela et al., 2006, Sitjà-Bobadilla and Palenzuela 2012). However, the relevance of other risks factors like farm management practices, and the epidemiological situation in gilthead sea bream farms, have not been thoroughly determined. In this study, a risk assessment questionnaire and cross-sectional epidemiological study was designed to get insights in the distribution and incidence of *E. leei* in more than 50 Mediterranean and Atlantic GSB farms throughout Western Europe.

Methods

A questionnaire for GSB cage farms was designed to ascertain information about farm background and self-awareness of risk relative to *E. leei* infection, as well as details on the facilities, production and farm management routines. In addition, the farms were invited to participate in a cross-sectional targeted epidemiological survey to test the presence of the parasite in their stock. The participant farms were recruited by local expert pathologists and consultants acting as nodes in each geographical region, and the samples and questionnaires were coded and studied blindly.

For those farms participating in the biological sampling, a protocol was submitted to sample one batch of fish at harvest time. The sampling was designed to detect prevalence higher than 9.5% with a 95% confidence. The intestinal rectal ampoules (portion with maximum predictive value for *E. leei* detection in GSB) were taken from at least 30 randomly selected fish during routine harvest from one or several cages. For the validation of the survey protocol, a land-based farm which is enzootic for the parasite was enrolled in the study and two different GSB lots were sampled as positive controls. The samples were fixed in 80-90 º Ethanol and paired into 15 tubes, which were shipped to the central diagnosis laboratory at IATS-CSIC.

The routine procedures for diagnosis of *E. leei* consist of a SYBR-green qPCR test with primers specific for the parasite against a standard curve containing known numbers of copies of the target gene. Upon reception at the laboratory the samples were individually processed and homogenised. DNA was extracted from an aliquot using a robotic system, its quantity and quality were evaluated, and samples were tested by qPCR at two different dilutions. They were considered positive when the cycle threshold (Ct) was <38.

Results and Discussion

A total of 45 cage farms from Croatia (12), Greece (12), Italy (13), Spain (7) and Malta (1) completed the questionnaires. Among them, 32% declared to have previous records of the parasite: 11 of them at some point in the last 5 years, 3 in the most recent production cycle, and 4 having infections recurrently. Among the farms with previous records, the disease was considered not relevant in 7, controllable in 7, and problematic in 4 sites.

(Continued on next page)

Country	# Farms	Infected (farmers opinion)	Infected (qPCR)
Croatia	9	0	7
Greece	9	8	9
Italy	12	5	11
Malta	1	1	1
Spain	5	2	5

Table 1: Comparison of infection rates estimated by farmers' self-awareness and by the cross-sectional study at harvest (for farms which supplied both questionnaires and samples).

Country	# Farms sampled	# Fish sampled	# Farms positive	# Farms negative	Minimum prevalence (range)
Croatia	9	270	7	2	0-33%
Greece	9	370	9	0	6-75%
Italy	12	390	11	1	0-47%
Malta	1	30	1	0	73%
Spain	7	210	7	0	3-93%
Control +	1	60 (2 lots)	1	0	20-100%
Total	**39**	**1330**	**36**	**3**	**0-100%**

Table 2: Results of qPCR analysis for *E. leei* in GSB farms.

A total of 40 cage farms sent a total of 1330 fish biological samples which were tested by qPCR. The results show that the infection persists unnoticed at many farms (Table 1), Famers were most aware of the presence of the infection in Greek waters. On the contrary, Croatian farmers were unanimously unaware of the presence of the parasite although it was detected at most of the tested sites. Prevalence of infection was generally lower in sites where the infection was not believed to be present, suggesting that low level infections, although widespread, do not cause any noticeable problems at many farms.

All countries sampled had the infection, and the only countries with farms in which the infection was not detected were Croatia and Italy. Prevalence was highest on average in Greece (Table 2).

The statistical analysis considered prevalence against farm level factors as well as batch level factors. For most variables no significant effect was observed. At a farm level, farms removing mortalities more frequently, and those with stronger water exchange have a lower prevalence of infection. The number of hatcheries supplying the farm and the size of fish at harvest showed the strongest positive association with prevalence. Larger farms with higher numbers of cages, cage sizes and annual production tended to have higher prevalence. At the batch level, fish which had experienced highest minimum temperatures and those introduced to the cages at larger sizes had higher prevalence, whereas shorter times on the farm and higher specific growth rates were associated with lower prevalence.

References

Palenzuela O (2006) Myxozoan infections in Mediterranean mariculture. Parassitologia 48 (1-2).

Sitjà-Bobadilla A, Palenzuela O (2012). *Enteromyxum* species In: Woo PTK & Buchmann (Eds) Fish Parasites: Pathobiology and Protection, p 163-176. CABI Publishing.

17α-METHYLTESTOSTERONE AND 17β-ESTRADIOL IMPLANT EFFECTS ON THE INDUCTION OF VITELLOGENESIS IN FEMINIZED EUROPEAN SILVER EELS (*Anguilla anguilla*)

Arjan P. Palstra [a]*, Lotte J. Bouwman [a], Pauline Jéhannet [a], Leo Kruijt [a], Henk Schipper [b], Marco H. Blokland [c], William Swinkels [d], Leon T.N. Heinsbroek [a,e], P. Mark Lokman [f]

[a] Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands
Email: arjan.palstra@wur.nl
[b] Experimental Zoology Group, Wageningen University & Research, Wageningen, The Netherlands
[c] Wageningen Food Safety Research, Wageningen University & Research, Wageningen, The Netherlands
[d] DUPAN Foundation, Bronland 12-D, 6700, AE, Wageningen, The Netherlands
[e] Wageningen Eel Reproduction Experts BV, Wageningen, The Netherlands
[f] Department of Zoology, University of Otago, Dunedin, New Zealand

Introduction

Successful assisted propagation of the European eel will lead to a closed production cycle supplying the aquaculture industry with glass eels. The current protocol for the propagation of European eel requires a long period of weekly hormonal injections with pituitary extract (Palstra et al., 2005) which is stressful for the brood stock eels and has negative impact on gamete quality and reproductive success. This procedure can at least partly be replaced by a single injection of a steroid implant (Thomson-Laing et al., 2019). In this study, we have tested the effects of 17α-methyltestosterone (17MT), as potent androgen activating the androgen receptor (Todo et al., 1999), and 17β-estradiol (E2), as inducer of vitellogenesis.

Materials and methods

Four groups of feminized eels (Chai et al., 2010) were subjected to a simulated migration (Mes et al., 2016) and subsequently injected with implants containing 17MT (T-group) or E2 (E-group); 17MT plus E2 (TE-group) to test the synergistic effect, or without any steroids as controls (C-group). Effects of a 2-month treatment on sexual maturation were investigated by assessment of the eye index (EI), hepatosomatic and gonadosomatic index (HSI and GSI, respectively), plasma steroids as determined by liquid chromatography mass spectrometry (LC-MS; Blokland et al., 2017), gonadal histology and rtPCR of androgen receptors a and b (*ara*, *arb*); estrogen receptor 1 (*esr1*); FSH receptor (*fshr*); vitellogenin receptor (*vtgr*) and aromatase (*cyp19*)(Jéhannet et al., 2019).

Results and discussion

After simulated migration, experimental eels initiated early previtellogenesis as shown by an increase in eye size (Fig. 1), GSI and oocyte diameter and a lower HSI. Plasma steroid levels also showed marked changes, among others an increase in 11KT and a decrease in E2. For many parameters, both the T and E group showed an increase vs. controls, with the TE group showing an even further increase indicating a synergistic effect: EI, GSI (3.4 for T and for E, 6.6 for TE), oocyte diameter (Fig.1) and *ara*, *arb* and *esr1* expression. Some parameters reflected 17MT or E2 specific effects. Only eels of the T-group showed increased expression of *cyp19* and of *fshr*, while *fshr* expression increased 44 fold in the TE group showing that 17MT in combination with E2 is most effective in raising fshr mRNA levels. Specific for eels of the E groups were vitellogenic changes such as an increase of HSI, plasma dehydroepiandrosterone (DHEA) and E2, and the presence of yolk in the oocytes of E and TE (Fig. 1), not for eels of the T group. Of note are further the effects of time and/or starvation occurring in the controls so independently from 17MT and E2 steroid action after simulated migration. Thus, EI, GSI, oocyte diameter, but also *fshr* and *vtgr* expression, increased after simulated migration, and independent of exogenous steroid treatment.

In conclusion, E2 is necessary to start vitellogenesis but 17MT has specific effects on *cyp19* and *fshr* expression. The combination is necessary for the full array of synergistic effects although 17MT implant treatment, followed up in time by E treatment, may result in a more natural sequence of 17MT-induced previtellogenic and E2-induced vitellogenic effects. As such, steroid implants could be applied in assisted reproduction protocols for European eel as slow release systems to initiate the vitellogenesis and to reduce the number of weekly hormonal injections, potentially improving oocyte quality and leading to production of more viable and robust larvae.

(Continued on next page)

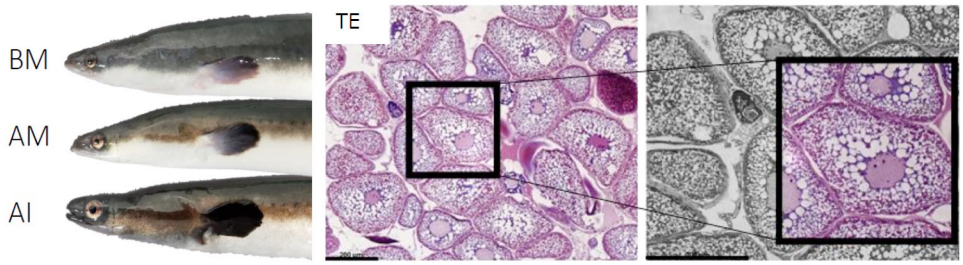


Figure 1. External changes in the same eel before and after simulated migration (BM/AM), after 8 weeks treatment with a 17MT plus E2 implant (AI) and showing the vitellogenic oocytes after implant treatment.

Acknowledgements: The authors thank the DUPAN foundation; The Dutch Ministry of Economic Affairs and the European Union, European Maritime and Fisheries Fund, and partners of the international EELRIC consortium (www.eelric.eu).

References

Blokland MH, Van Tricht EF, Van Ginkel LA, Sterk SS., 2017. Applicability of an innovative steroid-profiling method to determine synthetic growth promoter abuse in cattle. J Steroid Biochem Mol Biol 2017; 174:265–275.

Chai Y., Tosaka R., Abe T., Sago K., Sago Y., Hatanaka E., Ijiri S., Adachi S., 2010. The relationship between the developmental stage of oocytes in various seasons and the quality of the egg obtained by artificial maturation in the feminized Japanese eel *Anguilla japonica*. Aquaculture Sci. 58, 269-278.

Jéhannet, P., Kruijt, L., Damsteegt, E.L., Swinkels, W., Heinsbroek, L.T.N., Lokman, P.M., Palstra, A.P., 2019. A mechanistic model for studying the initiation of anguillid vitellogenesis by comparing the European eel (*Anguilla anguilla*) and the shortfinned eel (*A. australis*). Gen Comp Endocrinol 279, 129-138.

Mes, D., Dirks, R.P., Palstra, A.P., 2016. Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*). Aquaculture 452, 367–372.

Palstra, A.P., Cohen, E., Niemantsverdriet, P., van Ginneken, V., van den Thillart, G.E.E.J.M., 2005. Artificial maturation and reproduction of European silver eel: Development of oocytes during final maturation. Aquaculture 249: 533-547

Thomson-Laing, G., Damsteegt, E.L., Nagata, J., Ijiri, S., Adachi, S., Todo, T., Hiramatsu, N., Lokman, P.M., 2019. Synergistic effects of estradiol and 11-ketotestosterone on vitellogenin physiology in the shortfinned eel (*Anguilla australis*). Biology of Reproduction 100, 1319–1332.

Todo, T., Ikeuchi, T., Kobayashi, T., Nagahama, Y., 1999. Fish Androgen Receptor: cDNA Cloning, Steroid Activation of Transcription in Transfected Mammalian Cells, and Tissue mRNA Levels. Biochemical and Biophysical Research Communications 254, 378-383.

ASSESSMENT OF COMMON CARP WELFARE AND HEALTH UNDER ECO-INTENSIFICATION IN RAS BASED ON INTESTINE MORPHOLOGY, MICROBIOME COMPOSITION AND CHARACTERISTICS OF GUT-BRAIN AXIS

Panicz R.[1,*], Eljasik P.[1], Klopp C.[2], Sobczak M.[1], Sadowski J.[3]

[1] Department of Meat Science, Faculty of Food Science and Fisheries, West Pomeranian University of Technology in Szczecin, Poland
[2] Sigenae, UR875, INRAE, 31326 Castanet-Tolosan, France
[3] Department of Aquatic Bioengineering and Aquaculture, Faculty of Food Science and Fisheries, West Pomeranian University of Technology, Szczecin, Poland
*email: rpanicz@zut.edu.pl

Introduction

Common carp (*Cyprinus carpio* L.) is one of the most intensively farmed fish species globally (FAO, 2020). In Europe, traditional multi-pond production lasts depending on temperature of water approx. 33 months and include two wintering stages. In the autumn of the first and second year fish are harvested and transferred to deep (2 - 2.5 m) earthen ponds until all maintenance works are completed in the production ponds, the ponds are inundated and the appropriate level of water temperature is reached, usually in April or May. One of the GAIN project (https://www.gain2020.com/) objectives included ecological intensification of *C. carpio* farming by reducing the time needed to produce market size fish (1 – 1.5 kg) from 33 to 19 months. This has been possible by transferring common carp fingerlings to a RAS (Recirculating Aquaculture System) for overwintering (October – May) and afterwards moving the fish to on-growing ponds in the autumn of the second production year. However, wintering of common carp in RAS under an ambient temperature is a new concept and it is of paramount importance to assess health and welfare indicators in complement to zootechnical indices. Our studies revealed that molecular and histological characteristics of the intestine assured successful assessment of nutritional status in common carp and shed light on the importance of gut microbiota composition and intestine brain interplay as wellbeing and health parameters (Eljasik et al., 2021). Therefore, the aim of the multifaced study was to assess intestine morphology, gut-brain axis (GBA) gene activity and composition of gut microbiota in *C. carpio* overwintering in RAS compared to earthen ponds.

Material and methods

The study was conducted during 2019/2020 wintering period (October – May) in RAS system (6 tanks, 3 m^3 each, 100 fish per tank) under the tent and in earthen ponds (4.1 ha, density approx. 850 kg of carp per ha) of the Maliniec carp farm. Starting weight of fish in RAS and wintering ponds was approx. 45 ± 5 g. Fish in RAS were automatically fed two feed blends of different level of protein and fat, i.e., AL (42/12) and AG (30/9), while those in the ponds (ML) were not fed. Upon completion of the wintering AL, AG and ML fish (n = 5, each variant) were sacrificed, weighted and sampled, i.e. (i) proximate intestine to assess height of the mucosal folds (MS), width of lamina propria (LM), thickness of sub-epithelial mucosa (SM), number and area of goblet cells (GC), morphology of supranuclear vacuoles (SNV) and presence of eosinophilic vacuoles (EV); (ii) intestine and brain samples to assess health [*e.g. occludin*, *claudin-3c*, *interleukin 6* (*il6*), *mucin 5b* (muc5b), 70 kDa-heat shock protein (*hsp70*) in the intestine] and GBA-related welfare [*e.g. γ-glutamyl transpeptidase* (*ggt*), *cholecystokinin* (*cck*) in the intestine and *agouti-related protein* (*agrp*), *glucose transporter 2* (*glut2*), *orexin* in the brain] based on the expression of total 17 genes; and (iii) proximate intestine to characterise microbial communities by V3/V4 regions of the 16S rRNA gene sequencing with the Illumina MiSeq (300 PE), followed by raw data processing using DADA2 and analysis of composition of the gut microbiome using phyloseq in R package.

Results and Conclusions

Results from the histological evaluation showed that the AG fish had significantly (P = 0.01) longer MF (1108.9 ± 48,33 µm), bigger SNV sizes (23.2 ± 3.13 µm), lower LP sizes (19.3 ± 3.93 µm), lower GC numbers (28.0 ± 2.45) and lower SM thickness (45.1 ± 8.31 µm) comparing to AL (1012.8 ± 50.7; 18.8 ± 1.67; 24.5 ± 5.16; 57.6 ± 3.44; and 59.1 ± 7.07) and ML (898.3 ± 64.09; no SNV; 42.4 ± 11.69; 73.5 ± 7.77; 68.8 ± 13.23). The GC area was significantly (P = 0.01) larger among AG (50.7 ± 9.95 $µm^2$) and ML (50.7 ± 13.54 $µm^2$) fish comparing to AL (41.7 ± 10.63 $µm^2$) fish. All together histopathological indicators showed that AG fish overwintering in RAS were in better condition compared to AL common carp, and also unquestionably to ML fish from the typical wintering pond. The main reason for the poor health condition of the ML fish from the Maliniec farm was ceased feeding that eventually induced enteritis characterized, among other assessed parameters, by the absence of SNV and presence of eosinophilic vacuoles in the LP. Intestinal morphology in

(Continued on next page)

AL fish confirmed that common carp fed diet with higher fat content (12%) comparing to AG (9%) had lower condition presumably due to excessive amount of energy in the feed in relation to the needs during overwintering. Results of the gene expression analysis confirmed histological evaluation. Fish from the wintering ponds with clear symptoms of enteritis had the highest activity of *occludin* and *claudin-3c* both important in the assembly and maintenance of tight-junctions between enterocytes. Moreover, downregulated expression of *akp*, *ggt*, *muc5b* and *Na^+/K^+ ATPase* in ML fish showed low metabolic activity due to lack of feeding during the wintering period. Conversely, activity of those genes was significantly higher in AL common carp fed on a high-fat and -protein diet. However, the AL fish had also elevated expression values of *il-6*, *il1b, hsp70* and *claudin-3c* genes all together involved in inflammation process. In case of AG fish, results showed intermediate activity of genes involved in regulation of nutrients metabolism and functioning of the physical intestinal barrier, and low expression of pro-inflammatory genes. Our study, for the first time, characterised gut-brain axis in *C. carpio*. Activity of genes related to higher nutrient metabolism (*orexin*, *agrp*, *glut2*) had significantly higher expression in the brain of fishes from the AL group fed on a high-fat diet compared to fishes from the AG and ML groups. The analysis of microbiome based on V3/V4 regions revealed that composition of bacteria present in common carp gut was dominated by Proteobacteria and Firmicutes at the phylum level. Such microbial profile was previously described in literature for common carp (Liu et al., 2016), what may suggest no negative impact of wintering in RAS on common carp microbiota. Furthermore, on family level, microbiome was conquered by Enterobacteriaceae, particularly from genus *Escherichia-Shigella* and *Enterococcus*.

Concluding, wintering of common carp in RAS is a viable alternative to earthen ponds in which fish develop severe signs of enteritis, and thus lower condition before grow-out phase. The multifaceted study also provided first characteristics of the interplay within the gut-brain axis in common carp, crucial to assure optimal level of health and welfare.

Funding
This work was supported by the Horizon 2020 funding under grant agreement No. 773330.

References

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

Eljasik P., Panicz R., Sobczak M., Sadowski J., Tórz A., Barbosa V., Marques A., Dias J. 2021. Structural and molecular indices in common carp (*Cyprinus carpio* L.) fed n-3 PUFA enriched diet. Food and Chemical Toxicology, 112146.

Liu H., Guo X., Gooneratne R., Lai R., Zeng C., Zhan F., Wang W. 2016. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Scientific Reports, 6(1): 1-12.

LABELS ON SEAFOOD PRODUCTS IN DIFFERENT EUROPEAN COUNTRIES AND THEIR COMPLIANCE TO EU LEGISLATION

Simona Paolacci[1,2], Rogério Mendes[3] , Regina Klapper[4], Amaya Velasco[5], Graciela Ramilo-Fernandez[5], Marta Muñoz-Colmenero[5], Tavis Potts[6] Sandra Martins[7], Solene Avignon-[9], Julie Maguire[1,2], Enrique De Paz[1,2], Martin Johnson[1,8], Francoise Denis[9], Miguel A. Pardo[10], Dee McElligott[1,2], Carmen Gonzalez Sotelo[5]

[1] Bantry Marine Research Station, Ireland
[2] Indigo Rock Marine Research Station
[3] Portuguese Institute for the Sea and the Atmosphere, IPMA I.P.
[4] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish products, Palmaille 9, 22767 Hamburg, Germany
[5] Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain
[6] University of Aberdeen
[7] Instituto Português do Mar e da Atmosfera, I. P. (IPMA, I. P.)
[8] AquaTT Ireland
[9] Muséum National d'Histoire Naturelle, Station Marine de Concarneau, France
[10] AZTI Food Research, Basque Research and Technology (BRTA), Spain

Email corresponding author: spaolacci@bmrs.ie

Introduction

The increasing consumption of seafood products raised concerns over their sustainability and the conservation of marine resources. Seafood traceability, enabled by a regulated labelling system, is important to prevent overexploitation of these resources. Detailed seafood labels also help consumers to make informed choices about the products they purchase, enabling them to play a role in the conservation of marine resources. The regulations (EU) No.1169/2011 and (EU) No 1379/2013 are the European legislative tools that specify the mandatory information that must be present on seafood labels. The present study analysed the labels of seafood products found in fishmonger's shops and supermarkets of different European countries in order to verify the presence of mandatory information required by the EU regulations currently in place.

Material and Methods

The data were collected in 42 cities from 6 European countries: Portugal (PT), Spain (ES), France (FR), Germany (DE), United Kingdom (UK) and Ireland (IE). For each country, samples were collected in cities, in 3 supermarkets and 3 fishmongers' shops. Three groups of products were analysed (unprocessed non-prepacked, unprocessed prepacked and processed prepacked).

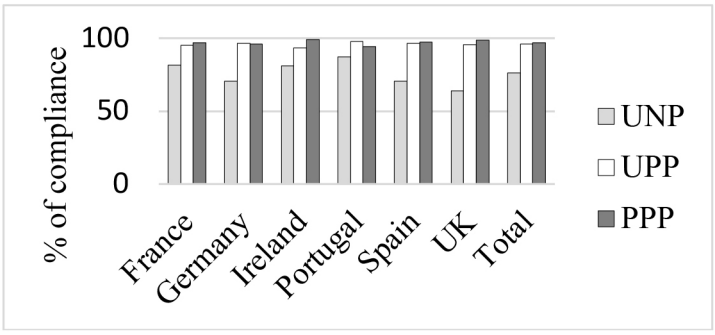


Figure 1. Compliance to labelling legislation for each country and for the three different groups of product assessed: Unprocessed Non-Prepacked (UNP), Unprocessed Prepacked (UPP) and Processed Prepacked (PPP).

(Continued on next page)

Results

The results show that there is a difference in compliance among groups of products and among countries. The country with the lowest level of compliance was United Kingdom (still part of EU in 2019, when the study was carried out), with an overall compliance of 63.7% (Fig. 1). The country with the highest level of compliance was Portugal (87.2 of compliance). Across all the countries analysed, supermarkets resulted more compliant than fishmonger's shops and processed prepacked products resulted more compliant, to the EU labelling legislation, than unprocessed non-prepacked products (Fig. 2).

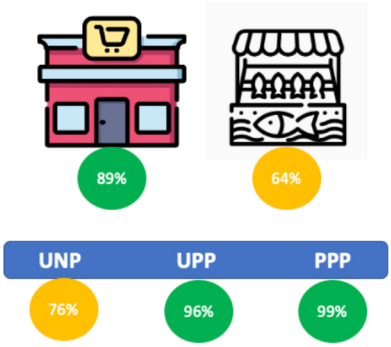


Figure 2. Compliance to the EU food labelling regulation in supermarkets and fishmonger's shops and in the three categories of product analysed.

DOES THE SCALE OF RAS MATTERS? COMPUTATIONAL FLUID DYNAMICS STUDY OF LARVAE PERFORMANCE IN RECIRCULATING AQUACULTURE SYSTEMS

Stepan Papacek[1], Karel Petera[2]

[1]University of South Bohemia in Ceske Budejovice, FFPW, CENAKVA, Institute of Complex Systems, 373 33 Nove Hrady, Czech Republic
Email: spapacek@frov.jcu.cz
[2]Czech Technical University in Prague, Faculty of Mechanical Engineering, Technicka 4, 166 07 Prague 6, Czech Republic

The improved design of aquaculture systems is needed facing the demand for increased production as well as increased concern of fish wellbeing. Here, we make another step towards use of computational fluid dynamics (CFD) for design and operation of recirculating aquaculture systems (RAS), see e.g. [1]. The proposed CFD based methodology allows the modeler (the designer) to have an overall picture about hydrodynamic conditions within the fish tank and to manipulate (*in silico*) all key phenomena involved, both physical (i.e., the tank geometry and operating conditions) and biological (fish density). Further optimization of the tank hydrodynamics, mainly to ensure the optimal rearing conditions of fish larvae and fast biosolids removal, is being expected.

The scale effect on larvae performance, i.e., the effect of different tank volumes on European sea bass *Dicentrarchus labrax* growth, survival and stress variables, was investigated by Lika *et al*. [2]. The cornerstone in modeling this phenomenon is consisting in fact that certain level of turbulence can enhance the feeding rate of the larvae, see [3]. However, the relation between feeding rate and the turbulence intensity is dome-shaped, see [4] and references within there. Consequently, it exists an optimal setting of the operating conditions (water recirculation rate or the liquid velocity in the inlet and all details concerning the boundary conditions) for a pre-established tank geometry, providing the optimal biological performance of larvae.

The big challenge resides in the optimization of both the biological performance of fish larvae and the self-cleaning capacity of RAS. Therefore, some assumptions must be undertaken. First, we can limit ourselves to maintain a sufficient water quality index (i.e., by defining a limit superior of the hydraulic retention time) while looking for an optimal biological performance. Second, the influence of fish larvae on the flow field can be neglected, i.e., we deal with the one liquid phase in CFD simulations only. Obviously, this is not the first work where the CFD is used for RAS hydrodynamics simulation. Nevertheless, to the best of our knowledge, there are only few works dealing with the multicriterial optimization with the CFD model, based on ANSYS Fluent code [5], embedded. Let us remind that experiments at laboratory scale or at field are laborious and time consuming. In this context, it becomes of utmost importance to develop a reliable CFD based methodology being able to provide reliable description of the flow field within RAS tank and an ease of performing a large range of parametric studies for optimization of both RAS scale and operating conditions.

Acknowledgements
The work of Stepan Papacek was supported by the MEYS of the Czech Republic - projects CENAKVA (No. CZ.1.05/2.1.00/01.0024), CENAKVA II (No. LO1205 under the NPU I program) and The CENAKVA Centre Development (No. CZ.1.05/2.1.00/19.0380). The work of Karel Petera was supported by the long-term strategic development financing of the CTU Prague.

References

[1] Papáček, Š.; Petera, K.; Císař, P.; Stejskal, V.; Saberioon, M. Experimental & Computational Fluid Dynamics Study of the Suitability of Different Solid Feed Pellets for Aquaculture Systems. Appl. Sci. 2020, 10, 6954.
[2] Lika, K.; Pavlidis, M.; Mitrizakis, N.; Samaras, A.; Papandroulakis, N. Do experimental units of different scale affect the biological performance of European sea bass *Dicentrarchus labrax* larvae? Journal of Fish Biology 2015, 86 (4), 1271–1285.
[3] Kristiansen, T.; Vollset, K.W.; Sundby, S.; Vikebø, F. Turbulence enhances feeding of larval cod at low prey densities. ICES J. Mar. Sci. 2014, 71, 2515–2529.
[4] Pécseli, H.L.; Trulsen, J.K.; Stiansen, J.E.; Sundby, S. Feeding of Plankton in a Turbulent Environment: A Comparison of Analytical and Observational Results Covering Also Strong Turbulence. Fluids 2020, 5, 37.
[5] ANSYS FLUENT product documentation, http://www.ansys.com/Products/Fluids/ANSYS-Fluent

THE DEVELOPMENT OF THE EYE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) REARED AT THREE DIFFERENT TEMPERATURES

Ioannis Papadakis[1]*, Maria Papadaki[1], Eirini Sigelaki[1], Chiara Vecchiatini[2], Nikolaos Mitrizakis[1], Constantinos C. Mylonas[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research
P.O. Box 2214, Iraklion, Crete 71003, Greece
papad@hcmr.gr
[2]University of Bologna Via Zamboni, 33, 40126 Bologna

Introduction

Larvae of most teleost fish are mainly visual predators. The distance from which fish can identify an object is called visual ability and depends on the overall organization of the eye, the developmental stage of the fish, and the light conditions that prevail in the rearing tanks.

Rearing water temperature is considered as the main factor that affects all physiological processes in poikilothermic organisms. Especially during larval rearing, rearing temperature significantly affects the ontogeny rates of various organs and systems that are being developed during this period. The present study aimed to evaluate the development of the optical system, focusing on the eye of sea bass larvae during the early stages of development under the influence of different rearing temperatures. The study of visual aspects in the early developmental stages in aquaculture is fundamental since it is associated both with the type, size, and quantity of food and with the appropriate prevailing light conditions that larvae should be provided with in their rearing tanks (Papadakis et al., 2018).

Materials and methods

Rearing of sea bass larvae was performed in duplicate 500-l tanks connected in a closed recirculated system according to the methodology of pseudo green water. Three different water temperatures (15, 17.5, and 20°C) were used during the rearing procedure. For the histological analysis of the eye, larvae at five different developmental stages (mouth opening, first feeding, flexion, all fins, metamorphosis, and juvenile) were sampled (n=3) and preserved in a buffer solution containing 4% formaldehyde and 1% glutaraldehyde. The larvae were embedded in methacrylate resin. Sections of 4 μm in thickness were obtained from a microtome and were stained with Methylene Blue, Azure II, and Basic Fuchsin. The examination of the sections was performed in bright field microscopy and photographed with a digital camera in X40 magnification. Images were obtained from the areas toward the olfactory nostril (Nasal, n) and toward the larval body (Temporal, t,) (Fig. 1a). Following this process, the pictures obtained from the histological sections were examined through an image analysis software (image j). The cones and rods in the retina were counted (Fig. 1b), and the diameter (mm) of the lens was measured. Finally, larval visual ability was assessed, based on the above calculations, according to the methodology established by Neave (1984).

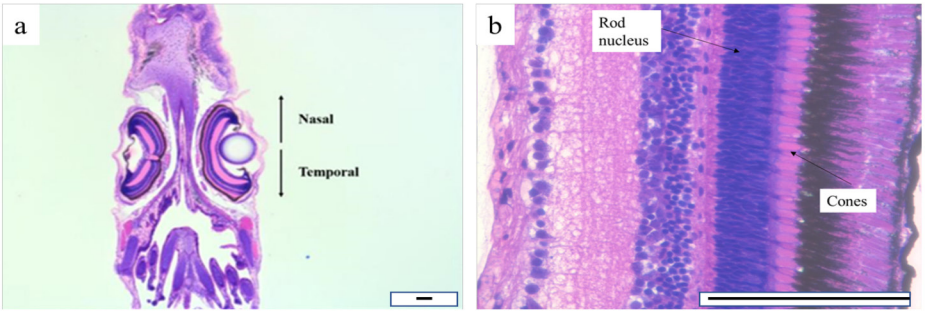


Figure 1. Histological sections of a sea bass larvae head, showing a) the Nasal and Temporal axes at the developmental stage of flexion and b) the cones and rods nucleus at the metamorphosis stage. Scale bar: 0.1 mm (black bar).

(Continued on next page)

Results and Discussion

The results of the study showed that temperature is a critical factor that causes significant differentiation in the number of cones and rods in the retina of the sea bass eye. The cones are the photoreceptors that are activated under high light intensities and are responsible for diurnal vision (Kusmic & Gualtieri, 2000). In sea bass, the diurnal vision abilities were differentiated according to the developmental stage and the rearing temperature. The number and the density of cones per unit of retina length were reduced at higher developmental stages. The lower number of cones after the flexion stage at 15ºC in comparison to 17.5ºC and 20ºC suggests that a lower visual acuity exists in larvae at lower temperatures, which means that at higher temperatures (17.5ºC and 20ºC) sea bass larvae can perceive a greater variety of different sized items. This offers new information related to the rearing protocol applied during larval rearing, concerning the size variation of preys - feeds that are included in the larval feeding protocol.

Differences also occurred in the number of rods in terms of the developmental stages and the rearing temperatures used. Rods are known to be structures that are responsible for nocturnal vision, activated under low light intensities. In most species the first appearance of rods occurs during the larval rearing period, and their number increases over time (Shand et al., 1999). In the present study, the rods appeared at the stage of flexion, and their number increased over time, which suggests that from this period onwards, the ability of objects perception under low light intensities was increased. The smaller number of rods at low temperature (15 ºC), indicates a lower ability to perceive objects under low light intensities. In contrast, at higher temperatures (17.5 ºC and 20 ºC), the ability of items perception under low light intensities was higher. Based on the above results, larvae reared in water temperatures close to 15ºC display reduced ability for small item visual perception.

The above data contribute to the knowledge for further optimization of feeding protocols (type - size of food) and the formation of visual conditions in the rearing tank (tank coloring). Additional studies on basic systems, such as the digestive and the visual system, focusing on the optimal relationship between the feeding protocols and the abiotic rearing conditions, such as lighting and temperature, will assist in the improvement of the larval rearing protocols, through the study of larval feeding behaviour.

References

Neave DA, (1984) The development of visual acuity in larval plaice (*Pleuronectes platessa* L.) and turbot (*Scophthalmus maximus* L.). Journal of Experimental Marine Biology and Ecology 78: 167-175

Papadakis IE, Kentouri M, Divanach P, Mylonas CC (2018) Ontogeny of the eye of meagre (*Argyrosomus regius*) from hatching to juvenile and implications to commercial larval rearing. Aquaculture 484: 32-43

Shand J, Arche, MA, Collin SP (1999) Ontogenetic Changes in the Retinal Photoreceptor Mosaic in a Fish, the Black Bream, *Acanthopagrus butcheri*. Journal of Comparative Neurology 412: 203-217

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727315 (MedAID). Call: SFS-23-2016 Improving the technical performance of the Mediterranean aquaculture.

EFFECT OF DIFFERENT DISSOLVED OXYGEN LEVELS ON GROWTH PERFORMANCE FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Ioannis Papadakis[1*], Lydia Katsika[1], Panagiota Tsoukali[1], Athanasios Samaras[1], Stavros Chatzifotis[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, P.O. Box 2214, Iraklion, Crete 71003, Greece
papad@hcmr.gr

Introduction

Among various physicochemical parameters, water temperature and dissolved oxygen (DO) play the most important role for optimum fish growth and maintenance of proper well-being and health status of fish. Low DO, (<40% saturation in the rearing water) combined with higher temperatures prevailing in summer months (>26 ^{o}C), reduces the fish's ability to feed (Mallekh and Lagardere, 2002). Therefore, fish farmers experience a significant economic loss. New systems are under development in the Mediterranean area, focusing on optimizing the DO levers during rearing in sea cage aquaculture. This study aims to gain knowledge on the management of dissolved oxygen in fish farms. An effort was made to estimate optimal DO levels under high rearing temperature (26.5 ^{o}C) for growth in European sea bass (*Dicentrarchus labrax*) culture.

Materials and methods

The study took place at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR) Heraklion, Greece. At the beginning of the trial, 315 fish were lightly anesthetized and individually weighted (88.00 ± 0.12g), and they were randomly distributed in 9 tanks. Sea bass (35 fish/tank, 105 fish/group) were placed in three recirculating aquaculture systems (RAS) with fully controlled rearing conditions in tanks of 500 liters (500l) in each RAS. Three different oxygen saturation levels were applied to each RAS (80-100%, 60-80% and 40-60%) at 26.5 ^{o}C and 38 ‰ salinity. Each experimental condition was tested in three replicates. The targeted oxygen saturation levels were maintained through an automatic oxygen monitoring system. Fish were hand-fed to apparent satiation three times a day for 7 days a week during the trial period. Fish weights were recorded monthly and at the end of the experiment, 5 fish per tank were sacrificed and their livers, visceral fat and viscera were weighed, and growth and somatic indexes were determined. Additionally, image analyses were performed on pictures from histological sections taken from different parts of the digestive system, such as the midgut, foregut, and liver. Concerning the liver's histological examination, the parameter related to lipids deposition was estimated by measuring areas covered by lipids inclusions on liver histological sections according to the methodology of Papadakis *et. al.* (2013).

Results and Discussion

The present study reinforced earlier observations in sea bass, and other species, about the negative correlation of oxygen saturation level with growth rates in marine fish (Pichavant *et al.*, 2001). The results showed (Table 1) that at 26.5 ^{o}C the best growth performance for sea bass was achieved at 80-100% followed by 60-80% and 40-60% O_2 saturation levels. The final fish body weight, the percentage growth of average body weight of fish and the specific growth rate were significantly lower ($p<0.05$) at the lowest oxygen saturation of 40-60%. Specifically, there was a gradation between the three levels with a positive correlation of oxygen level and the parameters of the final weight of fish, daily growth rate, specific growth rate and hepatosomatic index ($p<0.05$). The above results also agree with the histological analysis performed concerning the lipids deposition in the liver. Low hepatosomatic index values in fish are related to nutrition issues. The mesenteric fat index was significantly lower ($p<0.05$) in the group with 60-80% O_2 saturation than the other two groups.

The visceral somatic index was higher in the 80-100% O_2 saturation group and did not differ from the 40-60% O_2 saturation group, while a significantly lower value ($p<0.05$) appeared in the intermediate group of 60-80% O_2 saturation. No significant difference was observed in the feed conversion ratio among the three oxygen groups. However, the feed conversion ratio was numerically lowest at 80-100% DO. Furthermore, there were no significant differences ($p> 0.05$) among the three oxygen groups regarding the fish weight dispersion coefficient and the condition factor. Thus, the growth reduction observed in the present study (40-60%, 60-80% DO) can be associated with reduced feed intake at lower oxygen levels. The maximum growth rate can be achieved at the highest oxygen saturation levels of 80-100%.

(Continued on next page)

Table 1. Growth performance and meristic parameters of seabass reared in three different oxygen saturation levels.

Duration (95 days)	Oxygen Saturation level O_2		
	80%-100%	60%-80%	40%-60%
Initial body weight (g)	88.02 ± 0.22	88.26 ± 0.65	88.15 ± 0.46
Final body weight(g)	252.40 ± 2.59^{a}	230.68 ± 4.24^{b}	197.18 ± 0.44^{c}
Specific growth rate	1.11 ± 0.01^{a}	1.01 ± 0.03^{b}	0.85 ± 0.02^{c}
Daily growth rate	1.96 ± 0.04^{a}	1.69 ± 0.07^{b}	1.30 ± 0.05^{c}
Feed conversion ratio	1.41 ± 0.02	1.40 ± 0.04	1.44 ± 0.02
Daily food consumption	1.41 ± 0.02^{a}	1.29 ± 0.02^{ab}	1.21 ± 0.07^{b}
Visceral somatic index	9.98 ± 0.40^{a}	8.61 ± 0.29^{b}	9.51 ± 0.30^{ab}
Hepatosomatic index	1.68 ± 0.02^{a}	1.41 ± 0.04^{b}	1.16 ± 0.02^{c}
Condition factor	2.03 ± 0.02	1.97 ± 0.04	1.95 ± 0.02

References

Papadakis, I.E., Kentouri, M., Divanach, P., Mylonas, C.C. 2013. Ontogeny of the digestive system of meagre *Argyrosomus regius* reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile. Aquaculture, 388, 76-88

Mallekh, R., & Lagardere, J. P. (2002). Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. Journal of Fish Biology, 60(5), 1105-1115.

Pichavant, K., Person-Le-Ruyet, J., Bayon, N. L., Severe, A., Roux, A. L., & Boeuf, G. (2001). Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. Journal of Fish Biology, 59(4), 875-883.

Acknowledgments

The program is co-funded by the European Maritime and Fisheries Fund in Greece (EMFF OP) and the Hellenic Republic via the Operational Programme “Fisheries and Maritime 2014-2020” (EPALTH 2014-2020). Program MIS 5030044.

SPERM CRYOPRESERVATION OF THE ENDAGERED AUTOCHTHONOUS SICILIAN MEDIRRANEAN BROWN TROUT (*Salmo cettii*)

L. Proietti [1], L. Foglio [1], T. Bongiorno[1], D. Lo Monaco[2], P. Lamesa[3], G. Fortino[3], D. Carpinteri[2], V. Ciprì[2], C. Di Bella[2], K. Parati [1], V. Bornaghi[1]

[1]Istituto Sperimentale Italiano L. Spallanzani - Loc. La Quercia, 26027 Rivolta d'Adda (CR), Italy
[2]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" Area Sorveglianza Epidemiologica, Via Gino Marinuzzi, 3 – 90129 Palermo - Italy
[3]La Trota srl – c/da Pianette Noto (SR) - Italy

E-mail: katia.parati@istitutospallanzani.it

Introduction

Mediterranean trout (*Salmo cettii*) is an Italian sub-endemism, native to the Tyrrhenian peninsular regions, Corsica, Sardinia, Sicily and the western part of North Africa. In Italy the greatest number of populations is present in central-eastern Sardinia, south-eastern Sicily and a few in peninsular Italy. Over the last few decades, the Mediterranean trout has undergone a significant contraction, both numerical and areal, due to anthropogenic causes and hybridization with brown trout, leading to be included in the European Union Habitat Direct and considered a "vulnerable species" in Europe and "endangered" species in Italy. The guarantee of the survival of the species through planned and effective restocking plans is a relevant ecological objective, which can be also pursued by the application of aquaculture technologies, among which the production and storage of gametes, which could reduce the dependence on wild stocks, preserving at the same time the genetic resources of this species.

Sperm cryopreservation has been successfully performed in a number of important commercial aquatic species, particularly in some teleost fish and crustaceans (Diwan *et al*., 2020). The selection of pure breeders with high genetic variability and the storage of sperm obtained from these fish in a cryobank are important tools to make the management of the conservation of endangered species more efficient (Zuccon *et al*., 2017).

The purpose of this research was the development of a sperm cryopreservation protocol in order to create a cryobank for the conservation of milt obtained by pure breeders of Mediterranean trout (*Salmo cettii*) caught in west area of Sicily.

Materials and methods

During the the reproductivity season for Mediterranea trout (February – March 2021), 21 male breeders were caught in Cardinale torrent (located in the west of Sicily). The fish were previously anesthetized and then subjected to sampling of small fragment of adipose fin for genetic analysis and semen collection by squeezing for cryobank creation. The fish were also tested for the viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis virus (IHNV) indemnity.

Semen samples were diluted in StoreFish solution (IMV) and transferred on refrigerate condition (4-6°C) in laboratory. Then, they were diluted in FreezeSol solution (IMV), containing the cryoprotectants to prepare the semen for the cryopreservation step and finally, the samples were packaged in French straws of 0,5 ml and frozen by the programmable freezer MiniDigit cooler (IMV), following a specific freezing curve (data not shown). The frozen straws were conserved in liquid nitrogen. To evaluate the effect of cryopreservation on the sperm quality, each semen was analyzed for the determination of motility, before and after thawing, using both microscope and image analysis CASA systems (IVOS II – Hamilton Thorne).

Results and discussion

The taxonomic analysis, based on phenotypic observations on the livery carried out *in situ,* revealed the presence of 9.5% of hybrids with brown trout on the total of males caught. Genetic tests are in progress to confirm this data. Only the 58% of fish, classified as "genetic pure individuals", provided enough sperm quantity to carried out all the quality tests and, the 73% of these, showed sperm quality parameters suitable for subsequent cryopreservation (fresh sperm motility > 50%). Motility average value of fresh semen, before cryopreservation was 58.1±9.2% while, after thawing it decreased to 26.2 ±3.5%, (representing a yield thawing of 46±11.2%), with an activation time post-tawing of 32.5±5.7 seconds.

(Continued on next page)

This preliminary study allowed to set up a cryopreservation protocol for the semen of autochthonous Sicilian trout (*S. cettii*). Further study will be focused on the application of cryopreserved sperm on eggs to perform fertilization trials, in order to confirm the sperm quality after thawing and the effectiveness freezing protocol for the sperm cryobank implementation. This vanguard technology, in synergy with an extensive genetic study, a migratory behavior survey and a recovery of the altered habitat, represent an important tool for the restoration of native population of the Mediterranean trout.

Funding

This work has received funding from PoFeamp 2014-2018, measure 2.47 "Innovation in Aquaculture", art. 47-regulation (UE) n.508/2014 May, 15 2014 (project MEDIT REPRODUCTION).

References

Diwan Arvind D., Harke Sanjay N., Gopalkrishna, Panche Archana N. (2020) Cryobanking of Fish and Shellfish Egg, Embryos and Larvae: An Overview. *Frontiers in Marine Science* 7, doi: 10.3389/fmars.2020.00251.

Zuccon G., Wilson R., Ulheim J., Filonzi L., M., Piccinini A., Bilò F., Nonnis Marzano F. From genetics to cryogenetics: new strategies for the conservation of marble trout. Italian Journal of Freshwater Ichthyology, [S.l.], n. 4, p. 50-57, ott. 2017.

SENSITHAU PROJECT: RISK ANTICIPATION - FOUR SOURCES OF DATA TO BUILT RISK PREDICTIVE MODELS

Maxime Paris[1]*, Lucas Schaeffer[1], Romain Pete[2], Sébastien Mas[3], Florence Bouillé-Chada[4], Dimitri Trotignon[1]

[1] BIOCEANOR SAS, 1360 route des dolines, les Cardoulines B3, 06560, Valbonne, France
maxime.paris@bioceanor.com
[2] SMBT, 328 Quai des Moulins, 34200 Sète, France
[3] OSU-OREME, 2 Rue des Chantiers, 34200 Sète, France
[4] CLS, 11, rue Hermès, Parc Technologique du Canal 31520 Ramonville Saint-Agne, France

Introduction

Faced with the increase of harmful natural phenomena and infectious episodes, the shellfish and aquaculture industry in the world, has experienced major health crises since 2008 (Garcia et al. 2011). Several phenomena have disastrous consequences on the herds, such as microbial infections or anoxic crises (called "malaïgue") (Hamon et al. 2003) resulting in a rapid depletion of oxygen in the environment, vital for organisms. Beyond shellfish farming, lagoon environments such as Thau Lagoon (France) are real reservoirs of biodiversity, directly impacted by these phenomena. The SENSITHAU[1] project aims to integrate into the Lagoon Observation Network (ROL), an ecosystem database, for integrated management of the lagoon, combining ecology, health risks and shellfish production. Bringing together several key players in the Thau lagoon, the SENSITHAU project seeks the deployment of several *in situ* sensors measuring a wide range of physicochemical parameters in real time and at high frequency. Using data generated from *in situ* monitoring, sampling, and satellite, the project will be articulated around 2 axes:

- Deployment of an *in situ* telemetry network on various parameters of the lagoon water
- Development of predictive algorithms to anticipate risks associated with episodes of (i) malaïgue, (ii) microbial contamination and (iii) algal blooms.

Material and methods

The project takes place in the Thau Lagoon (Hérault, France), a semi-open environment.
Three datasets will be constructed to gather as many information as possible.

The 1st dataset is the physico-chemical data (temperature, dissolved oxygen, turbidity, salinity, and chlorophyll) monitored continuously (Aqurareal, Bioceanor) at 2 depth (surface and bottom).

The 2nd database is the water analysis from sampling at different strategic areas in the lagoon (where event occurs) of biotic (*Escherichia. coli* and enterococcus, phytoplankton) and abiotic (NO3, NH4, SO4, PO4, chlorophyll and phytoplankton diversity).

The 3rd database is the satellite images, exploring the analysis of 3 parameters (chlorophyll, backscattering coefficient of suspended inorganic matter and suspended particulate matter) using the Sentinel 2 and 3 satellite.

The 4th database includes other data such as wind, air temperature, rainfall level, flow rates of rivers and releases from wastewater treatment plants around the area.

The data is collected, harmonized, and stored for analysis. We will run preprocessing to identify the global behavior of different parameters (i.e., seasonality, extreme values). We will also analyze correlation between parameters to have a complete data exploration. At the end, we will build and compare various models: from statistical models to machine and deep learning methods to find the most performant one.

Results

The deployment of sensors and data collection in April 2021 constituted the first step for a global monitoring of the lagoon, including sensitives areas, susceptible for anoxic event, bacterial contamination, and blooms. All the sensors deployed in the lagoon represent a sentinel network for risk visualisation in real-time thanks to the continuous and connected devices. Different parameters monitored are redundant and measured at different scales (continuously with the autonomous devices, locally by sampling and by satellite). The data will be intercompared and will serve for statistical analysis. Those analysis will allow to identify key parameters and their variation overtime before, during and after an event of interest. Machine learning analyses will subsequently allow to anticipate events before they occur (Lafont et al. 2019). By the end of the project, correlation between *in situ* data and satellite images will give a role of sentinel for the satellite in the lagoon (Fig. 2). It will allow to alert the final user before an event occur.

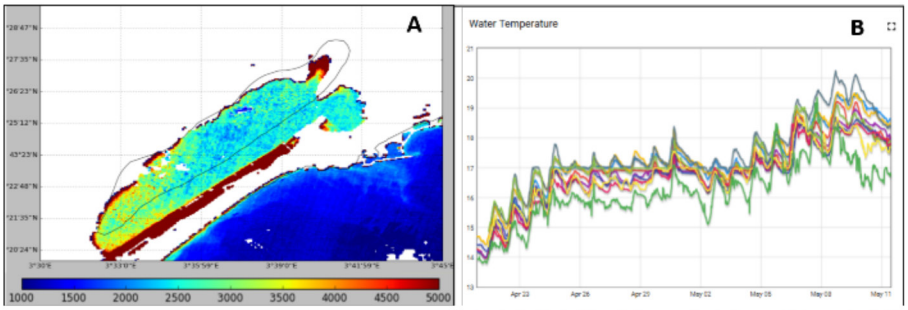


Figure 1: Example of data collected in the SENSITHAU project. A: Satellite data analysis for a-Chlorophyll (10^3 mg/m3) ; B: Water temperature monitoring thanks to autonomous devices (°C; 10 sites across Thau lagoon)

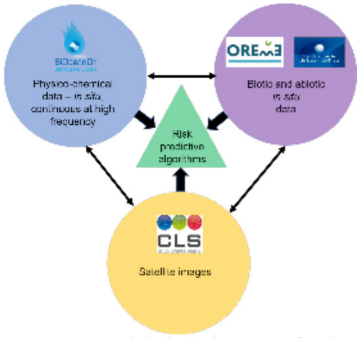


Figure 1: Global scheme of the construction of a large environmental data set

Discussion and conclusion

The development of IoT (internet of things) simplifies the collection of large amounts of data, in real time and at high frequency. Correlated to water sampling and data collections that remains impossible autonomously, it will allow to build large and more robust data sets. Using these data with machine learning opens the world of anticipation (Lafont et al. 2019). Different risks exist for the industries dependent of the water quality (i.e. oyster farming). Being able to anticipate those risks can give advantage to protect the farm and the business against phenomenon that can be disastrous (HAB, oxygen drop, bacterial contamination…). The SENSITHAU project propose to develop tools to anticipate those risks. In long term, satellite data and continuous monitoring will allow to have a survey of the lagoon 24/7 and automatically alert end-user to a potential risk. The development of this technology will be applicable all around the world and will benefit to many sensitive areas, where anticipation of exceptional events is essential.

References

Garcia C, Thébault A, Dégremont L, Arzul I, Miossec L, Robert M, Chollet B, François C, Joly J, Ferrand S, Kerdudou N, Renault T (2011) Ostreid herpesvirus 1 detection and relationship with Crassostrea gigasspat mortality in France between 1998 and 2006. Vet Res 42:1–11

Hamon P-Y, Vercelli C, Pichot Y, Lagarde F, Le Gall P, Oheix J. Les malaïgues de l'étang de Thau. Tome 1. Description des malaïgues. Moyens de lutte, Recommandations.

Trotignon D, El-Farsi A, Schaeffer L, Lafont M, Dupont S, Dupont C (2020). Machine learning for aquaculture, improving water quality management and use predictive maintenance to ensure data reliability. Aquaculture Europe 2020 conference.

Lafont M, Dupont S, Cousin P, Vallauri A, Dupont C (2019) Back to the future: IoT to improve aquaculture: Real-time monitoring and algorithmic prediction of water parameters for aquaculture needs. Global IoT Summit (GIoTS), 1-6

https://www.bioceanor.com/en/sensithau-project-launch

PERCEPTIONS AND INTERACTIONS OF MARINE LITTER AND OPEN-OCEAN AQUACULTURE FACILITIES: MADEIRA ISLAND AS CASE STUDY

Parretti P[1,2,*], Martins M[3], Monteiro J[1], Almeida S[1], Pombo A[3], Andrade C[4,5,6], Canning-Clode J[1]

1-MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI) Funchal, (Portugal)
2-CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory and Faculty of Sciences and Technologies, University of the Azores, Ponta Delgada (Portugal)
3- Escola Superior de Turismo e Tecnologias do Mar, Peniche (Portugal)
4-CIIMAR Interdisciplinary Centre of Marine and Environmental Research, Matosinhos(Portugal)
5-Madeira Oceanic Observatory – ARDITI/OOM, Funchal (Portugal)
6-Mariculture Centre of Calheta, Madeira, Portugal

Mail: paola.parretti@gmail.com

Introduction

Anthropogenic litter on the sea surface, beaches and seafloor has been significantly increasing over recent decades (Galgani et al. 2015). In present days, marine litter is worldwide distributed, and the Atlantic Island of Madeira is no exception (Álvarez et al. 2020). This accumulation of litter in the ocean is severely affecting ocean and coastal ecosystems in numerous ways, with impacts ranging from debris ingestion and entanglement that directly endangers marine life (i.e., seabirds, fishes, mussels, turtles and marine mammals) to smothering benthic communities (Kühn S et al. 2015) or spreading pest species and diseases (De la Torre et al. 2021; Lamb et al. 2018). In addition, there are also known economic impacts that may increase the cost associated with marine and coastal activities (Mouat et al. 2010). The aquaculture sector, widely recognised as an ocean-based source of marine litter (Bringer et al. 2021) but is also likely to be affected by marine litter. However, the impact of marine litter on open-ocean marine aquaculture facilities has received little attention. In this context, the present study combines information from video inspections of the cages, macro plastic in stomach contents and interview-based perception surveys to evaluate interactions between marine litter and open-ocean aquaculture of *Sparus aurata* in the south coast of Madeira Island.

Material and Methods

To assess the presence of marine litter in the proximity of offshore seabream aquaculture cages, video recording techniques were employed. The recorded videos were visually inspected and manually annotated to record presence of marine litter items and classify them following OSPAR hierarchical classification (OSPAR commission 2010). In addition, to assess correlation between macro plastic ingestion and the survivorship of aquaculture' fishes, gastrointestinal contents were inspected on specimens collected alive (n=164) and dead (n=233). Morphometric data from specimen was collected and plastic fragments were classified according to the Fulmar EcoCO methodology (MSFD 2013). Finally, a survey questionnaire was designed for aquaculture facilities' personnel to assess the perception of interference between marine litter and aquaculture activities. Data were gathered both through in-person and online interviews. Descriptive statistics were used to summarise results from the surveys, and statistical analyses were conducted using IBM SPSS statistic version 27.

Results

From 103 video surveys conducted, only 10.68% showed plastic debris around the offshore cages. A total of 12 plastic items was identified, being the category OSPAR2 (i.e. bags) the most frequent one (66.7%). While none of the specimens collected alive (n=164) contained macroplastic in their gastrointestinal tracts, a small percentage of individuals collected dead (5.15%, n= 12) contained plastics in their gastrointestinal content. All plastic debris found corresponds to the category "plastic sheet" of Fulmar EcoCO classification (e.g. remains from bag, agricultural sheets or rubbish bags). A maximum of 1 piece of debris was found in each individual of 0.96 ±0.59g (mean ±ST) and 9.19±3.81cm (mean ±ST). No particular relationship was found between fish and debris sizes (g nor cm). Overall, data gathered from the survey questionnaire showed low marine litter interference with daily aquaculture activities (7.2%). Respondents believed that presently, marine debris does not particularly affect the aquaculture sector in Madeira. However, it was the general opinion that marine litter can become a real threat to this activity in the future (96.3%). Respondents believe that marine litter represents a significant risk for the native fauna and flora in general and that local fisheries are the most affected economic activity. Respondents

(Continued on next page)

also identify fishing activities as the major source of marine litter and consider that aquaculture is currently an irrelevant source of marine litter production. Overall, respondents showed a weak willingness to pay for a tax on marine litter (55.6%), but they revealed a stronger willingness to participate in voluntary initiatives to reduce and collect marine debris (88.9%). Moreover, the majority of respondent (68%) believed that action to reduce marine litter should be done globally and further suggested that the aquaculture sector should reduce plastic use.

Discussion

The present case-study provides baseline information on the interactions and impacts of marine litter and offshore aquaculture facilities and activities. The results of this study are limited to the specificities of Madeira Island and existing facilities, warranting particular care in drawing general conclusions. The perception that marine litter currently has low interference with open-ocean aquaculture facilities is compatible and in line with the findings from video inspection of cages and gastrointestinal content examination. However, it is important to underline that the video inspection and ingestion assessments included in the present study were focused on macro-plastics and did not take into consideration the presence, abundance and effects of microplastic (<0.5 cm) contamination and their accumulation in the trophic chain. Finally, results of survey questionnaire highlight the need to develop a dedicated monitoring program to assess the management of waste generated from aquaculture activities.

References

Álvarez S, Gestoso I, Herrera A, Riera L, Canning-Clode J (2020) A Comprehensive First Baseline for Marine Litter Characterization in the Madeira Archipelago (NE Atlantic). *Water, Air, & Soil Pollution* 231(4): 182, DOI: 10.1007/s11270-020-04517-x

Bringer A, Le Floch S, Kerstan A, Thomas H (2021) Coastal ecosystem inventory with characterization and identification of plastic contamination and additives from aquaculture materials, *Marine Pollution Bulletin*, Volume 167,112286,ISSN 0025-326X, DOI: 10.1016/j.marpolbul.2021.112286

De la Torre GE, Dioses-Salinas DC, Pérez-Baca BL, Cumpa LAM, Pizarro-Ortega CI, torres FG, Gonzales KN, Santillán L (2021) Marine macroinvertebrates inhabiting plastic litter in Peru. *Marine Pollution Bulletin,* Volume 167,112296, ISSN 0025-326X, DOI: 10.1016/j.marpolbul.2021.112296

Galgani F, Hanke G, Maes T (2015) Global Distribution, Composition and Abundance of Marine Litter. In: Bergmann M, Gutow L, Klages M (eds) Marine Anthropogenic Litter. Springer, Cham. DOI: 10.1007/978-3-319-16510-3_2

Kühn S, Bravo Rebolledo EL, van Franeker JA (2015) Deleterious Effects of Litter on Marine Life. In: Bergmann M., Gutow L., Klages M. (eds) Marine Anthropogenic Litter. Springer, Cham. DOI: 10.1007/978-3-319-16510-3_4

Lamb JB, Willis BL, Fiorenza EA, Couch CS, Howard R, Rader DN, True JD, Kelly La, Ahmad A, Jompa J, Harvell CD (2018) Plastic waste associated with disease on coral reefs. *Science* V 359, 6374, pp: 460-462, DOI: 10.1126/science.aar3320

Mouat J, Lozano RL, Bateson H (2010) Economic Impacts of marine litter. KIMO International, pp. 105

MSFD Technical Subgroup on Marine Litter (2013) Guidance on monitoring of marine litter 540 in European seas. Joint Research Centre Scientific and Policy Reports. European 541 Commission

OSPAR Commission (2010) Guideline for monitoring marine litter on the beaches in the OSPAR maritime area

FINDING DOURADA: ASSESSING *Sparus aurata* ESTABLISHMENT FOLLOWING AQUACULTURE ESCAPES

Parretti P[1,2,*], Monteiro J[1], Gizzi F[1], Martinez-Escauriaza R[3,] Chebaane S[1], Nogueira N[3,4,5], Andrade C[3,4,5], Canning-Clode J[1]

1-MARE – Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI) Funchal, (Portugal)
2-CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory and Faculty of Sciences and Technologies, University of the Azores, Ponta Delgada (Portugal)
3-Oceanic Observatory of Madeira, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação. Funchal, (Portugal)
4-CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, (Portugal)
5- Mariculture Center of Calheta, Directorate for the Sea, Calheta (Portugal)

E-Mail: paola.parretti@gmail.com

Introduction

Gilthead seabream (*Sparus aurata*) is an important commercial species, greatly appreciated for consumption and one of the most used fishes in aquaculture (Jouvenel and Pollard 2001). Offshore aquaculture is a growing industry trying to face the increasing demand for seafood, however, fish escapes are cause for concern as they can represent a threat to the marine environment, especially in locations where the farmed species were not previously present (Ramirez et al. 2015). Although this seabream is native in the North-eastern Atlantic it is considered a non-indigenous species (NIS) in Madeira archipelago (Alves and Alves 2002). In Madeira Island, this species was introduced in 1997 for aquaculture purposes (Alves and Alves 2002) which currently hosts three open-ocean aquaculture farms breeding and producing *S. aurata*. Three years after introducing the species in the archipelago, a first sighting of 5 individuals in the wild was reported in the south coast of Madeira (Alves and Alves, 2002). Generally, unwanted escaping of fish from offshore cages often results from equipment malfunction, accidents and storms. As such, depending on the frequency and severity of such accidental incidents, the number of fishes escaping to the wild could result in the establishment of a local population. Recent studies highlight the presence of wild *S. aurata* along the coast of Madeira, being caught by recreational spearfishers from 2004 (Martinez-Escauriaza et al. 2020a) and representing the sixteenth most caught fish species by recreational shore anglers in 2017 (Martinez-Escauriaza et al. 2020b). In this context, this study aims to better understand the distribution and occurrence of *S. aurata* in Madeira coastal habitats and assess their invasiveness in the archipelago.

Material and Methods

Information on captures (i.e., species, date, location and size) during sport fishing contests held in Madeira between 2010 and 2019 (n=443) were compiled and analysed to assess *S. aurata* occurrences, distribution and trends. In addition, a custom designed online survey questionnaire targeting stakeholders from three maritime activities (1- SCUBA diving; 2-spearfishing; and, 3-fishing) was developed to assess the presence of *Sparus aurata* in the coastal waters of the Madeira archipelago. The survey was designed using the application 'Maptionnaire' (www.maptionnaire.com), that allows users to interact and report georeferenced data through a web-based interface. Data were later analysed and plotted with ArcGIS and IBS SPSS version 27. Finally, a risk analysis was conducted to assess invasiveness and evaluate possible impacts of *S. aurata* in Madeira habitats, under current and future climate change scenarios, using the Aquatic Species Invasiveness Screening Kit (AS-ISK, Coop et al 2016). Concerted scores for Basic Risk Assessment (BRA), Climate Change Assessment (CCA) and BRA+CCA were estimated from multiple screenings by users with different expertise. Concerted BRA and BRA+CCA scores were compared with thresholds for marine fishes in both temperate and tropical regions to assess invasiveness risk.

Results

Sport fishing contest data from 2010 confirmed the presence of *S. aurata* in the wild with capture in both south and north coasts of the island; whereas data from 2018 showcase that *S. aurata* specimens were captured almost every month. Similarly, the 73 validated respondents of the online survey reported sightings and captures in 120 sites around the island, most of which reporting small groups of 5 individuals (57%) but with larger groups of more than 20 individuals also being reported (14%). Moreover, respondents' perception is that seabream sightings in Madeira have been increasing since 2015. Concerted BRA and BRA+CCA scores rank high despite ranging confidence intervals, with average scores above thresholds for marine fishes in both tropical and temperate regions.

(Continued on next page)

Discussion

Considering the spatial distribution of *S. aurata* sightings and captures around Madeira Island, with reports from sites distant from aquaculture farms, and increasing trends in captures and sightings, it is reasonable that it has successfully established a local population. This is not surprising considering that Madeira island environmental conditions are within the range of conditions suitable for *S. aurata*. In addition, *S. aurata* averaged BRA and BRA+CCA scores are above the threshold for marine fishes, suggesting it may be a high or medium risk species. Such scores are not surprising as well, due to the high adaptability in food and habitat (Balart et al., 2009), *S. aurata* can potentially become a fierce competitor of local native species (e.g., *Pagrus* spp., *Diplodus* spp.). Overall, findings of this study warrant further investigations into local *S. aurata,* specifically to confirm its reproduction in the wild and assess impacts from its behaviour and feeding habits.

References

Alves FMA, Alves CMA (2002) Two new records of seabreams (pisces: sparidae) from the madeira archipelago. *Arquipélago* Life and Marine Science 19 A: 107-111

Balart E F, Pérez-Urbiola J C, Campos-Dávila L, Monteforte M, Ortega-Rubio A (2009). On the first record of a potentially harmful fish, Sparus aurata in the Gulf of California. *Biological invasions*, *11*(3), 547-550.

Copp GH, Vilizzi L, Tidbury H, Stebbing PD, Tarkan AS, Miossec L, Goulletquer P (2016) Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. *Management of Bioogical Invasions* 7:343–350. Doi: 10.3391/mbi.2016.7.4.04https://doi.org/10.3391/mbi.2016.7.4.04

Jardas I (1996) The Adriatic ichthyofauna. Školska knjiga, Zagreb, 535pp. *Croatian with English abstract*.

Jouvenel J Y, Pollard D A (2001) Some effects of marine reserve protection on the population structure of two spearfishing target fish species, *Dicentrarchus labrax* (Moronidae) and *Sparus aurata* (Sparidae), in shallow inshore waters, along a rocky coast in the northwestern Mediterranean Sea. *Aquatic Conservations* 11, 1–9.

Martínez-Escauriaza R, Vieira C, Gouveia L, Gouveia N, Hermida M (2020a) Characterization and evolution of spearfishing in Madeira archipelago, Eastern Atlantic. *Aquatic Living Resources*, 33, 15.

Martínez-Escauriaza R, Hermida M, Villasante S, Gouveia L, Gouveia N, Pita P (2020b) Importance of recreational shore angling in the archipelago of Madeira, Portugal (northeast Atlantic). *Scientia Marina*, 84(4), 331-341.

Toledo-Guedes K, Sánchez-Jerez P, González-Lorenzo G, Hernández AB (2009) Detecting the degree of establishment of a non-indigenous species in coastal ecosystems: sea bass Dicentrarchus labrax escapes from sea cages in Canary Islands (Northeastern Central Atlantic). *Hydrobiologia*, 623(1), 203-212.

Ramirez B, Ortega L, Montero D, Tuya F, Haroun R (2015) Monitoring a massive escape of European sea bass (Dicentrarchus labrax) at an oceanic island: potential species establishment. *Journal of Aquaculture Research and Development*. Vol. 6. doi: 10.4172/2155-9546.1000339.

A MACHINE LEARNING CLUSTERING ALGORITHM TO IDENTIFY GAPING BEHAVIOUR IN MYTILUS SPP UNDER CONTRASTING ENVIRONMENTAL CONDITIONS

Camilla Bertolini[1], Jacob J. Capelle[2], Tjeerd J. Bouma[3], Edouard Royer[1], Roberto Pastres[1*]

(1) DAIS, Ca' Foscari University of Venice, Via Torino 155, 30172, Mestre, Italy
(2) Wageningen marine research, 4401 NT Yerseke, The Netherlands
(3) EDS, Netherlands Institute for Sea Research, 4401 NT Yerseke, The Netherlands

Email: pastres@unive.it

Introduction

Lagoons and deltas, are highly heterogenous transitional systems, subject to multiple pressures. Species inhabiting these areas have adapted to cope with the natural heterogeneity (Bertolini et al. 2021) but local and global anthropogenic pressures, including climate change, may increase stress and in some cases lead to mortality. Studying behavioural responses can be the key to identify sub-lethal stress, as behaviour can have physiological links. Mussel gaping, for example, is a highly dynamic process, linked to key functions of metabolism, such as feeding and respiring: changes in its temporal pattern can affect energy intake and allocation, ultimately influencing growth and reproduction. This can be important in the context of sustainable cultivation of these species, where resource utilisation should be optimised by maximising the return from the input of seed, thus avoiding periods of metabolic suppression or energetically costly processes. The aim of this study was therefore to (1) test the use of a machine learning algorithm to identify key behaviours and (2) understand whether consistent patterns of behaviour could be linked to specific environmental conditions.

Methods

Biophys sensors (Bertolini et al. 2021) were deployed at multiple aquaculture sites of *Mytilus galloprovicnialis* within the Venice Lagoon (VL: 6 sensors, 3 sites, 1 year) and *M. edulis* in the Wadden Sea (WS: 30 sensors, 2 sites, 1 month). With the use of these electro-magnetic sensors the valve gaping amplitude (between 0: close and 1:open) can be obtained every minute, coupled with the temperature of the surrounding water.

Other environmental parameters likely to influence behaviour were measured in continuum (VL, 1 station: Dissolved Oxygen, Chlorophyll, Turbidity, sampling frequency 12 minutes ; WS, 2 stations: Chlorophyll, Turbidity, sampling frequency 5 minutes). A clustering algorithm (fuzzy k-means) was applied to daily patterns of gaping and to the environmental parameters measured. Within the days corresponding to a specified "environmental cluster", the occurrences of the different "gaping clusters" were counted in order to understand the prevalence of certain behaviours under specific combinations of environmental conditions.

Results

The algorithm led to identify the three distinct gaping clusters shown in Fig. 1, named "narrow", "mid", "wide" at all sites, except one, where the "mid" cluster was not found. Environmental variables also resulted in distinctive environmental clusters (VL: temperature and dissolved oxygen: 5, chlorophyll: 4 and turbidity: 3 ; WS: temperature: 3, chlorophyll and turbidity:2). At all sites in the VL mussels were more frequently widely opened (20 vs 10 days) at the highest temperature (cluster means 25-27°C) and when dissolved oxygen exhibited a more evident fluctuation. On the other hand, they were more frequently narrow (15 vs 5 days) when temperatures were in the mid-low (cluster means 10-12°C) and oxygen saturation mid-high. No relation could be found in VL with chlorophyll and the number of observations for medium and high turbidity were too low. In the WS there was a separation between the two sites, which exhibited distinct patterns of chlorophyll and turbidity. One site had low levels of chlorophyll and turbidity and mussels were more frequently narrow gaping (narrow: 35, mid:50, wide:18) while in the other site, where both chlorophyll and turbidity were higher they predominantly had medium and wider gaping (narrow: 30, mid: 80, wide: 50).

(Continued on next page)

Discussion

This study found that, an unsupervised consolidated clustering algorithm, allowed to identify patterns of behaviour and characterise some of its potential drivers. For example, at higher temperatures, mussels were consistently displaying a wider opening angle. This is consistent with shellfish physiology and formulations used in bioenergetic models, which predict an increase in oxygen demand with temperature. However, this behaviour could also increase the energy intake, which, on the other hand, is assumed to be maximum at the organism optimal temperature. Therefore, a more detailed analysis of these data could lead to improve shellfish bioenergetic models, which are being increasingly used in shellfish farming. Furthermore, mussels maintained a wide gape even in high turbidity conditions. At this site mussels have wider palps and grow less, suggesting adaptation to the conditions coming at a cost.

Figures

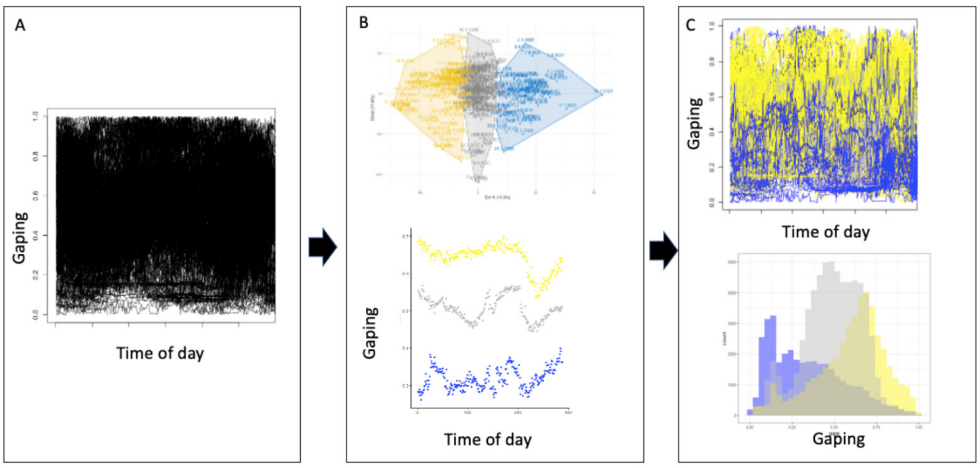


Fig.1 Example of processing. (A) raw data, (B) clusters identification (blue: narrow, gray: medium and yellow: wide), (C) validation

Acknowledgements

Scientific activity performed in the Research Programme Venezia2021, with the contribution of the Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia, provided through the concessionary of State Consorzio Venezia Nuova and coordinated by CORILA. The research leading to these results has also received funding from the GAIN project, European Union's HORIZON 2020 Framework Programme under GRANT AGREEMENT NO. 773330.

References

Bertolini C, Rubinetti S, Umgiesser G, Witbaard R, Bouma TJ, Rubino A, Pastres R (2021) How to cope in heterogeneous coastal environments: Spatio-temporally endogenous circadian rhythm of valve gaping by mussels. Sci Total Environ 768:145085

INHERITANCE OF SPERM CRYORESISTANCE IN COMMON CARP *(Cyprinus carpio)*

Bernadett Pataki[1], Béla Urbányi[1], Tímea Kollár[1], Ákos Horváth[1]

[1]Department of Aquaculture, Szent István University, Páter Károly u. 1, Gödöllő H-2100, Hungary
Email: pataki.bernadett@uni-mate.hu

Introduction
Conservation of aquatic genetic resources is crucial for both the preservation of biodiversity and for aquaculture. However there is not much information regarding the effects of the cryopreservation itself. Babiak et al. (2002) found that in the rainbow trout sperm of fish originating from eggs fertilized with cryopreserved sperm shows a higher cryoresistance in terms of fertilizing capacity. The present study evaluated the same method in common carp *(Cyprinus carpio)*.

Materials and methods
Six F1 full-sib families were created using the eggs of a single female which was fertilized with either 50 µL fresh or with 50 µL cryopreserved sperm from the same males (N=6). After sexual maturation spermiation of each F1 male was induced with Ovopel mammalian GnRH analogue. After 24 hours the males were spawned and the sperm was collected into 5-mL Eppendorf tubes. The fresh sperm parameters were measured with CASA (Computer Assisted Sperm Analysis) system. 100 µL of the samples were cryopreserved, 50 µL into each staws. Before cryopreservation the samples were diluted in grayling extender (200 mM glucose, 40 mM KCl, 30 mM Tris, pH: 8.0±0.2, Horváth et al. 2012) and methanol (10%) to the concentration of 10^9 spermatozoa per mL. The diluted samples were loaded into 0.5-mL plastic straws (Minitube GmbH, Tiefenbach, Germany). The samples were cryopreserved in the vapour of liquid nitrogen 3 cm above its surface for 3 minutes. After cryopreservation the samples were plunged into liquid nitrogen. The samples were thawed for 13 sec at 40 °C and post-thaw motility parameters (total motility, progressive motility, VCL, VAP, VSL, STR, LIN) were measured by CASA. A two-way ANOVA was conducted to investigate the main effects of sampling date and origin of the fish (fresh or cryopreserved sperm) on the motility parameters using the JASP software.

Results
It was found early on during the study that the family had no effect on the motility parameters of common carp sperm. Thus, our studies concentrated on the main effects of the origin of fish (fresh or cryopreserved sperm) and the sampling dates.

We found that the origin of fish ($p = 0.024$) as well as the sampling date ($p < 0.001$) had a singnificant main effect on the progressive motility of cryopreserved common carp sperm. The progressive motility of cryopreserved sperm from fish originating from eggs fertilized with cryopreserved sperm was 65 ± 15% while that of cryopreserved sperm from fish originating from eggs fertilized with fresh sperm was 59 ± 20%.

In case of all other parameters, no significant main effect of the origin of fish was detected. The sampling date had a significant main effect of motility parameters in all cases except for STR ($p = 0.206$) and LIN ($p = 0.051$).

Discussion
We have found that common carp originating from fertilization with cryopreserved sperm had a significantly higher post-thaw progressive motility measured by CASA than those originated from fertilization with fresh sperm. This is indirect proof that cryopreservation has a lasting effect on the reproductive characteristics of fish.

(Continued on next page)

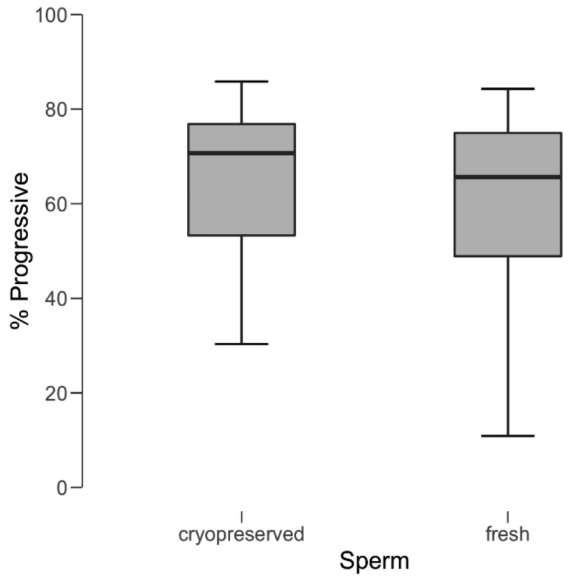


Fig.1. Progressive motility after cryopreservation of the sperm of males originated from fresh ($N = 63$) and cryopreserved sperm ($N = 46$). Significant differences can be found between the group originated from fresh and the group originated from cryopreserved sperm.

Acknowledgements

This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Subprogramme (TKP2020-IKA-12), the project K129127 of the National Research, Development and Innovation Office of Hungary and the EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union and the European Social Fund.

References

Babiak, I., Glogowski, J., Dobosz, S., Kuzminki, H., & Goryczko, K. (2002). Semen from rainbow trout produced using cryopreserved spermatozoa is more suitable for cryopreservation. Journal of Fish Biology, 60(3), 561–570.

Horváth, Á., Jesenšek, D., Csorbai, B., Bokor, Z., Raboczki, É., Kaczkó, D., Bernáth, G., Hoitsy, G., Urbányi, B., Bajec, S.S., Snoj, A., 2012. Application of sperm cryopreservation to hatchery practice and species conservation: A case of the Adriatic grayling (*Thymallus thymallus*). Aquaculture 358–359, 213–215.

SWEEPSTAKES REPRODUCTIVE SUCCESS FROM FERTILIZATION TO HATCHING IN A DOMESTICATED LINE OF *Oncorhynchus mykiss*

Paul, K.[1]*, Pelissier P.[2], Goardon L.[2], Dechamp N.[1], Dupont-Nivet M.[1], Phocas, F.[1]

1. Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France
2. INRAE, UE 0937 PEIMA, 29450 Sizun, France
E-mail : katy.paul@inrae.fr

Introduction

In a recent study, [1] showed that the INRA rainbow trout synthetic line, developed in the early 1980s by intercrossing several domesticated lines to create an experimental line with a large genetic variability, has a lower effective size than expected on the basis of its census size, while the population size has been kept constant over time by annually mating balanced numbers (60 to 80) of females and males. We hypothezise that it may be due to the well-known Sweepstakes Reproductive Success (SRS) phenomenon in fish [2], which results in very unbalanced parental contributions to the next generation. SRS may induce a large variance in reproductive success among individuals not only breeding in the wild, but also in multigenerational trout hatchery programs [3]. To validate this hypothesis, we carried out an experiment at the INRAE facilities (PEIMA, Sizun) to investigate the parental contributions and the offspring survival for a set of genotyped parents from the INRA synthetic line.

Materials and Methods

To assess the parental contributions to egg fertilization and offspring survival until first feeding, a full factorial mating plan was designed with 15 dams and 9 sires of the INRA rainbow trout synthetic line. All parents were genotyped for 57,501 SNP with the AxiomTM Trout Genotyping array. From the total spawn of each female, we retain 3 duplicate packages of 21 eggs each for parental contributions' study and 2 packages of 50 eggs for egg quality measurements. From the milt of 9 sires randomly divided into 3 groups of 3 males and the eggs of 15 dams also divided into 3 groups of 5 females, we fertilized ova according to a full factorial mating design made of 9 small factorial plans (each group of 5 females mated to each group of 3 males) generating 9 batches of 105 ova (duplicate batches). The eggs fertilization of each batch of 5 females was carried out male-by-male on a mixed set of 35 ova (7 ova from each of the dams). After fertilization, all eggs from a small factorial plan were grouped together in the same incubator until the eyed stage. In total for each duplicate batch, each sire could fertilized 105 eggs and each couple may have up to 7 offsprings. At eyed egg stage (20 dpf at 12°C), all embryos from the first duplicate batches were genotyped on a 96 SNP-array used for parental assignment. From the eyed egg stage until the end of the experiment (3 weeks after first feeding), all dead offsprings were collected each day and all dead and alive offsprings were genotyped with the AxiomTM Trout Genotyping array to perform both parental assignment and GWAS on offspring survival. Starting from the day of fertilization, offspring survival was evaluated at 3 stages: eyed egg stage (Se), hatching (Sh) and 3 weeks after first feeding (Sf). Parental contributions were assess at these 3 stages by counting the number of alive progeny for each parent and couple. Fisher exact tests were performed to assess imbalances in parental contributions to offspring survivals Se, Sh and Sf. In addition, time to death (TD in days) from eyed egg stage to hatching was analyzed under a GBLUP model including the fixed effect of the incubator, the random animal additive genetic effect and 3 covariates accounting for the offspring inbreeding coefficient (F) and its dam and sire inbreeding coefficients (F_{dam} and F_{sire}).

Results

Offspring survival rates were in average 91.0% for Se, 87.2% for Sh and 84.4% for Sf (Table 1). While no unbalanced sire contributions to the progeny cohort was detected, significant unbalanced dam contributions to offspring survival was observed at eyed egg stage and at hatching with values ranging from 71.4% to 100% for Se and from to 65.1% to 98.4% for Sh, depending of the dam (Table 1). After hatching, mortality was low and no strong variability was observed among the dams for their offspring survival beween hatching and the end of experiment. Therefore, we only investigate the genetic variability of offspring survival at hatching stage (TD). Heritability of offspring survival until hatching was low under a linear model (h^2=0.11±0.04). No significant QTL was detected. While dam and sire inbreeding levels did not significantly impact their offspring survival, offspring survival increased with its own inbreeding level (regression coefficient b = 11.2 ±3.4 d).

(Continued on next page)

Table 1. Dam size and reproduction mean performance and phenotypic correlations of dam characteristics with offspring survival rates.
FL: female fork length (mm); PW: female post-spawning body weight (g); SW: spawn weight (g); EW: average egg weight (mg); ED: average egg diameter (mm); CF: weight of coelomic fluid (g); F_{dam} : dam inbreeding coefficient. Se: Offspring survival from fertilization to eyed egg stage (22 days after fertilization); Sh: Offspring survival from fertilization to hatching (35 days after fertilization); Sf: Offspring survival from fertilization to 3 weeks after first feeding (64 days after fertilization).

Trait	Mean	Min	Max	Correlation with Se	Correlation with Sh	Correlation with Sf
FL	487,8	437	522	- 0.07	- 0.12	- 0.03
PW	2053,4	1382	2753	- 0.45 *	- 0.54 **	- 0.46 *
SW	267,1	177,8	406,5	0.50 *	0.54 **	0.58 **
EW	71,3	63,3	80,4	-0.09	- 0.31	- 0.38
ED	5,3	5,1	5,52	-0.17	- 0.24	- 0.29
CF	27,8	19,2	35,2	0.21	0.20	0.11
F_{dam}	0,18	0,11	0,42	0.22	0.19	0.18
F_{sire}	0.17	0.10	0.28	- 0.39	- 0.30	- 0.27
Se	91.0%	71.4%	100%	1	0.89	0.84
Sh	87.2%	65.1%	98.4%	-	1	0.97
Sf	84.4%	63.5%	95.2%	-	-	1

** p_value < 0.05 * p_value < 0.1

Investigating how dam characters correlate with offspring survival (Table 1), we only observed significant correlations with the dam post-spawning weight and her spawn weight, but in opposite trends. While high spawn weight was favourable to offspring survival, post-spawning weight of the dam was negatively correlated with offspring survival. Negative but not significant trends were also observed between egg quality traits (egg weight and diameter) and survival rates. Surprinsingly, it seems that the higher the number of eggs in the spawn is, the better the survival of each of them is.

Discussion and conclusion

Unbalanced dam contributions to the offspring cohort were identified and may explain the reduction in effective size of the INRA rainbow trout synthetic line over generations. Explanations for these unbalanced contributions remain unclear, but seem to relate to the dam fecundity and some intreseque egg quality traits that do not refer to egg size.

References

[1] D'Ambrosio et al.. 2019, Genetics Selection Evolution, 51:26.
[2] Hedgecock & Pudovkin. 2011, *Bullet in of Marine Science*
[3] Araki H. et al. 2007, *Mol Ecol*. 2007; doi:10.1111/j.1365-294X.2006.03206.x.

POLLUTION EFFECTS OF AQUACULTURE ACTIVITIES IN THE COASTAL MARINE ENVIRONMENT, Eastern mediterranean

A.Pavlidou*, Th. Zoulias, P. Zachioti, E. Rouselaki and I. Hatzianestis

Hellenic Centre for Marine Research, Institute of Oceanography, 46.7 Km Athens-Sounio Av., Anavyssos, 19013, Greece
aleka@hcmr.gr

Introduction
Marine finfish aquaculture industry is expanding, as the demand for seafood rises and cannot be met by wild catch fisheries. Greece dominates the Mediterranean aquaculture industry, largely based on the production of the Mediterranean sea bass and gilthead sea bream. However, aquaculture is associated with a wide range of environmental concern which need to be addressed for its sustainable development. Pollution problems caused by the application of antibiotics, chemicals and medicines, overstocking and overfeeding fishponds, fish waste etc, may affect the water quality, contribute to eutrophication problems and reduce dissolved oxygen concentration (Ahmed and Thompson, 2019). Eastern Mediterranean Sea (EMED) presents an oligotrophic character, thus, the rich protein food for feeding fish species such as the sea bream and sea bass, is an important source of nutrient inputs to coastal areas. The aim of this work is to investigate the effects of fish farms on the water column and the sediments, and assess the trophic status of the coastal marine environments affected by the fish cultures in relation to the undisturbed coastal waters.

Methodology
Water and sediment samples from eight coastal stations located very close to the floating finfish cages were taken monthly during two sampling periods: 2012-2013 and 2015-2016. Chemical parameters (e.g. dissolved oxygen, nutrients, total nitrogen, total phosphorus) were measured in the water column together with organic contaminants in sediments (aliphatic and polycyclic aromatic hydrocarbons), according to standard methods. The total amount of nitrogen and phosphorous released through the added food from the cages was evaluated, based on feed data from the aquacultures. Moreover, the trophic indexes developed for the oligotrophic waters of the Eastern Mediterranean were estimated (Pavlidou et al., 2015).

Results
Increased concentrations of inorganic and organic nitrogen have been recorded in the water column beneath the cages. It seems that the fish farms enrich the Greek coastal waters in inorganic nitrogen, mainly ammonium, but not in phosphorus, keeping the phosphorus limiting character of the EMED. Organic nitrogen and organic phosphorus predominated, whereas, ammonium was the predominant inorganic nutrient in the study areas. The organic nitrogen and phosphorus were higher during summer, leading to the relative downgrade of the trophic status of the water environment very close to the cages. The majority of stations has been classified into Good and Moderate trophic classes. Strong positive correlation between ammonium concentrations measured in the water column below the cages with the nitrogen and phosphorus released via feed, and negative correlation between dissolved oxygen and the released nitrogen and phosphorus was observed indicating probable effect of aquaculture activities in the marine environment.

Pollution from aliphatic and polycyclic aromatic hydrocarbons (PAH) was found in some of the aquaculture sediments. The predominant PAHs at the contaminated sites were of petrogenic origin, which is a quite unusual distribution for marine sediments, but it is similar to aquaculture sediments in other places of the world and is probably related to the fish feed (Wang et al., 2010; Tsapakis et al., 2010). Enrichment of some aquaculture sediments in organic carbon and phosphorus was also observed.

Conclusions
During summer the aquaculture activities seem to consist an important anthropogenic pressure for the surrounding marine environment, since the metabolic rate of the fish and the feed are increased. Ammonium and total nitrogen values were higher below and very close to the cages, compared to unaffected coastal waters, indicating the impact from the aquaculture to the marine environment, which however, is restricted close to the cages. Organic pollution from hydrocarbons was detected, which, even though lower than in other polluted areas (e.g. ports) should be considered as a pressure in the marine environment, thus PAHs should be included in any monitoring scheme regarding aquacultures. In order to meet the demand for food from a growing global population, aquaculture production should increase sustainably while at the same time its environmental impacts should be reduced considerably. Producing fish in an environmentally sustainable way is essential.

(Continued on next page)

References

Ahmed N and Thompson S, (2019). The blue dimensions of aquaculture: A global synthesis, Science of the Total Environment, 652: 851–861.

Pavlidou A., et al., (2015). Methods of eutrophication assessment in the context of the water framework directive: Examples from the Eastern Mediterranean coastal areas, Continental Shelf Research, 108: 156-168.

Tsapakis, M. et al., (2010). PAHs and n-alkanes in Mediterranean coastal marine sediments: aquaculture as a significant point source. Journal of Environmental Monitoring, 12:958-963

Wang, H.S, Liang, P, Kang Y, Shao D.D, Zheng G.J, Wu S.C, Wong C.K.C, Wong M.H., 2010. Enrichment of polycyclic aromatic hydrocarbons (PAHs) in mariculture sediments of Hong Kong. Environmental Pollution, 158:3298-308

IMPACT OF SEASONAL VARIATIONS ON THE CAROTENOID AND CHLOROPHYLL CONTENT OF *Fucus virsoides* FROM THE ADRIATIC SEA

S. Pedisić[a*], A. Dobrinčić[a], P. Lisica[a], Z. Zorić[a], Z. Čošić[a], Z. Pelaić[a], V. Dragović-Uzelac[a] and R. Čož-Rakovac[b,c]

[a]Faculty of Food Technology & Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia
[c]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[d]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
E-mail: spedisic@pbf.hr

Introduction

Marine macroalgae are valuable sources of bioactive compounds which are used in nutritional and/or pharmaceutical products. Marine algae can be classified based on pigment contents which have various biological activities such as antioxidant and anti-inflammatory activities (Catarino et al., 2018). Widely distributed Fucus genus of brown macroalgae is an excellent source of fucoxanthin carotenoid which contributing around 10% estimated total production of carotenoids in nature (Kim and Pangestuti, 2011). Chlorophyll a, is the most important chlorophyll for photosynthesis since allow the conversion of light into biological energy (Osorio et al., 2020). The inherent characteristics of each species and different environmental factors induced by seasonal variations in their normal ecosystem (temperature, light, salinity) controls the macroalgal growth rate in nature and may also affect their pigment contents (Marinho-Soriano 2012). However, the potential impact of seasonal variations in their bioactivity profile is often neglected in analytical studies. The brown alga *Fucus virsoides* is an endemic species in the Adriatic Sea and its typical habitat is in the intertidal zone, an extremely variable environment (Najdek et al., 2014).

Therefore, the aim of this study was to evaluate the effect of seasonal environmental variations on pigment content of *Fucus virsoides* from the Adriatic Sea.

Materials and method

Environmental parameters water and air temperature (°C) and salinity were measured with Hydrolab (DS5X).

Sampling of the brown alga *Fucus virsoides* was performed seasonally (Viz. January, May to October 2019) in coastal area of the Adriatic Sea (44°12 02´N to 15°28´51E, Croatia) at 0.5 m depth or along the surface of the sea. Samples were washed in seawater and then rinsed with distilled water, frozen at -60 °C, freeze dried (CoolSafe lyophilizer, Model: 55-9 PRO, Labogene, Denmark) and milled. Ultrasound-assisted extraction (Elmasonic 40H, Elma, Germany) was performed 20 minutes at 30 °C with acetone. Extracts were filtered and stored at 4 °C until analysis. Determination of pigments was performed using HPLC system (Agilent Infinity 1260 system) with UV/VIS PDA detection according to method described by Castro–Puyana et al. (2017). The identification of individual carotenoid and chlorophyll compounds was performed on the basis of their retention times and UV-VIS spectra and quantification was performed by the external standard method using calibration curves of the authentic standards (β-carotene, fucoxanthin, zeaxanthin, chlorophyll a and b). Data analyses were performed with the Excel and all values are expressed as mean±SD of three parallel measurements.

Results

Measured environmental parameters such as water and air temperature and salinity considerably differed in the winter period (13 °C, 8 °C and 33) compared to spring (17 °C, 19 °C and 38) and autumn (20 °C, 22 °C and 38) period. The highest pigment content was determined in acetone extract of brown alga *Fucus virsoides* sampled in January (35.6 mg/100 g) and lower in algae extracts sampled in October (27.14 mg/100 g) and May (26.79 mg/100 g) of 2019. Pigment content in all obtained extracts was mainly composed of carotenoids. Fucoxanthin was the predominant carotenoid (16.04–23.21 mg/100 g) followed by derivative of fucoxanthin (4.18-5.64 mg/100 g) and violaxanthin (2.80-4.09 mg/100 g). The β-carotene was determined in the lowest content in all samples (0.68-0.80 mg/100 g). Chlorophylls were determined in significantly lower contents and chlorophyll a was the most abundant (0.029-0.044 mg/100 g).

(Continued on next page)

Discussion and conclusion

In our study pigment composition and content was determined in the seasonally sampled brown alga *Fucus virsoides* located in Adriatic Sea. Carotenoids are the major pigments in *Fucus virsoides* and approximately 60-65% of the total carotenoid content corresponds to xanthophyll fucoxanthin what is in accordance with literature data (Catarino et al., 2018). Chlorophyll a is major chlorophyll in all samples. Results showed that pigment content was significantly higher in algae extracts sampled in winter (January of 2019) than in spring (May 2019) and autumn (October 2019) period. Pigment content decreased in the spring and then slightly increased again in autumn. Loss of pigment content during spring and autumn compared to winter period is probably related to environmental conditions such as light, temperature and salinity (Osório et al., 2020). Measured environmental parameters such as water temperature, air temperature and salinity were considerably lower in the winter period while in spring and autumn period slight differences were observed. According to literature data, pigment content especially of xanthophylls decreased in algae if the light intensity was increased due to increase in temperature (Ismail & Osman, 2016). Salinity and temperature have significant effects on photosynthetic pigments also (Ding et al., 2013).

In conclusion, the present study showed that the most suitable period for collection of *Fucus virsoides* from the Adriatic Sea with high pigment content is winter.

References

Castro-Puyana, M., Pérez-Sánchez, A., Valdés, A., Ibrahim, O.H.M., Suarez-Álvarez, S., Ferragut, J.A., Micol, V., Cifuentes, A., Ibáñez, E., and García-Cañas, V. 2017. Pressurized liquid extraction of *Neochloris oleoabundans* for the recovery of bioactive carotenoids with anti-proliferative activity against human colon cancer cells. Food Research International 99, 1048-1055.

Catarino, M.D., Silva, A. M. S., Cardoso S.M. 2018. Phycochemical Constituents and Biological Activities of Fucus spp. Marine Drugs 16, 249-283.

Ding, L., Ma, Y., Huang, B., Chen, S. 2013. Effects of seawater salinity and temperature on growth and pigment contents in *Hypnea cervicornis* J. Agardh (Gigartinales, Rhodophyta). BioMed Research International ID 594308, 10.

Kim, S-K., Pangestuti, R., 2011. Chapter 9- Biological Activities and Potential Health Benefits of Fucoxanthin Derived from Marine Brown Algae. Advances in Food and Nutrition Research, 64, 111-128.

Marinho-Soriano, E. 2012. Effect of depth on growth and pigment contents of the macroalgae *Gracilaria bursapastoris*. Brazilian Journal of Pharmacognosy 22(4), 730-735.

Najdek, M., Iveša, Lj., Paliaga, P., Blažina, M., Čelig, A. 2014. Changes in the fatty acid composition of *Fucus virsoides* J. Agardh in response to the type of substratum. Acta Adriatica 55(1), 19 – 30.

Osório, C., Machado, S., Peixoto, J., Bessada, S., Pimentel, F.B., Alves, R.C., and Oliveira, M.B.P.P. 2020. Pigments content (Chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. Separations 7, 1-14.

SEASONAL CHANGES OF ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF THREE MACROALGAE FROM THE PORTUGUESE COAST

Pedro, J.[ac*], Cardoso, C.[ab], Afonso, F.[c], Bandarra, N. M.[ab]

[a] Division of Aquaculture, Upgrading and Bioprospection (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, IP), Avenida Alfredo Magalhães Ramalho, 6, 1495-006 Lisbon, Portugal
[b] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal
[c] CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal.
E-mail: joana.pedro12@gmail.com

Introduction
Macroalgae are natural products rich in bioactive compounds with antioxidant and anti-inflammatory activities supplying bioactive substances with a wide range of applications, including human and animal nutrition. However, from the edible seaweed of European Atlantic shores, there are still many species that call for further investigation. Bearing this in mind, the three macroalgae *Bifurcaria bifurcata*, *Carpodesmia tamariscifolia* and *Codium sp*. were selected and harvested in two different seasons. The main goals of this work were to assess the *in vitro* bioactivities variation with the season of these understudied seaweed species from the Portuguese coast and consequently disclosure their potential interest in animal feed and human diets.

Material and Methods
The brown macroalgae *Bifurcaria bifurcata* and *Carpodesmia tamariscifolia*, previously classified as *Cystoseira tamariscifolia*, and the green macroalgae *Codium sp.* were harvested in the proximities of Farol do Cabo Raso, Portugal (38°42'38.7"N 9°29'09.7"W), in August and November of 2020. After immediate transport, the fronds of the three seaweeds were rinsed with potable water, freeze-dried, ground and stored at -80ºC until further analysis. Free fucose was determined by the cysteine-sulphuric acid method for methyl pentoses (Xing et al., 2013) and total polyphenol content of ethanol extracts was determined by the Singleton and Rossi (1965) method, using the Folin-Ciocalteu reagent. As for the antioxidant activity, the DPPH radical (Miliauskas et al., 2004), Ferric Ion Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1996) and ABTS radical cation (Re et al., 1999) assays were applied. Anti-inflammatory activity was quantified, by testing ethanol extracts at 1 mg/ml, using a commercial cyclooxygenase (COX) inhibitory screening assay kit (Cayman Chemical Company, USA).

Results
Results showed that the highest total polyphenol level was found in *B. bifurcata* regardless of the season, varying between 10.13-11.01 mg GAE/g dw seaweed. On this species, seasonality showed no effect, however on *C. tamariscifolia* and *Codium sp.* there was an increase of phenolic content from Winter to Summer. Fucose was not detected in *B. bifurcata* and the overall results were low except for the *Codium sp.* Summer extracts which reached 4.68± 10.42 mg/g dw seaweed.

As for the antioxidant activities, by the DPPH method, the ethanol extracts of *B. bifurcata* were more antioxidant than the extracts from *C. tamariscifolia* and season affected both species. However, values were higher in the Summer extracts of *B. bifurcata* and Winter extracts of *C. tamariscifolia*. Moreover, the antioxidant levels assessed by the method FRAP showed high levels for the *Codium sp.* extracts, these being 20% more antioxidant in the Summer than in the Winter. On the other hand, *Codium sp.*, when compared to the other species, showed the lowest antioxidant levels according to the ABTS method, including in the Summer.

In addition, the extracts from all the species showed inhibition of the COX-2, the most anti-inflammatory extract being from the *C. tamariscifolia* harvested in Summer (94.2 ± 4.0 %). Season affected both *B. bifurcata* and *C. tamariscifolia*, where Summer extracts were more anti-inflammatory than Winter extracts, and there was no difference in the extracts from *Codium sp.*, that showed the lowest anti-inflammatory activity (13.6-15.5 %).

Overall, the results attained from the three species harvested in Summer and Winter showed a significant biotechnological potential, especially the studied brown macroalgae.

(Continued on next page)

References

Benzie, I.F.F. & Strain, J.J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, 239(1), 70–76.

Miliauskas, G., Venskutonis, P.R. & Van Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, 85(2), 231–237.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237.

Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.

Xing, R., Liu, S., Yu, H., Chen, X., Qin, Y., Li, K. & Li, P. (2013). Extraction and Separation of Fucoidan from *Laminaria japonica* with Chitosan as Extractant. *BioMed Research International*, 2013, 1–4.

CHRONIC INFLAMMATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES FED TRYPTOPHAN- SUPPLEMENTED DIETS

D. Peixoto[1,2*], I. Duarte[1,2], A. Ricardo[1,2], P. Santos[1,3], M. Machado[1], R. Azeredo[1], B. Costas[1,2]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal
[2] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[3] MARE-Politécnico de Leiria – Centro de Ciências do Mar e do Ambiente, Peniche, Portugal
*E-mail: dpeixoto@ciimar.up.pt

Introduction

Amino acids (AA), such as tryptophan, play several regulatory functions on key metabolic pathways that may be important, among other functions, to immune and neuro-endocrine responses. The inflammatory process is responsible for an increase of the metabolic needs which results in the depletion of essential nutrient provisions. For this reason, a higher nutrient demand has to be supplied in order to avoid the risk of an inefficient response and damage by self-mechanisms. Therefore, dietary supply of specific nutrients such as AA could be a strategy to be applied in specific situations when requirements are higher, such as stressful conditions and inflammation. This study aimed to contribute to this endeavour by assessing the immunological profile and inflammatory response of European seabass (*Dicentrarchus labrax*) fed dietary tryptophan supplementation undergoing a chronic inflammatory condition.

Materials and methods

European seabass juveniles (33.0 ± 3.78 g) were randomly distributed in 12 tanks and maintained in two recirculated seawater systems (temperature 20 ± 0.5°C; salinity 32; photoperiod 10h:14h D:L). At the beginning of the trial, a group of fish was sampled (undisturbed group). Afterwards, half of the fish were intraperitoneally injected with 100 µL of either Freund's Incomplete Adjuvant solution (FIA) to induce a peritoneal inflammation, while the other half was injected with Hanks' Balanced Salt solution (HBSS) and served as a sham group. In a complete randomized design, two dietary treatments were evaluated in triplicate groups: a control diet (CTRL), meeting the indispensable amino acids profile established for seabass, and a CTRL-based diet supplemented with tryptophan (0.3 % DM basis; TRP). Fish were fed these diets for 4 weeks, twice a day with a daily average ration of 2 % body weight. Sampling at 0 (undisturbed), 1, 2, 3 and 4 weeks (n=9) allowed the collection of data regarding haematological profile, plasma immune parameters (i.e. peroxidase, lysozyme and bactericidal activities) and gut immune and oxidative stress parameters (i.e. lipid peroxidation, reduced glutathione: oxidized glutathione ratio, peroxidase, bactericidal, superoxide dismutase and catalase activities). For all five sampling times fish were euthanized by anaesthetic overdose with 2-phenoxyethanol.

Results

Total peritoneal leucocyte counts increased in fish injected with FIA compared to HBSS group regardless diet and sampling point. Compared to the undisturbed group, FIA-injected fish augmented total white blood cells and this parameter increased in this group from 1 to 2 weeks. Red blood cells increased at 3 weeks post-injection and remained high until the end of the experiment, irrespective of dietary treatment or stimulation. FIA-injected fish decreased gut peroxidase and bactericidal activities compared to those injected with HBSS. However, gut bactericidal activity increased from 2 to 3 weeks, remaining high at 4 weeks regardless stimuli and diet. Regarding oxidative stress, superoxide dismutase activity, total glutathione and oxidized glutathione contents peaked at 2 weeks in FIA-injected fish gut, regardless diet, but decreased to basal levels at 4 weeks. Finally, regarding humoral immune parameters, bactericidal activity decreased after 1 week post-injection irrespective of dietary treatment or stimulation, while no significant differences were detected in either lysozyme and peroxidase activities.

(Continued on next page)

Discussion and conclusion

Preliminary data from the present study illustrate the effect of FIA-induced chronic inflammation, with both cellular response and the oxidative stress being enhanced in the presence of the phlogistic agent. Moreover, a clear time-dependent response was observed in most of the analysed parameters, evidencing their regulation over a prolonged timeframe, as expected in a chronic inflammatory setting. Tryptophan immune modulation has been shown to be closely associated to its role in the neuroendocrine response (Azeredo et al., 2017; Machado et al., 2019). The absence of tryptophan-mediated effects might be related to the neuroendocrine status of the fish throughout the experiment, where besides the intra-peritoneal injection and the development of an inflammatory response, there was no particular stressful condition. Nevertheless, future plasma cortisol measurements as well as brain and head-kidney neuroendocrine- and immune-related molecular analyses will assist in better understanding the interactive effects of tryptophan nutrition and chronic inflammation.

Acknowledgements

This work was supported by project INFLAMMAA (PTDC/CVT-CVT/32349/2017), financed by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020, and through national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal). DP and BC were supported by FCT, Portugal (UI/BD/150900/2021 and IF/00197/2015, respectively).

References

Azeredo R, Machado M, Afonso A, Fierro-Castro C, Reyes-López FE, Tort L, Gesto M, Conde-Sieira M, Míguez JM, Soengas JL, Kreuz E, Wuertz S, Peres H, Oliva-Teles A and Costas B (2017) Neuroendocrine and Immune Responses Undertake Different Fates following Tryptophan or Methionine Dietary Treatment: Tales from a Teleost Model. Front. Immunol. 8:1226. https://doi.org/10.3389/fimmu.2017.01226

Machado M, Azeredo R, Domingues A, Fernandez-Boo S, Dias J, Conceição LEC, Costas B (2019) Dietary tryptophan deficiency and its supplementation compromises inflammatory mechanisms and disease resistance in a teleost fish. Scientific Reports 9:7689. https://doi.org/10.1038/s41598-019-44205-3

DIETARY MICROALGAE INCLUSION CAN BOOST GROWTH PERFORMANCE AND HEALTH CONDITION OF SENEGALESE SOLE (*Solea senegalensis*) POST-LARVAE

D. Peixoto[1,2*], M. Hinzmann[1], W. Pinto[3], R. Nogueira[3,6], J. Silva[4], J. Navalho[5], J. Dias[3], L.E.C. Conceição[3], B. Costas[1,2]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal
[2] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[3] SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[4] ALLMICROALGAE - Natural Products, SA, Pataias, Portugal
[5] Necton S.A., Belamandil s/n, 8700-152 Olhão, Portugal
[6] Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
*E-mail: dpeixoto@ciimar.up.pt

Introduction

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species targeted for aquaculture in Southern-European countries. This species, as most farmed fish, is potentially subjected to stress and pathogens due to environmental factors. Functional feeds are fortified with selected ingredients/additives, rich in natural molecules and/or vital nutrients that improve fish health status, reducing infection and severity during an outbreak of infectious diseases. Also, can improve immunocompetence prior to or during vaccination counteracting immunosuppression caused by stress and contaminants. Microalgae have been included in diets for farmed aquatic animals due to their antioxidant capacity and bioactive compounds, promoting optimal growth and health. For these reasons, this study intended to evaluate the effects of dietary microalgae inclusion in both health status and growth performance of Senegalese sole post-larvae.

Materials and methods

Senegalese sole post-larvae with 34 days after hatching (DAH) were randomly distributed by 15 tanks with an initial density of 2650 post-larvae/m^2 and three isonitrogenous (60% crude protein) and isolipidic (17% crude lipids) diets were randomly distributed by triplicate groups of tanks. The experimental diets comprised a control (CTRL) diet based on marine protein sources (i.e. fish meal, squid meal and krill meal) and 3 % of cellulose filler, whereas four other diets were based on the control diet but replaced cellulose by 3 % *Isochrysis galbana* (ISO), *Nannochloropsis sp*. (NANO), *Skeletonema* sp. (SKELO) and *Tetraselmis striata* (TETRA). The experimental diets were supplied through automatic feeders set up to supply 8 meals in a 24 h period. At 65 DAH, 30 post-larvae/tank were sampled for analyses of parameters related to health status. The total length, dry weight, feed conversion ratio, relative growth rate, and survival were also assessed.

Homogenates of 10 individuals were performed for the analyses of immune (i.e. lysozyme and bactericidal activities) and oxidative stress (i.e. reduced glutathione: oxidized glutathione ratio, catalase, lipid peroxidation, glutathione S-transferase and superoxide dismutase activities) related parameters. Visceral cavity plus head of 12 individuals from each treatment were used for gene expression analysis (i.e. hepcidin, complement component 3, interleukin-1β, interlukin-10, g-type lysozyme, toll-like receptor 1 and toll-like receptor 5).

Results

Survival, relative growth rate and feed conversion ratio were not altered by the dietary treatments. However, post-larvae fed ISO presented lower dry weight compared to the other dietary treatments whereas the total length of post-larvae fed NANO dietary treatment increased compared to those fed the ISO diet.

Regarding immune status, an increase in lysozyme levels and bactericidal activity against *T. maritimum* of the whole post-larvae in fish fed TETRA and SKELO, respectively, it was observed. Catalase and superoxide dismutase activities presented lower values in fish fed NANO, TETRA and SKELO when compared to CTRL and ISO dietary treatments. Reduced glutathione: oxidized glutathione ratio was shown to be lower in post-larvae fed ISO and NANO dietary treatment, but contrary to NANO, post-larvae fed ISO increased their levels of glutathione S-transferase. The mRNA expression levels of hepcidin, interlukin-10, toll-like receptor 1 and toll-like receptor 5 were up-regulated in fish fed NANO dietary treatment compared to the other dietary treatments.

(Continued on next page)

Discussion and conclusion

Results from the present study seem to be in line with the widely known recognition of microalgae in terms of antioxidant capacity and growth promotion, which usually translates into good welfare of farmed fish. Senegalese sole post-larvae fed dietary treatments presented normal ranges for growth performance and survival. Fish homogenates reveal that in general NANO improves the antioxidant system, whereas SKELO and TETRA showed improvements in fish immune status. Nonetheless, molecular biomarkers in fish fed NANO suggested that this microalga seems to also improve the Senegalese sole immune status by the up-regulation of immune related genes, such as toll-like receptor 1, toll-like receptor 5, hepcidin and interleukin-10. This up-regulation can be related to the described immunostimulatory characteristics of *Nannochloropsis* sp., improving the innate defense mechanisms in the fish and thereby providing enhanced resistance to pathogens. According to results from the present study, *Nannochloropsis sp*., *Skeletonema* sp. and *Tetraselmis striata* seem to be promising candidates for inclusion in microdiets for Senegalese sole post-larvae.

Acknowledgements

This work was supported by VALORMAR project (POCI-01-0247-FEDER-024517) funded by Compete 2020, Lisboa 2020, Algarve 2020, Portugal 2020 and the European Union through ERDF. BC was supported by FCT - Foundation for Science and Technology (IF/00197/2015).

PRO-HEALTH FEEDS TO REDUCE *Sparicotyle chrysophrii* IN SEA BREAM (*Sparus aurata*). IDENTIFICATION AND SELECTION OF NATURAL DIETARY COMPOUNDS WITH POTENTIAL ANTIPARASITIC EFFECTS

N. F. Pelusio[1]*, L. Parma[1], A. Di Biase[2], M. Fioravanti[1], A. Gustinelli[1], E. Brini[1], F. Dondi[1], M. C. Sabetti[1], G. Bignami[1], A. Marchi[1], A. Bertini[1,3], G. Cenci[1], A. De Marco[1], L. Morsiani[1], L. Mariani[1], S. Busti[1], P. P. Gatta[1], A. Bonaldo[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna, Italy
[2]Veronesi Holding S.p.A., Via Valpantena 18/G, 37142 Quinto di Valpantena, Verona, Italy
[3]Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38010 San Michele all'Adige, Italy
E-mail: nicole.pelusio2@unibo.it

Introduction
Nowadays Mediterranean farmed gilthead sea bream (*Sparus aurata*) production and health is threatened by the hematophagous gill parasite *Sparicotyle chrysophrii* (Sitjà-Bobadilla et al., 2010). To counteract this problem, natural functional feeds could be a sustainable solution to promote fish health, to help the animal ward off pathogens, and to reduce the use of chemical treatments thus minimizing environmental impact (Rigos et al., 2016; Firmino et al., 2020). To this aim, the present study explored the effect of two different natural functional feeds at two different dosages on gilthead sea bream growth and welfare conditions.

Materials and methods
Five iso-energetic and iso-proteic diets (42% crude protein, 18% crude fat, digestible energy 17.3 MJ/kg) were tested in triplicated fish groups: one as control, and other four containing natural additives - caprylic acid (CA), and a blend of organic compounds (MIX) - at two different dosages (5 and 10 g/kg MIX as MIX5 and MIX10, 15 g/kg and 30 g/kg as CA15 and CA30, respectively). Fish (initial body weight: 71.7 g) were fed to visual satiation twice a day over 79 days. To evaluate both additive and dosage effects, samplings occurred at the beginning (T0) and at the end of the trial (T1). Growth performances, feed utilization and carcass composition were estimated. To access fish welfare response, gill arches were sampled from 3 fish per tank for histomorphology evaluation and plasma biochemistry and haematocrit were performed from 5 fish per tank.

Growth and proximate composition data were analysed by one-way ANOVA, while plasma biochemistry and haematocrit were analysed by two-way ANOVA followed by a Tukey's multiple comparison test. Differences among treatments were considered significant when P value was lower than 0.05.

Results
Significant diet effect on growth (final body weight FBW, weight gain WG, specific growth rate SGR) was detected, with highest values for animals fed MIX10 and the lowest for ones fed CA30. Significant differences were found on feed intake (FI) and feed conversion rate (FCR) with lowest values in specimens fed CA30 and highest in ones treated with MIX10. Concerning nutritional indices, significant diet effect was found on protein and lipid efficiency ratios (PER and LER, respectively), with most elevated values in animals fed CA15 and CA30. On proximate body composition, no significant diet effect occurred. Among most relevant plasma analytes, iron content and haematocrit displayed significant diet effect, with an overall increase for all treatments at T1. On gill arch histomorphology, no significant differences were found among treatments.

Discussion and conclusion
Preliminary growth results suggested that MIX10 additive exerted a major growth in sea bream rather than CA dosages. On the other hand, protein and lipid utilization were slightly improved by CA added diets. Furthermore, the comparable iron level in plasma and haematocrit increased in all diets at T1 with no morphological alteration of gill arches. In particular, MIX5 and MIX10 seemed to display a stronger increment in plasma Fe compared to the other diets results that may suggest the potential of the tested additives to alleviate anaemic conditions caused by *Sparicotyle* infections (Sitjà-Bobadilla and Alvarez-Pellitero, 2009). In conclusion, this study provided a preliminary investigation concerning the growth and pro-health effect of natural functional additives necessary for a further in-field investigation in order to alleviate *Sparicotyle chrysophrii* outbreaks in gilthead seabream.

(Continued on next page)

Acknowledgements
This research was fully supported by ERC (European Research Council) in NewTechAqua project (New technologies Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture), Call H2020-EU.3.2.3.2. (Developing competitive and environmentally-friendly European aquaculture), Grant Agreement n. 862658. Feed additives were kindly provided by Adisseo, Dendermonde, Belgium (contact for further information: Merce Isern I Subich, mariamerce.isern@adisseo.com).

References

Firmino, J. P., Vallejos-Vidal, E., Sarasquete, C., Ortiz-Delgado, J. B., Balasch, J. C., Tort, L., Estevez, A., Reyes-López, F. E., Gisbert, E. (2020). Unveiling the effect of dietary essential oils supplementation in Sparus aurata gills and its efficiency against the infestation by Sparicotyle chrysophrii. Scientific reports, 10(1), 1-23.

Rigos, G., Mladineo, I., Nikoloudaki, C., Vrbatovic, A., Kogiannou, D. (2016). Application of compound mixture of caprylic acid, iron and mannan oligosaccharide against Sparicotyle chrysophrii (Monogenea: Polyopisthocotylea) in gilthead sea bream, Sparus aurata. Folia parasitologica, 63, 027.

Sitjà-Bobadilla, A., Alvarez-Pellitero, P., (2009). Experimental transmission of *Sparicotyle chrysophrii* (Monogenea: Polyopisthocotylea) to gilthead seabream (*Sparus aurata*) and histopathology of the infection. Folia Parasitologica 56, 143–151.

Sitjà-Bobadilla, A., Redondo, M., Álvarez-Pellitero, M. (2010). Occurrence of *Sparicotyle chrysophrii* (Monogenea: Polyopisthocotylea) in gilthead sea bream (*Sparus aurata* L.) from different mariculture systems in Spain. Aquaculture Research 41, 939–944.

UTILIZING CYBER-PYSICAL-SYSTEMS TO ENABLE SAFE AND SUSTAINABLE AQUACULTURE FARMING

C. Peppler[1*], M. von Stietencron[2], M. Lewandowski[3], A. Schiller[3], J.-F. Uhlenkamp[2], S.Bruns[1], J. Coordes[3]

[1]Polyplan-Kreikenbaum Gruppe GmbH, Überseetor 14, 28217 Bremen, Germany
[2]BIBA - Bremer Institut für Produktion und Logistik GmbH at the University of Bremen,Hochschulring 20, 28359 Bremen, Germany
[3]SWMS Consulting GmbH, Donnerschweer Str. 4a, 26123 Oldenburg, Germany
E-Mail: peppler@polyplan-kreikenbaum.eu

The work presented here is one of 11 so-called “application experiments” (AE) embedded in a consortium of more than 30 partners in the EU-project DIH4CPS (digitial innovation hubs for cyber-physical systems). Our AE titled “Smart Aquaculture” is about digital innovations in an Indoor Shrimp Farm in Germany (www.die- landgarnele.de). Indoor aquaculture is a growing sector of food production but still in a young stage of development, to be enhanced by using the opportunities of digital technologies.

One central issue is the determination of the optimum daily feed dose, which is related tothe live biomass in a tank. So far the biomass largely is an estimate based on assumptions on growth and survival rate of the cultured organism. This can easily lead tounderfeeding or overfeeding, both may have negative effects: either reduced growth or reduced water quality, in turn creating reduced growth. Accordingly, a sustainable production requires the knowledge of how much live organisms are present in a tank at any time, giving reliable values for their feed demand.

Still, the operation of an indoor aquaculture plant largely is based on manual data assessment and data evaluation. Thus, important information is not readily available in situations when rapid decision-making on adequate measures to positively influence theproduction process is required. This leads to significant inefficiencies and overall to a poor performance of the plant.

As a first step to support the operator in decision-finding all data must be brought together, which is achieved by an IoT platform. The PLC data of the plant are transferreddirectly to the platform. Some parameters cannot be measured online and are acquired, e.g. by manual sampling or laboratory tests. These values have so far been stored in separate protocol files, but not returned to the system. In this regard, an app was developed to speed up manual data collection. These data are now stored directly in theIoT platform.

Data visualization is another step to support the operator by providing an overview aboutwhat is going on in the plant at any time. For a comprehensive understanding of all collected data these are processed to give a clearly arranged visualization. A pending task is the interpretation of this data collection by applying AI.

In future steps, the collected data will be analysed by AI and trends as well as correlations will be searched for. Through the further analyses, detrimental developments and errors can be detected and prevented at an early stage. This way the operation of an aquaculture plant can be simplified, and the yield can be increased as astep towards a more sustainable way of producing animal proteins for human consumption.

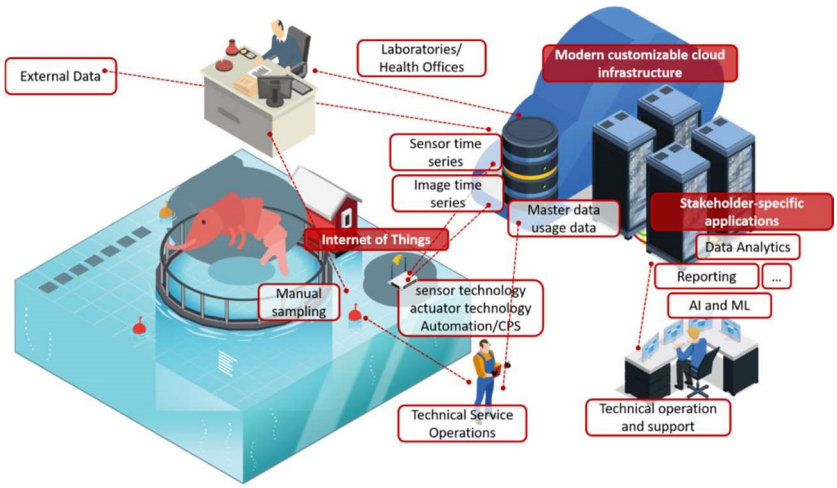


Fig. 1 Project Concept of AI-supported Aquaculture

(Continued on next page)

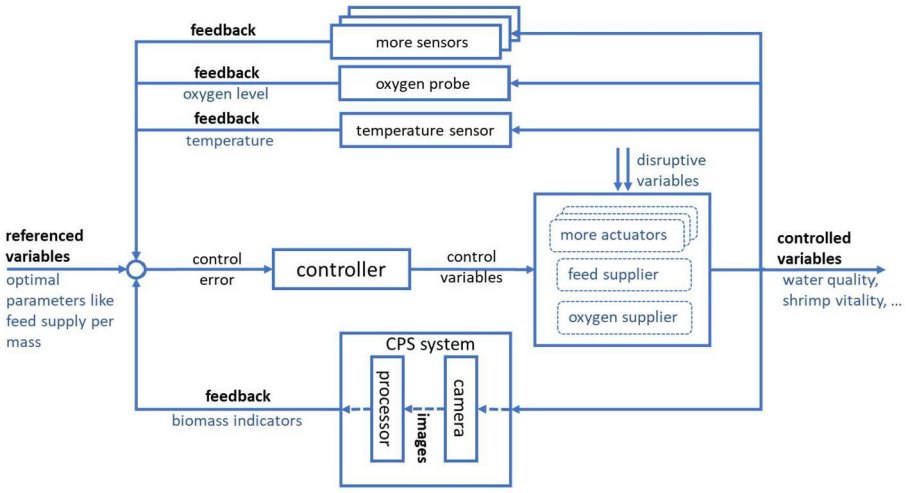


Fig. 2 Advanced Control and Feedback Loop

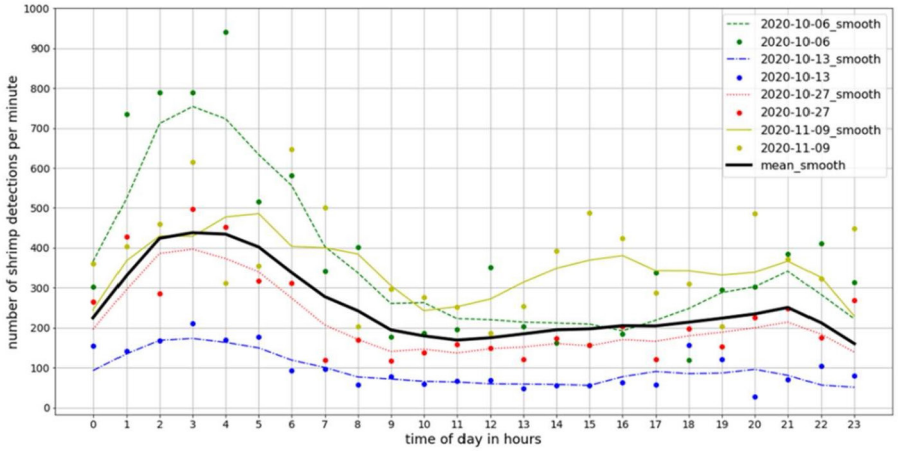


Fig. 3 The pattern of counting events indicates a diurnal rhythm of activity as only swimming shrimp are detected.

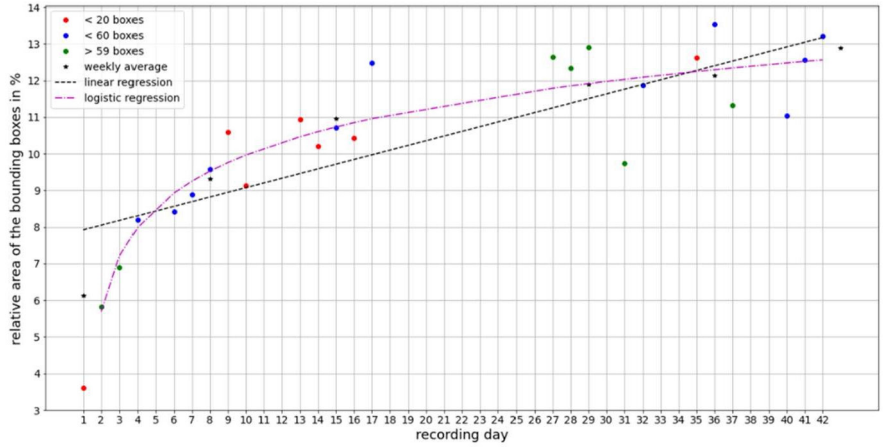


Fig. 4 Our AI-based image evaluation identifies a continuous growth trend.

Acknowledgement
The research presented in this poster is result of the project DIH4CPS, which receives funding from the European Union's Horizon 2020 research and innovation programmeunder grant agreement No 872548. It reflects only the authors' view, and the Commission is not responsible for any use that may be made of the information it contains.

DOES INDUSTRIAL PROCESSING AFFECT HEALTH AND NUTRITIONAL PROPERTIES OF AQUACULTURE SEAFOOD PRODUCTS?

Irene Peral*[1], Raquel Llorente[1]

[1]AZTI, Food Research, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160 Derio, Bizkaia, Spain
*E-mail: iperal@azti.es

Introduction

Aquaculture is one of sectors for seafood production with higher potential of growth which allows the suitable maintenance of fish consumption in the world, which make up 60% of total fish consumption, but is expected to continue rising (FAO, 2018). Thus, new food products based on aquaculture products are required on the market to fill the consumers´ needs. For this reason, AZTI (Spain) has successfully developed four new food products based on aquaculture species (seabass, gilthead seabream and meagre) in the frame of H2020 MedAID project.

Global health authorities recommend consuming fish on a regular basis as part of a healthy diet due to their rich source of nutrients including protein, minerals and micronutrients. Particularly, dietary recommendation for fatty fish indicates a consumption of one to two portions per week (ISSFAL, 2004). Farmed gilthead seabream, seabass and meagre supply a high level of omega-3 long-chain polyunsaturated fatty acids, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Llorente, 2020).

However, when these raw materials are employed by food industry to develop other final products, their nutritional composition might change due the fish flesh content and the ingredients employed in the product formulation and depending on the industrial technology applied. So, the aim of this study was (i) to analyse how the nutritional composition varies in the new aquaculture seafood product elaborated in H2020 MedAID project from seabass, gilthead seabream and meagre raw materials and (ii) to determine the potential nutritional and health claims might be made on these aquaculture products.

Materials and methods

Fillet samples from fresh aquaculture seabass, gilthead seabream and meagre and the final products derived from them were analysed in quadruplicate for moisture, ash, crude protein, fibre and fat content employing AOAC methods. Salt content was calculated theoretically using USDA Nutrient database. The sugar profile was analysed by gas chromatography mass spectrometry GC/MS and fatty acid profile by gas chromatography and flame ionisation detector (GC–FID) extracting and converting fatty acids to methyl esters. Total Energy (kcal/kJ) was calculated theoretically using conversion rates of Food Regulation (EU) 1169/2011.

Taking into account the nutritional composition obtained previously, nutritional and health claims related to protein and fat content in final products developed were evaluated according to Food Regulations (EU) 1924/2006 and (EU) 432/2012.

Results

The nutritional composition obtained in fresh fish and processed products is described in Table 1. Differences among raw material and final products can be explained by the fish flesh percentage in the final product and other ingredients employed in food formulation, but not by the industrial processes applied. Such processes as cutting, chopping, mixing, stuffing, pasteurization and freezing did not affected nutritional composition due to an optimization of processing parameters and food ingredients selection.

Nutritional claims linked to the high protein content and fatty acid profile are presented in Table 2. Due to their high composition of unsaturated fatty acids, health claims could be included in the marketing of this product. Specially, in the case of unsaturated fatty acids, EPA and DHA, those related to the heart and brain function, normal vision and maintenance of normal blood cholesterol levels.

Conclusions

Aquaculture seafood products with high quality nutritional profile can be developed by food industry as a healthy option for consumer due to their high protein content and fatty acid profile, specially DHA and EPA. Good formulation and technological practices assure the nutritional value of them.

(Continued on next page)

Table 1. Nutritional composition in raw meagre, seabass and seabream and processed products.

Nutrition facts	Raw fish			Aquaculture products			
Per 100gr	Meagre mince	Seabream fillet	Seabass fillet	Grilled seabass	Seabream with cous-cous	Meagre burger	Seabream breaded bites
Energy (calories)	187kcal 781kJ	160kcal 669kJ	136kcal 568kJ	168kcal 703kJ	170kcal 710kJ	200kcal 836kJ	187kcal 784kJ
Protein	18g	19.78g	20.77g	16.31g	12.99g	17.39g	15.33g
Total Carbohydrates:	0g	0g	0g	5.01g	10.87g	0.24g	16g
*Total sugars	0g	0g	0g	0.15g	1.01g	0.17g	0.52g
Total fat:	12.75g	8.99g	5.86g	9.19g	8.18g	11.81g	6.45g
* Saturated	3.32g	2.02g	1.32g	2.06g	1.84g	2.59g	1.42g
* Monounsaturated	6.04g	4.56g	2.99g	6.42g	4.11g	6.05g	3.17g
* Polyunsaturated	3.38g	2.41g	1.55g	1.44g	2.10g	3g	1.86g
Salt *	0.17g	0.15g	0.17g	0.98g	0.47g	0.15g	0.9g
Dietary fiber	0g	0g	0g	0.1g	0.35g	0.1g	1.37g
EPA	381	201	126	97	201	288	146
DHA	725	578	337	247	451	607	393
Omega 3 (EPA + DHA) (mg)	1106	779	463	344	652	895	539

Table 2. Nutritional claims in aquaculture processed food products, percentage of the energy value (%), and omega-3 fatty acids (mg).

Nutritional claims EC 1924/2006	Grilled seabass	Seabream with cous-cous	Meagre burger	Seabream breaded bites
High protein. At least 20 % of the energy value of the food is provided by protein.	39%	31%	35%	32%
High unsaturated fat: more than 20 % of energy value of the product	86%	76%	77%	78%
High monounsaturated fat: more than 20 % of energy of the product	70%	50%	51%	49%
High omega-3 fatty acids: more than 80mg of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal	895 mg	652 mg	895 mg	539 mg

Acknowledgments

MedAID (Mediterranean Aquaculture Integrated Development) project, H2020 grant agreement number 727315 (http://www.medaid-h2020.eu/).

References

FAO, 2018. The state of World Fisheries and Aquaculture 2018. Meeting the sustainable development goals. Rome. Retrieved from Rome: http://www.fao.org/publications/sofia/en/

ISSFAL, 2004. Report of the Sub-committee on Recommendations for Intake of Polyunsaturated Fatty Acids in Healthy Adults. Brighton

Llorente, R., Peral I., Ibargüen, M. and Naranjo, A. 2020. New aquaculture fish products from consumer to the market. Conference, Eposters & e-Market. Aquaculture Europe 2020. Pag 323.

Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers.

MODULATORY EFFECTS OF ALGAE AND GRAPE SEED DIETS ON IMMUNE AND OXIDATIVE STRESS RESPONSES OF SENEGALESE SOLE (*Solea senegalensis*) POST-LARVAE CHALLENGED AGAINST *Tenacibaculum maritimum*

S. Pereira[1,3*], M. Hinzmann[1], D. Peixoto[1], M. Morais[2], W. Pinto[2], L. Conceição[2], B. Costas[1,3]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Av. General Norton de Matos S/N, 4450-208 Matosinhos (Portugal)
[2]SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão (Portugal)
[3]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto (Portugal)
e-mail: susana.pereira@ciimar.up.pt

Introduction

Nutrition can have significant health implications for animals. Grape seed are reportedly rich in polyphenols [1], whereas some micro and macroalgae genus are rich in carotenoids and vitamins [2]. Some of these bioactive compounds have the ability to modulate the oxidative status and immunity of several fish species and it is reasonably studied [3]. Tenacibaculosis is an ulcerative disease caused by *Tenacibaculum maritimum* that affects diverse fish species, including *Solea senegalensis* [1]. This work aimed to study the effects of dietary supplementation with micro- and macro-algae, and a grape seed extract on immune and oxidative stress responses of Senegalese sole (*Solea senegalensis*) post-larvae subjected to a bacterial challenge assay against *Tenacibaculum maritimum*.

Materials & Methods

Post-larvae of Senegalese sole were reared at SPAROS facilities (Olhão, Portugal) from 30 to 63 days after hatching (DAH) to assess the effects of two experimental diets (Blend and GS). A commercial diet (COMM) served as control, Blend was supplemented with a micro- and micro-algae mixture at 3% of inclusion, while GS was supplemented with a grape seed extract (GSE). All diets were formulated and manufactured by SPAROS with premium marine and plant-based ingredients. Each diet was administered to 3 random replicates (n= 425 /tank), divided in 8 meals in a 24-hour period. Larvae with 63 DAH were transferred from SPAROS to CIIMAR facilities and were immediately subjected to a bath challenge assay with *Tenacibaculum maritimum*. Bath infection was performed for 12 hours in 20 L with 5 x 10^2 cfu/mL of bacteria. Larvae from 9 tanks were separated in unchallenged (n= 50) and challenged treatments (n= 100), where both treatments had equal handling conditions, except for unchallenged groups where marine broth was used instead of bacteria. Feeding was not provided during infection but continued with the same scheme after that period. After 1 and 3 days post-infection (DPI), pools of larvae (n= 15 per tank) were sampled to assess immune parameters and oxidative stress biomarkers.

Results & Discussion

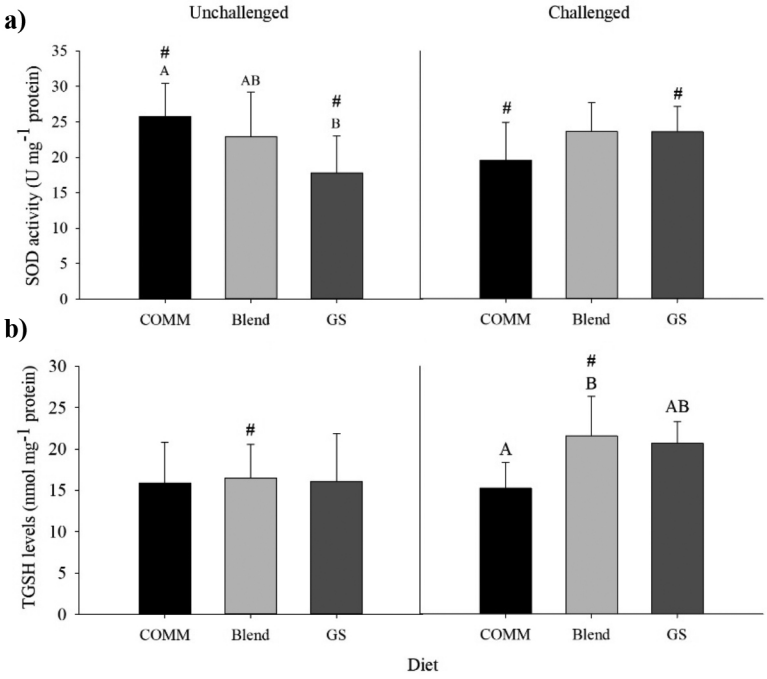
Regarding immune parameters, peroxidase activity increased in unchallenged larvae fed GS compared to COMM after 3 DPI, also shown in [4] with different doses of GSE. Considering oxidative stress responses, superoxide dismutase (SOD) activity decreased in unchallenged larvae fed GS compared to COMM after 1 DPI, this trend also found in other fish species supplemented with various doses of *Vitis vinifera* oil [5]. However, in our study SOD activity increased in challenged larvae fed GS. Total glutathione (GSH) levels increased in challenged larvae fed Blend compared to COMM diet after 1 DPI. Those levels were also higher than unchallenged larvae fed Blend. These results could suggest boosting of GSH synthesis influenced by alga extracts supplementation, unlike what described by [2], that showed its decrease. Moreover, catalase (CAT) activity increased in challenged larvae fed GS compared to unchallenged ones fed with the same diet after 1 DPI, hinting that possibly the bioactive compounds of grape seed extract elicited a boost in the production of CAT. After 3 DPI, alterations were only found in GSH:GSSG ratio, where challenged larvae had significant lower ratios in Blend and GS diets than the unchallenged ones. These results might indicate higher amounts of oxidized GSH (GSSG) in larvae.

Conclusions

In conclusion, diets supplemented with grape seed extract and a blend of micro- and macro-algae seem to benefit oxidative stress responses in sole. Still, gene expression analyses are in progress to allow a better understanding of the health-related effects of these diets in post-larvae of Senegalese sole.

(Continued on next page)

Fig. 1 a) SOD activity and b) total GSH levels of larvae of Senegalese sole challenged against *T. maritimum* after 1 DPI and fed with three diets (n = 15). Letters and cardinal show significant differences between dietary and challenge treatments, respectively (two-way ANOVA; Tukey test, $P \leq 0.05$).



Acknowledgments
We would like thank the EPPO team of Instituto Português do Mar e da Atmosfera (IPMA) for the sole larvae supply. This work was supported by project FEEDMI (39948), financed by COMPETE 2020 and CRESC Algarve 2020 in the framework of Portugal 2020.

References
1. Guardiola, F.A., et al., Fish Shellfish Immunol, 2019. **87**: p. 744-754.
2. Batista, S., et al., J. of Applied Phycology, 2020. **32**(3): p. 2041-2059.
3. Fumanal, M., et al., Fish Shellfish Immunol, 2020. **100**: p. 186-197.
4. Al-Atbee, I., Biological and Applied Environ. Res., 2021. **5**(1): p. 130-140.
5. Arslan, G., et al., Aquaculture Research, 2018. **49**(6): p. 2256-2266.

SOCIAL ACCEPTABILITY OF AQUACULTURE IN ANDALUSIA (SPAIN). A FOCUS ON INSTITUTIONAL ISSUES AND GOUVERNANCE

José Antonio Pérez Agúndez[1], Pascal Raux[2], Manuela Vieira Pak[3], Marianna Cavallo[2], Loeiza Lancelot[1]

[1]Ifremer, Université Brest, CNRS, UMR 6308, AMURE, Unité d'Economie Maritime
UEM, rue Dumont d'Urville, 29280 Plouzané, France
E-mail : jose.perez@ifremer.fr

Aquaculture development in the Mediterranean is limited by several constraints relying on zootechnical, ecological, economic and social issues. Social acceptability (SA) is one of the main bottlenecks to aquaculture development. This concept is complex to apprehend since it includes considerations which are vague and intangible. SA is the result of social interactions that lead to the acceptance or opposition to private or public decision-making processes from one or several social groups. The increasing complexity of interactions between ecosystems and social systems in coastal and marine areas exacerbates this problem mainly in the case of new activities development. Aquaculture is a recent activity that is trying to find a place in the blue economy but often must to face social opposition from other existing users or by citizens in general.

Many recent works addresses this issue through different perspectives. A large majority of them focuses on the analysis of public opinion to identify the key drivers of SA. In this work, the authors focuses on SA from the institutional and governance perspective with an emphasis on the impacts from social interaction processes.

To investigate this issue, this work analyse the social acceptability of aquaculture development in Andalusia (Spain) in the framework of the H2020- MedAID project. A joint collaboration with the regional agency of Andalusia in charge of aquaculture planning was built to explore the local conditions on coastal areas for the social support of aquaculture development. This concern was considered a key issue to address in the regional plan of aquaculture development that each European Region must to build in the framework of the Common Fisheries policy.

The work conducted emphasises the existence of institutional gaps between the high institutional levels, which build political strategies that are not always suitable to be implemented by local institutions, given the social complexity of the territories. It is also highlighted the lack of frameworks, protocols and means to effectively conduct participatory processes in the context of coastal planning, especially in the case of aquaculture development plans. These shortcomings result in social conflicts, particularly due to the lack of transparency and trust with the Administration as a result of the poor inclusion of stakeholders in decision-making processes. The fieldwork carried out showed that the conditions are not yet in place to conduct participatory processes, even in the framework of an experimental exercise.

HISTOLOGY REVEALS THE TISSUE FACTORS COMPROMISING THE DIFFERENTIAL GROWTH RATE AND PHYSIOLOGICAL PERFORMANCES OF THE MUSSEL *Mytilus galloprovincialis*

Maitane Pérez-Cebrecos[*1,3], Daniel Prieto[1], Irrintzi Ibarrola[1], Urtzi Izagirre[2,3]

[1]Dept. Genetics, Physical Anthropology and Animal Physiology and [2]Dept. Zoology and Animal Cell Biology. CBET Research groups. Fac. Science and Technology, University of the Basque Country (UPV/EHU), Bilbao, Basque Country, Spain
[3]Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Plentzia, Basque Country, Spain
E-mail: maitane.perez@ehu.eus

Introduction

One of the traits of bivalves that affects aquaculture, and specially mytiliculture, consists in the existence of large differences in growth performances between individuals. A great proportion of such differences appear to be genetically determined and hence to be heritable, and they are turned into measurable differences in the energy balance of individuals. However, genetic clues are not clearly identified and more studies are needed at different biological organization levels to better manage the mussel production. Under high organic content rations, the differences between fast and slow growing individuals are determined by distinct inter-individual capacities to acquire and process food particles, with a higher metabolic efficiency[5]. The size of the digestive gland has also been reported to constitute a gross measurement of the digestive[6] and growth[7] performances, and different gill-surface areas seem to point out a different food acquisition capacity[5]. Yet the underlying structures are still unknown. The aim of these study was to stablish the histological bases of the differential physiological performance between differentially growing individuals of *Mytilus galloprovincialis*.

Methods

Juvenile mussels (shell length of 10.98 ± 0.52 mm) sampled from an intertidal rocky shore were maintained in the laboratory under conditions of continuous feeding with *I. galbana*. After three months, mussels were subdued to a two-week acclimation under two different food-rations: high (H: 50 000 part · mL^{-1}) and low (L: 10 000 part · mL^{-1}). To analyse the differential growth of mussels, the largest 40 (fast-growers – F), the 40 medium-sized mussels (intermediate growers – I) and the smallest 40 (slow-growers – S) were selected and characterized in terms of energetic physiology and histology. The main components of the energy balance (clearance rate -CR- ($L \cdot h^{-1}$) and routine metabolic rate -RMR- (mL $O_2 \cdot h^{-1}$)) were measured following standard procedures[6]. Once the physiological measurements were completed, dissections were performed for histological examination. To determine the status of the digestive gland, the mean epithelial thickness (MET), mean luminal radius (MLR), mean digestive diverticula radius (MDR), and the connective to diverticula ratio (CTD) were quantified. In addition, the adipogranular cell density and the gill integrity were calculated.

Results

Fast mussels were 3.5 times heavier than S ones (1.35 ± 0.21 g *vs*. 0.37 ± 0.08 g), and had almost 70 % larger shell lengths (24.73 ± 1.11 mm *vs*. 14.57 ± 1.11 mm).

Physiological measurements. Both growth condition (F, I or S) and food ration (H or L) exerted a significant effect on the CR of mussels: CR was two times higher at low food ration (0.411 ± 0.16 $L \cdot h^{-1}$) as compared with high food ration (0.233 ± 0.15 $L \cdot h^{-1}$); and fast growers attained two times higher values than slow growers (0.429 ± 0.19 *vs*. 0.216 ± 0.12, respectively). Medium growers (I) had intermediate CR values that were not significantly different to those in F and S mussels. In good correspondence with CR values, the gill surface-area of F mussels was significantly higher than that of S ones, irrespective of the ration. However, no significant effects on RMR were recorded.

(Continued on next page)

Histological assessment. Significant differences in the gills were associated with the growth condition factor. F and I mussels had a higher organization level of the epithelium with an evident higher frontal and latero-frontal cilia density than S mussels. In addition, irrespective of the ration, S mussels had lower MDR and MET values than F and I ones (MDR: 23.53 ± 0.89 *vs*. 26.27 ± 1.23 and 25.72 ± 1.91; MET: 7.50 ± 2.45 *vs*. 13.44 ± 1.98 and 12.29 ± 2.09). Mussels fed high food ration achieved significantly higher MDR and MLR of the digestive diverticula than those fed with low ration. The adipogranular cell index was two times higher in F and I mussels than in S individuals, and no significant effect of food ration was recorded.

Discussion

Both fast and slow growing individuals showed a similar regulatory response to the particle concentration increase through CR reduction, a phenomenon that has been comprehensively addressed and interpreted in bivalves as a mechanism allowing the regulation of ingestion rate and gut passage time[1,2]. In this context, the food acquisition and processing capacities of fast and slow growers differ considerably. This has to do, to a great extent, with the different capacity of their gills, digestive gland and energy-storage capacity, as observed herein. Previous studies[3,4,5] have shown that both inter-specific and size-related intra-specific differences in CRs may be explained by corresponding differences in gill surface areas. The data from the present study not only agrees with that, but also has revealed that gills of slow growers possess a lower cilia density and a disorganised structure overall. Histological analysis showed that F individuals have a greater digestive capacity, thanks to a higher digestive diverticula to connective tissue proportion, and larger and thicker digestive diverticula, which could lay bare higher intracellular digestive and absorption capacities. In addition, fast growers showed a higher level of investment in storage in the form of adipogranular tissue, which is expected to reflect an increase in gamete production. These evidences could be of a mayor importance since the selection of animals with enhanced digestive capacity and growth conditions could be an improvement in aquaculture management.

References

[1]Bayne, B. L., Hawkins, A. J. S., & Navarro, E. (1987). *J. Exp. Mar. Biol. Ecol*., 111, 1-22.
[2]Navarro, E., Iglesias, J. I. P., Ortega, M. M., *et al*. (1994). *Physiol. Zool*., 67, 468-496.
[3]Pouvreau, S., Jonquières, G. & Buestel, D. (1999). *Aquac*., 176, 295-314.
[4]Honkoop, P. J. C. (2003). *Oecol*., 135, 176-183.
[5]Prieto, D., Urrutxurtu, I., Navarro, E., *et al*. (2018). *Mar. Environ. Res.,* 140, 114-125.
[6]Ibarrola, I., Etxeberria, M., Iglesias, J. I. P., *et al*. (2000). *J. Exp. Mar. Biol. Ecol*., 252, 199-219.
[7]Albentosa, M., Sánchez-Hernández, M., Campillo, J. A., *et al*. (2012). *Comp. Biochem. Phys. A.,* 163, 286-295.

PHYSIOLOGICAL BASIS FOR INTER-FAMILY AND INTER-INDIVIDUAL GROWTH VARIABILITY IN THE SPAT OF CLAM *Ruditapes decussatus:* LONG-TERM AND ACUTE GROWTH

Maitane Pérez-Cebrecos[*1,2], Daniel Prieto[1], Irrintzi Ibarrola[1], Kristina Arranz[1], Iñaki Urrutxurtu[1], Miren Bego Urrutia[1] and Enrique Navarro[1]

[1]Dept. Genetics, Physical Anthropology and Animal Physiology. Fac. Science and Technology, University of the Basque Country (UPV/EHU), Bilbao, Basque Country, Spain
[2]Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Plentzia, Basque Country, Spain
E-mail: maitane.perez@ehu.eus

Introduction

In bivalve populations reared either under controlled laboratory conditions or under environmental conditions, inter-individual growth rates attain an outstandingly high variability[1,2,3,4]. This variation indicates the existence of endogenously determined differences in the physiological capacity of individuals to acquire and metabolize food energy, which happens to be of great interest in aquaculture, setting a potential for the selective breeding of commercial species of bivalves. Hence, sibling specimens (families) constitute an excellent biological material to test such dissimilarities. The carpet shell clam *Ruditapes decussatus* is one of the most gainful molluscs in European aquaculture. Indeed, the FAO pointed out a global production of 5389 tonnes for this species in the year 2018. In this context, the present study was designed to identify the physiological parameters responsible for the differences between four families (inter-family differences) and between sibling individuals (inter-individual differences) in the growth potential of the clam *Ruditapes decussatus*.

Methods

Four different families (Fam 1, Fam 2, Fam 3 and Fam 4) of the clam *Ruditapes decussatus* were produced simultaneously at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) (Montbui, Catalunya) and maintained under constant immersion and food supply for 12 months. When arrived at our laboratory, clam spat was classified into two different size-groups based on shell-length (long-term growth): fast growers (F) (> 11 mm) and slow growers (S) (5-7 mm). After three months under identical feeding conditions, again large inter-individual differences were found within those two groups (acute growth), which enabled a new selection by growth condition of fast (F) and slow (S) growing individuals, resulting in four experimental groups within each family: F_F, F_S, S_F and S_S. Six individuals from each experimental group within all the families were employed to measure the complete set of parameters taking part in the energy balance: clearance rate (CR: $L \cdot h^{-1}$), routine oxygen consumption (RMR: mL $O_2 \cdot h^{-1}$), absorption efficiency (AE: decimal units), absorption rate (AR: $mg \cdot h^{-1}$), ammonia excretion rate (U: µg $NH_4 \cdot h^{-1}$), and scope for growth (SFG: $J \cdot h^{-1}$).

Results

After the acute growth, the live-weight of fast growers was around six-fold higher than that from slow growers (Fam 1: 425.2 ± 155.0 *vs*. 61.8 ± 10.9; Fam 2: 402.2 ± 170.8 *vs*. 73.9 ± 22.4; Fam 3: 421.9 ± 146.8 *vs*. 67.2 ± 16.5; Fam 4: 464.9 ± 229.4 *vs*. 72.7 ± 21.3), and the shell-length two-fold larger (Fam 1: 13.6 ± 1.9 *vs*. 6.9 ± 0.5; Fam 2: 13.2 ± 1.7 *vs*. 7.4 ± 0.9; Fam 3: 13.5 ± 1.6 *vs*. 7.0 ± 0.6; Fam 4: 13.8 ± 2.1 *vs*. 7.3 ± 0.9).

Inter-family differences. Both for CR and AR, family 2 attained virtually two-times higher values than family 4 (3.40 ± 1 $mg \cdot h^{-1}$ *vs*. 1.99 ± 0.86 $mg \cdot h^{-1}$). The same pattern was true for U. Such higher clearance capacity was not translated though into higher RMR for family 2; indeed, family differences in the metabolic cost fall to the lower values of family 1, which showed a 63.5 % lower rate. Yet it was family 1 the one with the lowest absorption efficiency. Consistently with CR, AR and U, the SFG of family 2 is roughly 60 % higher than in family 4 (2.37 ± 0.95 *vs*. 0.95 ± 0.56).

Intra-family differences. Irrespective of the growing period (long-term or acute), CR values were persistently higher in the fast-growing groups, without affecting the AE. AR and U followed the same trend recorded for CR. Besides, oxygen consumption rates were not different between sizes or growth conditions, indicating a higher metabolic efficiency for those growing faster. However, the SFG was only affected by the size factor; in other words, the acute growth of clams did not have an effect on their growth potential.

(Continued on next page)

Discussion

The physiological differences underpinning inter-family differences between the faster and slower growing families (2 and 4, respectively), turn out to be similar to those causing intra-familiar differences between fast and slow-growing siblings: higher clearance rates are linked to higher metabolic expenditures and ammonia excretions that, as a balance, give place to an enhanced energy budget. A conceptual framework where the results obtained herein fit together, and that utterly explains the fast-growing behaviour reported at long-term growth, acute growth and family-level (Fam 2 *vs*. Fam 4), is the acquisition model proposed by Bayne[5,6]. This model is supported by a good number of studies[7,8,9], which show that individuals with a high growth-rate, or fast-feeders in this model, hold up their advantage in the capacity to develop higher filtration rates without causing an evident lessening in the absorption efficiency. Nevertheless, the non-significant differences at acute growth or family level in the SFG, evince that the inter-individual growth differences at long-term are such that almost overshadow the effects that the other factors presumably exert upon the growth of individuals. The results obtained imply that not only selective breeding of fast-growing individuals should be encouraged in clam production, but also that the choosing of families that behave as fast growers should be weighed up.

References

[1]Brown, J. R. (1988). *Mar. Ecol. Prog. Ser., 45*, 225-236.
[2]Gaffney, P. M. and Scott, T. M. (1984). *Aquac., 42*, 289-302.
[3]Tamayo, D., Ibarrola, I., Urrutia, M. B. and Navarro, E. (2011). *Aquac., 321*, 113-120.
[4]Tamayo D., Ibarrola, I., Urrutxurtu, I. and Navarro, E. (2014). *Mar. Biol., 161*, 1627-1637.
[5]Bayne, B. L. (2000). *J. Exp. Mar. Biol. Ecol., 251*, 185-203.
[6]Bayne, B. L. (2004). *Integr. Comp. Biol., 44,* 425-432.
[7]Tamayo, D., Ibarrola, I., Cigarría, J. and Navarro, E. (2015). *J. Exp. Mar. Biol. Ecol., 471,* 92-103.
[8]Prieto, D., Urrutxurtu, I., Navarro, E., Urrutia, M. B. and Ibarrola, I. (2018). *Mar. Environ. Res., 140,* 114-125.
[9]Pace et al 2006

FISH MEAL-FREE DIETS SUPPLEMENTED WITH HEALTH PROMOTERS SUPPORT OPTIMAL GROWTH IN GILTHEAD SEA BREAM, WITH BENEFITIAL CHANGES IN GENE EXPRESSION, INTESTINAL MICROBIOTA AND IMPROVED INTESTINAL DISEASE RECOVERY

M.C. Piazzon[1], F. Naya-Català[2], G.V. Pereira[3], I. Estensoro[1], R. Del Pozo[1], J.A. Calduch-Giner[2], W.G. Nuez-Ortín[4], O. Palenzuela[1], A. Sitjà-Bobadilla[1], J. Dias[3], L.E.C. Conceição[3], J. Pérez-Sánchez*[2]

[1]Fish Pathology Group and [2]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, CSIC, Spain. [3]Sparos Lda, Olhão, Portugal. [4]Adisseo, Dendermonde, Belgium
E-mail: jaime.perez.sanchez@csic.es

Introduction

The exponential growth of the aquaculture sector requires the development of sustainable aquafeeds with less dependence on marine products. Tolerance to fish meal (FM) and fish oil replacement in the economically important gilthead sea bream (*Sparus aurata*) is being extensively studied with many products emerging as alternative feed ingredients. It has been demonstrated that alternative diets influence the composition of intestinal adherent microbial populations, which have a key role on host metabolism, health and disease resistance. In addition, low fish meal diets showed an increased susceptibility to enteric parasites (Piazzon et al., 2017). Clearly, differences in diet have an impact on the overall health and metabolism of the fish and many parameters have to be taken into account when studying alternative diets for their use in aquaculture. In this study we evaluated the effect of a novel feed formulation (NoPAP SANA) with total replacement of FM by insect meal and bacterial fermentation biomass, and supplemented with the health-promoter additive SANACORE®GM (Palenzuela et al., 2020), on growth performance, gene expression, intestinal microbiota and disease resistance in gilthead sea bream.

Methods

Tagged gilthead sea bream of mean weight 21.3 g were distributed in two open-flow tanks (160 fish/tank) and fed *ad libitum* during 34 days with control or NoPAP SANA diets. Twelve fish/diet were sacrificed and head kidney (HK), liver (L) and posterior intestine (PI) were taken for RNA extraction. From the same fish, the adherent bacteria of PI were collected and immediately used for DNA extraction. RNA from HK, L and PI was used to run three customized PCR-arrays including genes of interest for each tissue, with markers of performance and metabolism (L), immune system (HK and PI), epithelial integrity, nutrient transport and mucins (PI). Using the bacterial DNA, the V3-V4 region of the 16S rRNA of each individual sample was 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. Metagenome prediction and pathway analysis were performed using Piphillin. Differential gene expression and OTU presence and abundance correlations were studied using the corrplot R package. From the remaining fish, 70 fish/group were challenged with the intestinal parasite *Enteromyxum leei* by effluent exposure and the remaining fish were used as controls. The challenge lasted 78 days, including a non-lethal diagnosis sampling at day 40. At the end of the challenge all fish were sampled for histological and molecular diagnosis. Biometric values from all fish were taken in all sampling points.

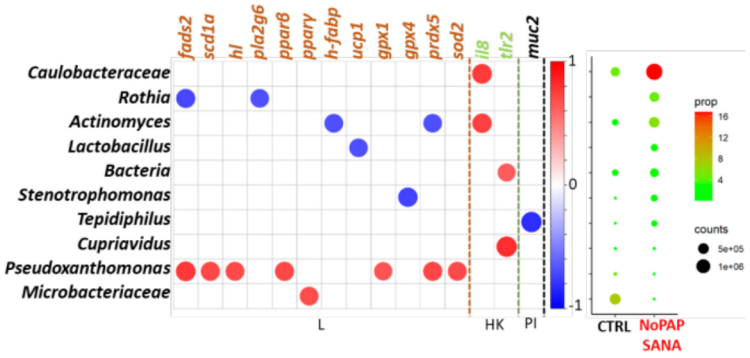


Figure 1: Correlation plot showing significant correlations between OTUs and liver (L), head kidney (HK) and posterior intestine (PI) differentially expressed genes. Positive and negative correlations are represented in red and blue, respectively. On the right, dotplot depicting the relative abundance of the respective OTUs in proportion (colour scale) and normalized counts (dot size).

(Continued on next page)

Results

A slight decrease in condition factor and specific growth rate was detected in the NoPAP SANA group. However, all fish grew efficiently considering gilthead sea bream standards. NoPAP SANA group showed differential expression of 17 out of 44 genes in L, two out of 29 in HK, and 4 out of 44 in PI. The bacterial composition at the PI showed no major differences in diversity or at the phylum level. However, 29 abundant (>1%) OTUs significantly changed with the diet. From these, 10 OTUs were significantly correlated with differential expression of genes in the different tissues, highlighting *Pseudoxanthomonas* which was positively correlated with the expression of seven L genes, or *Actinomyces*, significantly correlated with the expression of L and HK genes (Fig. 1). Inferred metagenome analyses revealed that the altered microbiota with NoPAP SANA diet could account for changes in 15 metabolic pathways. The intensity and prevalence of infection after the parasite challenge was not significantly different between diets. In fact, infected fish from both groups showed similar recovery rates.

Conclusions

NoPAP SANA promoted good growth parameters and efficient conversions arising as a good alternative for a FM-based diet in gilthead sea bream diets. This diet modulated the expression of several genes in L showing the capacity to reduce lipogenesis, mitochondrial activity and the risk of oxidative stress and, at the same time, promoting an anti-inflammatory gene expression profile in HK and PI. Changes were also detected in the adherent bacterial populations of PI, with significant changes of OTUs that could potentially account for significant metabolic alterations. The correlations between presence and abundance of intestinal bacteria with changes in gene expression of different tissues, together with the pathway analysis results, show that microbiota changes can have an impact on host metabolism at a systemic level, and *vice versa*. Clearly, the changes induced by this novel FM-free diet supported an accelerated growth with an overall feed conversion ratio close to 1 and no increased susceptibility against this intestinal parasite, as often observed in studies when replacing a FM-based diet.

References

Palenzuela O., Del Pozo R., Piazzon M.C., Isern-Subich M.M., Ceulemans S., Coutteau P., Sitjà-Bobadilla A. 2020. Effect of a functional feed additive on mitigation of experimentally induced gilthead sea bream *Sparus aurata* enteromyxosis. *Diseases of Aquatic Organisms*, 138: 111-120.

Piazzon M.C., Calduch-Giner J.A., Fouz B., Estensoro I., Simó-Mirabet P., Puyalto M., Karalazos V., Palenzuela O., Sitjà-Bobadilla A., Pérez-Sánchez J. 2017. Under control: how a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegetable diets. *Microbiome*, 5:164.

Acknowledgements: GAIN (EU-H2020 #773330); RYC2018-024049-I/AEI/10.13039/501100011033.

ONTOGENETIC SHIFT AND LATERALIZED SWIMMING BEHAVIOR IN ATLANTIC HALIBUT (*Hippoglossus hippoglossus)*

P. Perrichon[1*], Ø. Sæle[2], T. Furmanek[2], T. Harboe[1], B. Norberg[1]

[1] Institute of Marine Research, Austevoll Research Station, NO-5392 Storebø, Norway
[2] Institute of Marine Research, NO-5817 Bergen, Norway
* E-mail: prescilla.perrichon@gmail.com

Introduction

Atlantic halibut (*Hippoglossus hippoglossus*) is a large, long-lived flatfish inhabiting boreal and subarctic waters and is an attractive demersal species of high commercial value (Haug, 1990). Despite a slow success in farming halibut in 1980's, juvenile production has increased by 5.4-fold from <300 000 in 2015 to ca 1 500 000 in 2019 and continues to grow. A key component in aquaculture is the production of good quality larvae, which is inherently tied up with the metamorphosis phases in flatfish species (Harboe et al., 2009). Flatfish metamorphoses from bilaterally symmetrical larvae to asymmetric juveniles (body pigmentation and eye migration), with a change in swim posture angle to transit from pelagic to a benthic lifestyle (Figure 1; Sæle et al., 2004).

Processes driving metamorphosis and settlement are important for understanding connectivity between life history stages and to solve major problem related to larval fish health. Variation in life traits is often related to environmental variables (i.e. temperature, turbidity, light intensity, photoperiod, or life feed organisms). Illumination has been shown to be an important factor in control of metamorphosis asymmetry through the endocrine regulation and activation of phototransduction pathway in flatfish skin (Alves et al., 2016; Power et al., 2001; Shao et al., 2017). The body tilting and related lateralized behavior initiated before metamorphosis raise questions about when tissue programming and preparation of the larvae for tilted swimming and metamorphosis are initiated and how these both processes are linked (Schreiber, 2006). This work explored the ontogenetic dynamic of lateralized swimming and the key developmental trigger driving settling side behavior (dependent or independent of light) in Atlantic halibut larvae.

Materials and Methods

Halibut larvae were reared in a 1100 L first-feeding tank on enriched brine shrimp (*artemias*) during 53 day under natural photoperiod. Light were used as a factor influencing the metamorphosis process. Therefore, two rearing light conditions were tested: i) a standard condition with regular top light ("control") and ii) an extra underwater illumination with regular top light. The underwater illumination started after 5 days post-first-feeding. Lighting conditions were tested in triplicates.

A morphological screening was performed at the end of metamorphosis, by manually scoring eyes migration and pigmentation phenotypes, as well as general biometric analyses. Lateralized swimming behavior of larvae was monitored every day during first-feeding in a production tank, using a video system comprised of 4 underwater cameras. Videos were then digitalized using Tracker, a video analysis and modeling tool, and the body tilting angle of larvae were extracted. Comparative RNA sequencing was also conducted in the head regions of larvae to examine the potential mechanisms triggering this lateralized swimming.

Results and Discussion

The developmental window of tilting swimming was identified and preceded the morphological remodeling in Atlantic halibut. The genetic basis for this settling side behavior is ongoing and might contribute to understanding of factors necessary for metamorphosis success in flatfish. Understand biological processes in fish species should be further investigated to better estimate larval welfare and health in aquaculture.

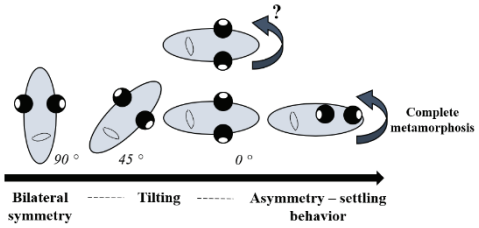


Figure 1. Schematic of the lateralized behavior during metamorphosis in flatfish

(Continued on next page)

References

Alves, R.N., Gomes, A.S., Stueber, K., Tine, M., Thorne, M.A.S., Sráradóttir, H., Reinhard, R., Clark, M.S., Rønnestad, I., Power, D.M., 2016. The transcriptome of metamorphosing flatfish. *BMC Genomics* 17, 1–24.

Harboe, T., Mangor-Jensen, A., Moren, M., Hamre, K., Rønnestad, I., 2009. Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles. *Aquaculture* 290, 250–255.

Haug, T., 1990. Biology of the Atlantic Halibut, *Hippoglossus hippoglossus* (L., 1758), *Advances in Marine Biology*.

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Björnsson, B.T., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* 130, 447–459.

Sæle, Solbakken, J.S., Watanabe, K., Hamre, K., Power, D., Pittman, K., 2004. Staging of Atlantic halibut (*Hippoglossus hippoglossus* L.) from first feeding through metamorphosis, including cranial ossification independent of eye migration. *Aquaculture* 239, 445–465.

Schreiber, A.M., 2006. Asymmetric craniofacial remodeling and liberalized behavior in larval flatfish. *J. Exp. Biol.* 209, 610–621.

Shao, C., Bao, B., Xie, Z., Chen, X., Li, B., Jia, X., Yao, Q., Ortí, G., Li, W., Li, X., Hamre, K., Xu, J., Wang, L., Chen, F., Tian, Y., Schreiber, A.M., Wang, N., Wei, F., Zhang, J., Dong, Z., Gao, L., Gai, J., Sakamoto, T., Mo, S., Chen, W., Shi, Q., Li, H., Xiu, Y., Li, Y., Xu, W., Shi, Z., Zhang, G., Power, D.M., Wang, Q., Schartl, M., Chen, S., 2017. The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nat. Genet.* 49, 119–127.

EVALUATION OF ENVIRONMENTAL AND ECONOMICAL IMPACTS OF NOVEL FEED INGREDIENTS FROM AQUACULTURE SIDESTREAMS

M. Perucca*, S. Anegalla, S.Truffa

Project Hub 360, Corso laghi, 13, 10090, Buttigliera Alta (TO), Italy.
E-mail: massimo.perucca@project-sas.com

Introduction

The innovative approach of Sidestream project in secondary bio-production of high value compounds by utilization of low trophic marine invertebrates (polychaete worms and gammarid shrimps) and bacteria, which are reared on aquaculture side streams, allows to assess the environmental and economic consequences. In attempts to achieve zero waste following sustainability and circular principles, life cycle assessment (LCA) and life cycle costing (LCC) methods play a major role. Due to the lack of Aquaculture datasets for LCA, these analysis in project gives an opportunity to build database for aquaculture systems. The assessment takes into account the entire life cycle: starting from pre-production stages to the final disposal. The outcomes of the analysis are intended to clarify the methodological choices made, identify possible data gaps, hotspots and provide recommendations in the direction of environmental and economical sustainability.

Methods

The LCA analysis corresponds to the ISO 14040/14044:2006 standards[1] with 11 global, local and toxicological impacts. From a full analysis of methodology in LCA, a modular approach has been accounted for the whole sidestream exploitation routes with specifications in system boundaries, functional units, assumptions and allocations. The analysis also accounts for performing comparisons of project products with the benchmark. The LCA inventory results are evaluated under design for costing through the LCC in order to keep the same or possibly lower the costs for the processing and exploitation of side streams. The analyzed modules are integrated through a synthesis, renormalization and weighting process with reference to the identified functional units allocations (output products) in order to obtain assessment of the whole functional system.

Results

The LCA results are the evaluations of the potential impacts associated with inputs and outputs of a product/process. The inputs section often requires more attention when dealing with material input coming from a sidestream as allocation factors and data sourcing are critical. The following fig.1 shows the preliminary assessment of processing aquaculture sludge which then fed as primary feed to Polychaetes (at lab scale).

Conclusion

The amount of input energy utilized for modular (lab scale) processes should be noted as they determine a relevant contribution to impacts. For the upscaling of the aquaculture sludge processing specific attention has to be paid to the energy efficiency and direct energy sources to comply with the sustainability requirements. Apart from this, developments in the fields of aquaculture databases and data quality for LCA are foreseen to enhance the assessments involved and also allows to perform comparisons with benchmark.

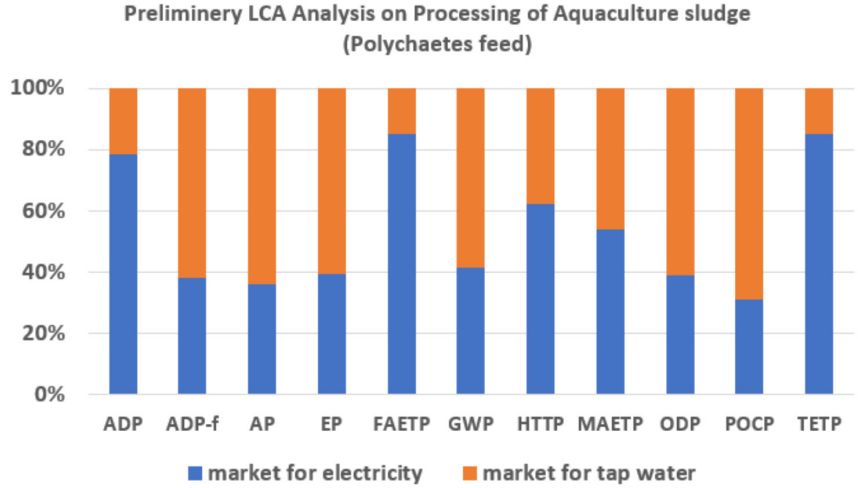


Fig 1: Preliminary LCA analysis on processing aquaculture sludge (model has been developed for 5kg sludge as reference technical Unit).

(Continued on next page)

Acknowledgement
This work is part of Sidestream project which is funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement no.817992. www.sidestream.info

References

1.ISO - International Organization for Standardization. Environmental management life cycle assessment—Principles and framework (ISO 14040:2006) requirements and guidelines (ISO 14044:2006).

MARINE HEATWAVES SIGNIFICANTLY IMPAIR THE PERFORMANCES OF MANILA CLAM *Ruditapes philippinarum* AT MULTIPLE PHYSIOLOGICAL LEVELS

Peruzza L.[1*], Bonsembiante F.[1], Panin M.[2], Poli F.[2], Smits M.[1], Manuzzi A.[1], Dalla Rovere G.[1], Babbucci M.[1], Milan M.[1], Gelain M. E.[1], Bargelloni L.[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Padova, Italy
[2]Department of Biology, University of Padova, Via U. Bassi 58/b, Padova, Italy
Email: luca.peruzza@unipd.it

Introduction:

Climate extreme events such as Heat Waves (HWs) are a serious threat for marine ecosystems since they can lead to massive mortality of benthic organisms, to biodiversity loss, ecosystem changes and extensive damage for human activities, e.g. aquaculture. One of the most threatened activities from HWs is bivalve aquaculture, since bivalves are particularly susceptible to HWs and are mostly farmed in coastal ecosystems where the effects of HWs will be more intense. However our knowledge regarding the effects of HWs on bivalves at physiological, immune and molecular level is still limited. In order to fill this gap, we used the Manila clam *Ruditapes philippinarum* (the most farmed clam species worldwide and an important ecosystem engineer) as a model species to characterise in detail the responses of clams when challenged by an HW.

Materials and Methods:

A population of 240 Manila clams (shell length 23 – 31 mm) was purchased from SATMAR, one of the biggest hatcheries in Europe and acclimated to lab conditions for 15 days (20 ˚C and 32 psu). After acclimation half population was exposed to HW conditions (30 ˚C for one month) and the remaining half was kept at 20 ˚C as control. After the exposure a series of physiological performances (growth, condition index, hepato-somatic index, clearance rate, burying speed, total haemocyte count) were evaluated in order to reveal the effects of HWs on the physiology of this species.

Results:

No increased mortality in HW-exposed clams was observed during the experiment. Our results indicated a significant decrease in growth, condition index and hepato-somatic index of HW-exposed animals, suggesting that energy reserves were diverted to counteract stressful conditions. Further, the burying speed was significantly increased in animals after the exposure to HW. Interestingly, clearance rate was higher in HW-exposed animals, while no change has been found in total haemocyte count or in the composition of the haemocyte subpopulations (e.g. granulocytes, hyalinocytes).

Conclusions:

Overall, the exposure to HW is able to induce a series of important changes to the physiology of clams, by impairing fundamental processes such as the burying velocity or the energy reserves in the hepatopancreas, thus posing a great concern for the aquaculture sector. Further in depth characterisation of the molecular responses triggered during HW-exposure are currently in place in order to reveal the transcriptomic and metabolomic responses of HW-exposed animals and provide a comprehensive picture of the impact of HWs on the physiology and ecology of the species and on its aquaculture.

COMPARATIVE TRANSCRIPTOMICS OF SUSCEPTIBLE AND RESISTANT NNV-INFECTED GILTHEAD SEA BREAM LARVAL STAGES REVEALS THE PUTATIVE MOLECULAR MECHANISMS OF TOLERANCE TO NNV INFECTION

Peruzza L.[1*], Pascoli F.[2], Dalla Rovere G.[1], Franch R.[1], Ferraresso S.[1], Babbucci M.[1], Biasini L.[2], Abbadi M.[2], Panzarin V.[2], Toffan A.[2], Bargelloni L.[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Padova, Italy
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy
Corresponding author: luca.peruzza@unipd.it

Introduction:

The gilthead sea bream is an important marine fish for Mediterranean aquaculture. This species has historically been considered resistant to viral nervous necrosis (VNN), a devastating fish disease affecting several major aquaculture species. However, with the emergence of RGNNV/SJNNV reassortant strains, also sea bream hatcheries are now seriously threatened, as these viral strains severely affect larvae and juveniles. While host response to betanodavirus in adult fish has widely been investigated, little is known regarding the most sensitive early life stages and currently the transcriptomic response of gilthead sea bream to RGNNV/SJNNV reassortant is still limited. Interestingly, early life stages (i.e. larvae younger than 35 days) are susceptible to VNN, while later stages (i.e. larvae older than 35 days) are resistant. This offers a unique opportunity for a comparative approach in order to understand the transcriptomic mechanisms that are triggered in susceptible vs resistant larvae, understand the genes and pathways that are activated in resistant larvae and pinpoint the molecular bases of resistance to RGNNV/SJNNV.

Materials and Methods:

This study reports the first time-course RNA-seq analysis on 21-day old (susceptible) and 35-day old (resistant) sea bream larvae experimentally infected with a RGNNV/SJNNV strain. Infected and mock samples from each developmental stage (i.e. 21 and 35 days) were collected at 6h, 12h, 24h, and 48h post infection (hpi). Four biological replicates, of five pooled larvae each, were analysed at all the time points.

Results:

Results highlighted a large set of significantly regulated genes, especially at 6hpi and 12hpi in 21-day old larvae. Particularly, several heat shock protein encoding transcripts were up-regulated (e.g. *hspa5*, *dnaj4*, *hspa9*, *hsc70*), while many immune genes were down-regulated (e.g. *myd88* and *irf5* at 6hpi, *pik3r1*, *stat3*, *jak1*, *il12b* and *il6st* at 12hpi). A gene set enrichment analysis (GSEA) was implemented to identify altered pathways/processes. Interestingly, many immune processes were found down-regulated in susceptible larvae and up-regulated in resistant larvae. In particular, the neutrophil degranulation process was down-regulated at 6hpi, 12hpi and 24hpi in susceptible larvae, while it was up-regulated at 6hpi, 12hpi and 24hpi in resistant larvae; the autophagy pathway was down-regulated at 6hpi in susceptible and resistant larvae, while it was up-regulated at 12hpi and 24hpi in resistant larvae only; the peroxisome, an important component of the antiviral immunity, was down-regulated at 6hpi in susceptible larvae, while there was no impairment of this process in resistant larvae; the immune system process was up-regulated at 12hpi and 24hpi in resistant larvae while it was not differentially regulated in susceptible larvae.

Conclusions:

Differently from previous studies in which it was proven that the RGNNV strain is able to induce mortality only in sea bream larvae infected by intramuscular route (while no mortality nor severe NNV signs are observed in older fish), our data confirms the hypothesis that the reassortant RGNNV/SJNNV induces transcriptional changes in the early larval stages of this species putatively by interfering with important immune processes and ultimately leading to the death of infected larvae.

DIETARY ROTIFERS REQUIREMENT FOR ZEBRAFISH OPTIMAL LARVAL GROWTH AND SKELETAL DEVELOPMENT

K. Pes[1,*], G. Martins[1], A. Carletti[1,3], M. Tarasco[1], P. Diogo[2], P. J. Gavaia[1,3]

[1] Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal
[2] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
[3] Necton, S.A., Belamandil, Olhao, Portugal

[*] Email: kpes@ualg.pt

Introduction

Currently, saltwater rotifers (e.g., *Brachionus plicatilis*) represent an excellent live food to be used for zebrafish (*Danio rerio*) larval feeding. They are considered better than *Artemia salina* nauplii for several reasons: smaller size, higher reproductive rate, adaptability to salinity and temperature changes, and slower swimming behaviour (Lawrence et al., 2015). Even though their nutritional profile is suboptimal for fish larvae, they are filter feeders and can be enriched through bioencapsulation, generally with microalgae containing a balanced nutritional value (such as *Nannochloropsis* sp.). This method allows the improvement of rotifers nutritional profile according to the predator's requirements (Rodríguez et al., 1996). Since the 1970's, rotifers have been intensively used in zebrafish facilities, yet a standardized feeding protocol (i.e., rotifers concentration, bioencapsulation, etc) to optimize zebrafish growth and larval quality has not been established. Several authors and studies used different concentrations of rotifers, thereby making it impossible to cross-check and make comparative assessments (Allen et al., 2016; Aoyama et al., 2015; Hernandez et al., 2018; Martins et al., 2016; Monteiro et al., 2018).

The aim of this study was to estimate the optimum concentration of rotifers that meets the zebrafish nutritional requirements during larval development, and the optimization of resources in zebrafish production.

Materials and Methods

Wildtype zebrafish embryos (AB) were obtained from an in-house breeding program. At 5 days post fertilization (dpf), larvae were selected and divided in 15 tanks in a static condition, in 1L of water with a density of 98.3±7.3 larvae L^{-1}. At 15 dpf, water level was increased to 2 L and larval density adjusted at 38.4±5.47 larve·L^{-1} in order to maintain biomass density and promote growth. Salinity was adjusted daily to 4 ppt, with the scope of maintaining salinity within the tolerance range of both zebrafish larvae and rotifers, without impacting the swimming behaviour of live preys. Photoperiod was set as 14h light :10h dark. Five different rotifer concentrations were added daily to each tank for 25 days (25, 50, 100, 150 and 200 rotifers·mL^{-1}). Rotifer concentrations and health of the culture was evaluated on a daily basis. Prior to feeding, rotifers were enriched with 2mL·mL^{-1} of *Nannochloropsis* sp. liquid concentrate (19% microalgae dry weight, Green Formula, Necton SA, Olhão, Portugal) for 2h. A 100% water renewal was performed daily in fish tanks. Zebrafish growth parameters (i.e., total length, body depth, wet and dry weight) were assessed at 15 and 30 dpf. In particular, in order to assess zebrafish total length and body depth, whole larvae were imaged using a Leica MZ10F stereoscope and analysed using ImageJ 1.53v software. To assess dry weight, zebrafish were dried for 24h at 60°C in VWR VENTI-line® drying oven and weighted with a Sartorius (MSA36S-000-DH) microbalance. Finally, to evaluate the impact of the different treatments on zebrafish skeletal development, fish from the second sampling were fixed in PFA 4% and stained with alizarin red S (0.05%). The mineralization of bone structures and incidence of skeletal deformities was determined through stereo microscopical observation.

Results and Discussion

Our results showed a significant difference in growth among the groups, with a statistically significant higher growth in terms of total length, body depth and weight in fish fed with 100, 150 and 200 rotifers mL^{-1} at 15 dpf (Figure 1 A), while only fish fed with 150 and 200 rotifers mL^{-1} had a significantly higher growth at 30 dpf (Figure 1 B). Analysis of the skeletal anomalies revealed that lower rotifer concentrations did not induce a higher incidence of skeletal anomalies or affected skeletal development and larval quality, although it showed a reduction of growth and mineralization status of the larvae. These results indicate that it is possible to feed zebrafish larvae using a lower number of rotifers than previously reported (Aoyama et al., 2015; Hernandez et al., 2018; Lawrence et al., 2015).

(Continued on next page)

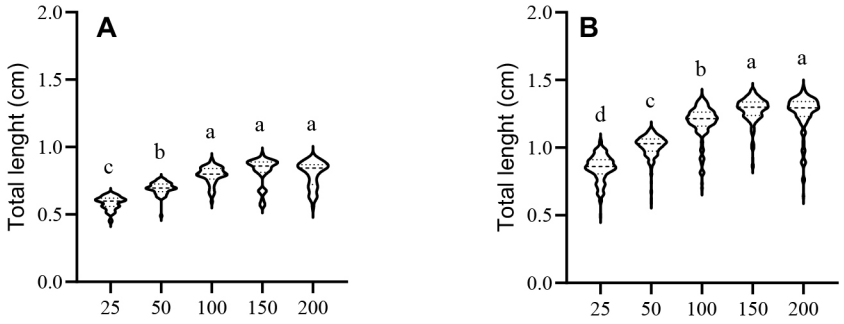


Figure 1. Growth in length in zebrafish fed with different concentration of rotifers per mL (25, 50, 100, 150, 200). Total length at 15 (A) and 30 (B) days post fertilization. Violin plots show frequency distribution of the data. Different letters at the top of the bars indicate significant differences among experimental groups (one-way ANOVA on ranks followed by Dunn's multiple comparison test, $P < 0.05$).

Fundings

This work was funded by the project ZEBRABLOOM-ALG-01-0247-FEDER-039896.

Bibliography

Allen, R. L., Wallace, R. L., Sisson, B. E. 2016. A rotifer-based technique to rear zebrafish larvae in small academic settings. Zebrafish, 13(4), 281-286.

Aoyama, Y., Moriya, N., Tanaka, S., Taniguchi, T., Hosokawa, H., Maegawa, S. 2015. A novel method for rearing zebrafish by using freshwater rotifers (*Brachionus calyciflorus*). Zebrafish, 12(4), 288-295.

Hernandez, R. E., Galitan, L., Cameron, J., Goodwin, N., Ramakrishnan, L. 2018. Delay of initial feeding of zebrafish larvae until 8 days postfertilization has no impact on survival or growth through the juvenile stage. Zebrafish, 15(5), 515-518.

Lawrence, C., James, A., & Mobley, S., 2015. Successful replacement of Artemia salina nauplii with marine rotifers (*Brachionus plicatilis*) in the diet of preadult zebrafish (Danio rerio). Zebrafish, 12(5), 366-371.

Lawrence, C., Sanders, E., Henry, E. 2012. Methods for culturing saltwater rotifers (*Brachionus plicatilis*) for rearing larval zebrafish. Zebrafish, 9(3), 140-146.

Martins, S., Monteiro, J. F., Vito, M., Weintraub, D., Almeida, J., Certal, A. C., 2016. Toward an integrated zebrafish health management program supporting cancer and neuroscience research. Zebrafish, 13(S1), S-47.

Monteiro, J. F., Martins, S., Farias, M., Costa, T., Certal, A. C. 2018. The impact of two different cold-extruded feeds and feeding regimens on zebrafish survival, growth and reproductive performance. Journal of developmental biology, 6(3), 15.

Rodríguez, C., Pérez, J. A., Izquierdo, M. S., Cejas, J. R., Bolanos, A., Lorenzo, A. 1996. Improvement of the nutritional value of rotifers by varying the type and concentration of oil and the enrichment period. Aquaculture, 147(1-2), 93-105.

THE EFFECT OF ALTERNATIVE FEED INGREDIENTS ON GROWTH AND STRESS RESPONSE OF EUROPEAN SEABASS *(Dicentrarchus labrax)* KEPT IN RECIRCULATING AQUACULTURE SYSTEMS

J. Petereit[1]*, C. Hoerterer[1], A A. Bischoff[2] , L.d Conceição[3], R. Pastres[4] B. H. Buck[1, 5]

[1] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven (Germany)

[2] University of Rostock, Aquaculture and Sea-Ranching, Justus-von-Liebig-Weg 6, 18059 Rostock (Germany) [3] SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão (Portugal)
[4] Ca' Foscari University of Venice, Via Torino 155, 30172 Mestre (Italy)
[5] University of Applied Sciences Bremerhaven, Bremerhaven (Germany)

Email: Jessica.petereit@awi.de

Introduction

Aquaculture feeds are urgently needing alternative sources of protein for the declining fish meal supplies as well as their mostly unsustainable origin (Ceccotti et al., 2019). Most of these current alternatives consist of plant-based ingredients, whereby these alternative protein sources often lead to a decreased digestion rate, health as well as welfare of the fish across species (Booman et al., 2018; Pelletier et al., 2018).

In our study, we investigated the effect of alternative feed formulation on growth characteristics, feed utilization and stress response of European seabass (*Dicentrarchus labrax*).

Material and Methods

From June to September 2020 experiments were conducted with European seabass in a recirculating aquaculture system (RAS) at the Alfred Wegener Institute (Bremerhaven, Germany). Fish were kept at standardized conditions (20 ± 0.9 °C, 95.21 ± 6.71% oxygen saturation, salinity 49.95 ± 0.87 mS cm^{-1}, pH 7.7 ± 0.04) in 700 L tanks (1 m^2 bottom area) throughout the entire trial.

A total of 375 fish were individually pit-tagged and randomly distributed to 15 tanks (25 fish/tank). The mean fish weight was about 320 g ± 72.4 g as well as the mean fish length about 30.5 ± 2.1 cm. Fish were fed twice a day (at 9:00 a.m. and 2:00 p.m.). Feeding at 9:00 a.m. was based on approximately 50 g pellets/tank and the feeding at 2:00 p.m. was *ad libitum*.

Five experimental diets (Sparos Ltd, Olhão, Portugal) were tested in four replicate tanks: (i) control (CTRL) consisting of 18 % fish meal and 10 % poultry meal; (ii) no processed animal protein (NOPAP) consisting of approx. 3 % fish meal from hydrolysates and 35 % insect meal, fermented biomass and algae; (iii) processed animal protein (PAP) consisting of 3 % fish meal from Hydrolysates and 31 % animal and insect meal; (iv) PLUS consisting of 23 % fish meal and was used as a positive control for the best growth performance; and (v) MINUS consisting of 3 % fishmeal and 34 % animal protein and was used as a negative control for growth performance (see Table 1).

Every four weeks all fish were weighted and measured in length to identify the growth performance. Additionally, blood and organs were sampled of five fish per tank at the end of the trial to identify the health and welfare parameters. Further, another five fish per tank were sampled at the end of the trial to identify the sensory quality of the fish filet.

Table 1 Diet formulation for 4 alternative diets in European seabass and the commercial CNTRL feed.

Ingredients (%)	CTRL	NOPAP	PAP	PLUS	MINUS
Fish meal	18.0	3.0	3.0	23.0	3.0
PLANTs					
PAPs	10.0		21.0		34.0
Insect meal		15.0	10.0	13.5	
Fermentation biomass		20.0	15.0	16.0	
Macroalgae & Microalgae		2.8	2.8	2.8	2.8

(Continued on next page)

Results and Discussion

The parameters for the growth performance showed no difference between the alternatively fed diets, neither for final weight (lowest MINUS group 459 ± 104 g; highest NOPAP group 475 ± 102 g), nor for voluntary feed intake or condition factor (lowest MINUS group 1.18 ± 0.006; highest PLUS group 1.21 ± 0.02). Significant differences were found in the relative growth rate between the control group and the fish fed NOPAP, MINUS and PLUS diets. This indicates that the fish fed the control group still had the best growth performance. However, this trend was not observed for the FCR, as the control group was not significantly different from the fish fed the alternative diets.The results showed slightly decreased health parameters for the PAP and MINUS groups with a significant increase in the viscerosomatic index between the fish fed the control/PLUS diet and the fish from the MINUS group.

The biochemical analyses of the fish plasma was conducted for a better interpretation of the growth parameter results as well as the fish stress response (Papaharisis et al., 2019). Lactate dehydrogenase showed no significant differences, ruling out diet-dependent effects on the inflammatory response of the fish. Blood parameters glucose and lysozymes did not show significant differences between fish fed the alternative diets, but interestingly, total protein content showed significant differences. Further analysis of the data is needed to better interpret the alternative diets in regard to welfare parameters.
Sensory analysis of the fillet showed no significant differences in taste, odor, juice deposition, protein deposition, and fat deposition. Post-cooking consistency was affected by the alternative diets, with fish fed the control being significantly firmer to the bite than fish fed the MINUS and PLUS diets.

Overall, these preliminary results seem to support the hypothesis that the NOPAP and PLUS diets are viable options for feeding European seabass compared to the commercial diet (CNTRL). However, results showed slightly decreased health parameters for the PAP and MINUS groups, while sensory function was not significantly affected by any of the diets tested (except for consistency after cooking). Interestingly, fish fed the PLUS diet had significantly lower total plasma proteins and significantly firmer-to-bite filets after cooking.

References

Booman, M., Forster, I., Vederas, J. C., Groman, D. B., & Jones, S. R. (2018). Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). *Aquaculture*, *483*, 238-243

Ceccotti, C., B. S. A. Al-Sulaivany, O. A. M. Al-Habbib, M. Saroglia, S. Rimoldi and G. Terova (2019). "Protective Effect of Dietary Taurine from ROS Production in European Seabass under Conditions of Forced Swimming." Animals (Basel) **9**(9).

Papaharisis, L., T. Tsironi, A. Dimitroglou, P. Taoukis and M. Pavlidis (2019). "Stress assessment, quality indicators and shelf life of three aquaculture important marine fish, in relation to harvest practices, water temperature and slaughter method." Aquaculture Research **50**(9): 2608-2620.

Pelletier, N., Klinger, D. H., Sims, N. A., Yoshioka, J. R., & Kittinger, J. N. (2018). Nutritional attributes, substitutability, scalability, and environmental intensity of an illustrative subset of current and future protein sources for aquaculture feeds: Joint consideration of potential synergies and trade-offs. *Environmental science & technology*, *52*(10), 5532-5544

EFFECT OF PLANT AND ANIMAL PROTEIN SOURCES AT TWO SUBSTITUTION LEVELS ON GROWTH AND FEED PERFORMANCE OF MARKET SIZED TURBOT (*Scopthalmus maximus*)

C. Hoerterer[1]*, J. Petereit[1], G. Lannig[1] , L. Conceição[2], J. Johansen[3], B. H. Buck[1, 4]

[1] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven (Germany)
[2] SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão (Portugal)
[3] Salten Havbrukspark, 8120 Nygårdsjøen (Norway)
[4] University of Applied Sciences Bremerhaven, Bremerhaven, Germany

Email: Christina.Hoerterer@awi.de

Introduction

To conserve and sustainably use aquatic resources, the reduction of the environmental footprint of aquaculture feeds has become a high priority (Hardy and Barrows 2003; Olsen and Hasan 2012). The challenge is to feed farmed fish with diets that are nutritious but at the same time economically and environmentally sustainable (Glencross et al. 2020). In Europe, turbot (*Scophthalmus maximus*) aquaculture has a high potential for sustainable production but the low tolerance to fishmeal replacement in the diet represents a big issue (von Danwitz et al. 2016). Therefore, this study investigated the effects of more sustainable feed formulations on growth and feed performance of market sized turbot in recirculating aquaculture systems.

Material and Methods

In a 16-week feeding trial (trial registration number 500-427-103-1/2019-1-19) with 301 ± 10 g turbot, one control diet (CTRL) and four experimental diets were tested. The CTRL was based on a commercial formulation and in the experimental diets, 30% and 60 % of fishmeal was substituted with insect meal and fermentation biomass. Furthermore, the plant protein content in the control was replaced by sustainable terrestrial plant proteins (PLANT) or processed animal proteins (PAP) (see Table 1). 400 fish were individually marked with pit tags and distributed randomly into 20 tanks with 4 tanks per diet. During the trial period the turbot were fed twice a day and measured and weighed every four weeks.

Results and Discussion

Turbot from the CTRL group had on an individual basis (n=65) a significantly higher weight gain (210 ± 87 g) and growth rate (0.46 ± 0.17 % d^{-1}) than the PAP30, PAP60 and the PLANT60 group (158 ± 63 g, 0.37 ± 0.13 % d^{-1}; 149 ± 72 g, 0.35 ± 0.13 % d^{-1} and 159 ± 75 g, 0.37 ± 0.14 % d^{-1} respectively). The FCR was significantly lower in the CTRL (1.3) compared to the PAP30, PAP60 and the PLANT60 group (1.7; 1.9 and 1.7 respectively); with no significant differences to the NOPAP30 group (1.5). The weight gain (184 ± 66 g), growth rate (0.42 ± 0.11 % d^{-1}) and FCR (1.5) in the NOPAP30 group did not differ significantly from the CTRL.

In general, the PLANT diets with insect meal and fermentation biomass performed significantly better than PAP in market sized turbot. Furthermore, the performance of the diets significantly decreased with the substitution level, with no interaction between the protein source and substitution level. In conclusion, the PLANT diets are more suitable, but the lower costs for diets with PAPs might be an economic benefit for turbot farmers outbalancing the slightly poorer growth and feed performance of turbot.

Table 1 Formulation of the five experimental diets for marked sized turbot

Ingredients (%)	CTRL	PLANT 30	PAP 30	PLANT 60	PAP 60
Fishmeal	40.0				
Fishmeal from by-product		28.0	28.0	16.0	16.0
Fish protein hydrolysate	4,0	4,0	4,0	4,0	4,0
PLANTs	42.0	30.0	12.0	30.0	12.5
PAPs			21.0		21.0
Insect meal		9.0	9.0	14.0	14.0
Fermentation biomass		9.0	9.0	14.0	14.0
Krill meal		3.5	3.5	3.5	3.5
Macroalgae & Microalgae		2.5	2.5	2.5	2.5
Lipids	11.5	11.0	10.0	11.5	10.0
Vitamins, Minerals, AAs etc.	2.5	3.0	2.0	4.5	2.5

(Continued on next page)

References

Glencross BD, Baily J, Berntssen MHG, Hardy R, MacKenzie S, Tocher DR. 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Reviews in Aquaculture 12:703-758.10.1111/raq.12347.

Hardy RW, Barrows FT. 2003. Diet Formulation and Manufacture. Pages 505-600 in Halver JE, Hardy RW, eds. Fish Nutrition. San Diego: Academic Press.

Olsen RL, Hasan MR. 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science & Technology 27:120-128.10.1016/j.tifs.2012.06.003.

von Danwitz A, van Bussel CGJ, Klatt SF, Schulz C. 2016. Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (Psetta maxima L.). Aquaculture 450:405-411.10.1016/j.aquaculture.2015.07.026.

A WELFARE SCORING TOOL FOR EUROPEAN SEA BASS AND GILTHEAD SEA BREAM BASED ON OPERATIONAL WELFARE INDICATORS

T. Petochi[1*], A. Feraldi[1], F. Cardia[1], M.G. Finoia[1], V. Donadelli[1], F. Padrós[2], G. Marino[1]

[1] ISPRA Italian Institute for Environmental Protection and Research, Rome, Italy
[2] Fish Diseases Diagnostic Service, Universitat Autònoma de Barcelona, Spain
E-mail: tommaso.petochi@isprambiente.it

Introduction

Animal welfare represents a key issue in global and European strategies for sustainable food production, including aquaculture (Keeling et al., 2019; Green Deal, F2F). The strategic guidelines for EU aquaculture highlight the need to improve the welfare of farmed fish, focusing on good farming practices and the use of common, validated, species-specific and auditable welfare indicators (COM(2021) 236 final). Sea bass and sea bream are major marine species farmed in Mediterranean with over a billion of specimens harvested every year (www.fishcount.org.uk). Operational welfare indicators (OWIs) and benchmarking systems are thus necessary to measure the welfare of these species during farming cycles. This work describes the *Bass&Bream Welfare Scoring Tool (BBW-Tool)*, a methodology developed in PerformFISH project (http://performfish.eu/) with the aim to assist the Mediterranean Marine Fish Farming sector in evaluating fish welfare and improving welfare standards.

Materials and Methods

The BBW-Tool has been developed following the composite indicators approach which consists in a wise aggregation of a certain number of elementary indicators representing different issues of the same multi-dimensional phenomenon (Alaimo & Maggino, 2020). The tool measures the welfare of sea bass and sea bream in different sea cage systems across the Mediterranean Sea during the grow out phase.

i) *Selection and validation of OWIs* – a set of 9 OWIs was derived by the 9 Welfare-KPIs validated by the MMFF industry for the PerformFISH KPI-benchmarking system (ISPRA, 2018). Additional 15 OWIs were identified through a PerformFISH online welfare survey and the expert judgment (Petochi et al., 2019). A total of 24 animal-based and environmental/management based OWIs make up the BBW-Tool.

ii) *Development of W-Index*: the fish W-Index applies the Adjusted Mazziotta–Pareto Index (AMPI). It requires data normalization and equal weighting of the indicators and is based on a min–max transformation, consisting in re-scaling individual indicators to two 'goalposts', i.e. a min and a max value of each variable across time periods and units (e.g. batches, farms, companies, geographical areas). The normalized values and the aggregated composite indicator fall in the range 70-130 (Mazziotta and Pareto, 2018), allowing the evaluator to identify batches and farms with higher or lower level of fish welfare than the average.

iii) *Testing the BBW-Tool*: the tool has been tested in 12 farms for sea bass and in 10 farms for sea bream. The analysis was performed by individual batch (a group of homogenous rearing units) analysed within the period 2016-2020, for a total of 61 and 54 batches of sea bass and sea bream respectively accounting for about 49 million of stocked fish. Production data have been provided anonymously by companies through the KPI-VRE hosted in PerformFISH Gateway D4 Science (ISPRA, 2018). A subset of 11 OWIs (fish mortalities total, by diseases, at 3 and 10 days after transport-stocking; vaccinated fish; antiparasitic and antibiotic treatments; feed intake; stocking density at harvest; discharged fish at slaughter; oxygen depletion days) was measured at two stages of production cycle: from stocking up to 50g and on whole cycle up to harvest-800g.

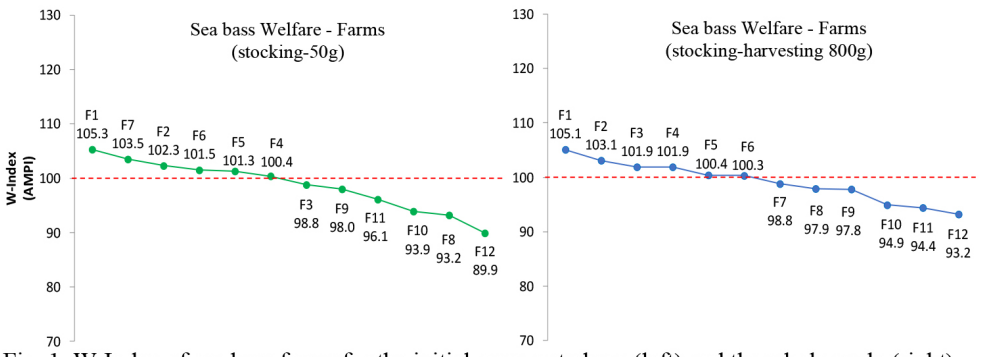


Fig. 1. W-Index of sea bass farms for the initial grow out phase (left) and the whole cycle (right)

(Continued on next page)

Results

Consistently with the assumption of the model, W-Index values for all batches and farms fall in the range 70–130. For sea bass, the W-Index at farm level for the whole production cycle ranges from 93.2 to 105.1. The best performing farm F1 keeps its advantage from stocking and confirms the top of the ranking at harvest. Similarly, the farm F12 shows the worst W-Index, being at the bottom in both ranks. Most of the other farms show improvement or worsening of W-Index between the two phases (Fig.1). Similar patterns result for sea bream data. Additionally, a multivariate linear regression model with W-Index as depend variable and the OWIs as explanatory variables reveals that the most relevant OWIs which negatively affect the W-Index in both species are mortality (total, by diseases, first 3 days) and number of treatments. The "percentage of discarded fish at slaughter" is a key OWI in W-Index, mainly reducing the welfare score in sea bream while "feed intake" increases the W-Index in sea bass. The "percentage of vaccinated fish" positively affects the W-Index in both species.

Discussion and conclusion

Two main challenges in assessing the welfare of farmed fish are: i) construction of a set of reliable welfare indicators and ii) their synthesis in a holistic welfare index. The Adjusted Mazziotta–Pareto Index (AMPI), already applied to measure complex multidimensional phenomena in the field of quality of life and well-being of citizens, has been successfully developed in PerformFISH to provide robust and repeatable assessments of the level of welfare of sea bass and sea bream from stocking to harvest. The BBW-Tool has been tested on 115 batches of sea bass and sea bream in different culture systems across the Mediterranean, and represents a promising non-invasive methodology to assess the overall well-being of sea bass and sea bream for:

- Company self-evaluation and benchmarking; audits, official controls
- Code of Conduct and certification standards
- Improving social acceptability of Mediterranean aquaculture products

Acknowledgments

The study has received funding from the European Union's Horizon 2020 research and innovation program under Grant Agreements No 727610 (PerformFISH). This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein.

References

Alaimo, L.S., Maggino, F. (2020). Sustainable development goals indicators at territorial level: Conceptual and methodological issues-The Italian perspective. Soc. Indic. Res., 147(2), 383-419.

Keeling, L., Tunón, H., Olmos Antillón, G., Berg, C., Jones, M., Stuardo, L., Swanson, J., Wallenbeck, A., Winckler, C., Blokhuis, H. (2019). Animal Welfare and the United Nations Sustainable Development Goals. Front. Vet. Sci., 6.

ISPRA, 2018. PerformFISH Deliverable 7.1 Set of KPIs for MMFF Sector. http://performfish.eu/deliverables/

Mazziotta, M., Pareto, A. (2018). Measuring well-being over time: The adjusted Mazziotta–Pareto index versus other non-compensatory indices. Soc. Indic. Res., 136(3), 967-976.

Petochi T., Tamburrini M., Fabris A., Tort L., Padrós F., Montero D., Donadelli V., Marino G. (2019). A survey to identify operational welfare indicators (OWIs) in farmed European sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata*. Aquaculture Europe. Berlin, Germany. 7-10 October 2019.

SYSTEMIC AND CUTANEOUS MYCOBACTERIOSIS IN GILTHEAD SEA BREAM *Sparus aurata* FARMED IN ITALY

T. Petochi[1*], D. Florio[2], L. Di Renzo[3], P. Di Marco[1], K. Varello[4], V. Donadelli[1], A. Longobardi[1], A. Gustinelli[2], M. Caffara[2], F. Di Giacinto[3], M. L. Fioravanti[2], G. Marino[1]

[1] ISPRA Italian Institute for Environmental Protection and Research, Rome, Italy
[2] Department of Veterinary Medical Sciences, University of Bologna, Italy
[3] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy
[4] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy
E-mail: tommaso.petochi@isprambiente.it

Introduction

Disease outbreaks caused by different nontuberculous mycobacteria (NTM) in Mediterranean farmed fish show an increasing trend in recent years (Mataragka et al., 2021; Mugetti et al., 2020, 2021) affecting productions and posing risks for potential zoonosis. This work reports on the first record of systemic mycobacteriosis characterized by cutaneous granulomas occurred in gilthead sea bream (*Sparus aurata*) farmed in land-based systems in Italy.

Materials and Methods

Farming systems: sea bream farmed in land-based recirculating (RAS) and flow-through (FT) tanks were examined for the occurrence of cutaneous granulomatosis episodes on July 2020 and February 2021. Both culture systems are served by wells with water temperature in the range 22-23°C and pH 6.9-7.2. Water salinity was 9.5‰ in RAS and 38‰ in FT. Dissolved oxygen was kept around saturation levels. Fish were analyzed for: i) *Anatomo-histopatology*: a total of 150 fish (200 g) from RAS and 100 fish (400g) from FT farms were examined for the presence/absence of external and visceral nodules. *S*amples of skin, muscle, spleen, liver, kidney, stomach, intestine and brain from symptomatic fish were fixed in 10% buffered formalin and processed through routine histological techniques. Sections were stained with Hematoxylin & Eosin and Ziehl-Neelsen; ii) *Bacterial isolation and identification*: samples from cutaneous lesions, spleen of fish from RAS were inoculated on Löwenstein-Jensen medium and bacterial isolates identified by molecular analysis. Additionally, samples from skin lesions, spleen, kidney and intestine were cultured at 25°C for 24h on TSB and TSA media and bacteria characterized by vitek iii) *Blood chemistry*: after fasting, 30 fish were sampled from RAS and anaesthetized (150mg/l MS 222 Pharmaq) for blood withdrawal. Serum samples were stored at -80°C for analysis of cortisol, osmolality, minerals and metabolic, liver and kidney profiles according to Di Marco et al. (2017).

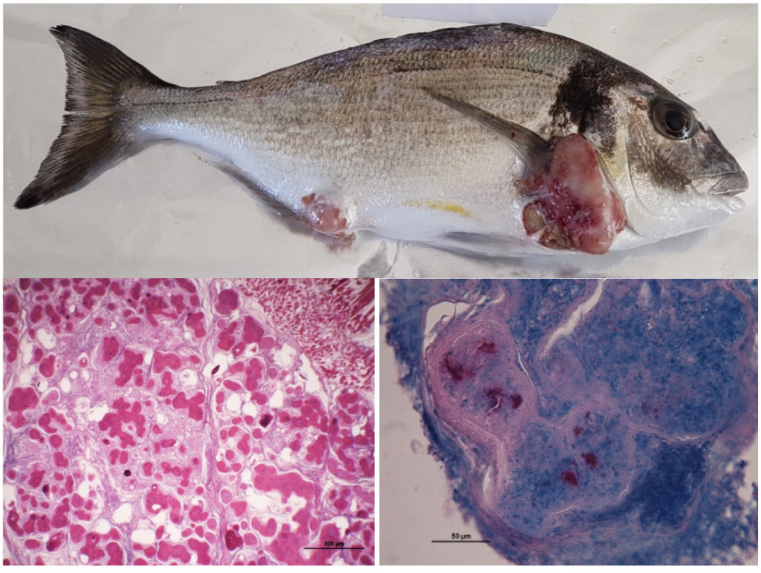


Fig. 1. Skin lesions caused by NTM in sea bream (top). Multiple granulomas in dermal tissue (H&E, bottom left); acid-fast bacilli stained in red inside granulomas (ZN, bottom right).

(Continued on next page)

Results

Prevalence of fish with cutaneous granuloma differed between farming systems, being 69% in RAS and 3% in FT. Clinical signs of disease were characterized by reddish-pinkish colored hyperplastic nodules with different degree of severity, ranging from pinhead-sized lesion (79%) to larger granuloma (21%), with necrotic foci. Lesions were observed on the lateral side, at the base of fins and tail, head, eyes and operculum (Fig. 1). No multifocal nodules were observed in the visceral organs and only two fish from RAS displayed a small single subcapsular nodule on spleen.

Histological analysis showed severe chronic inflammation with multinodular granuloma with a necrotic centre and epithelioid organisation in dermal, hypodermal and muscle tissues and other visceral organs, with the exception of brain. All lesions revealed acid-fast bacilli by ZN staining (Fig. 1). *Mycobacterium marinum* was isolated and identified from skin and spleen. Morover opportunistic pathogenic Gram-negative bacteria (*Sphingomonas paucimobilis*; *Aeromonas hydrophila/caviae)* were isolated from the skin lesions.

Serum cortisol, glucose and triglycerides were significantly lower and albumin significantly higher in symptomatic fish. No significant differences were found for other parameters.

Discussion and conclusion

The cutaneous granulomatosis described in the present study is comparable to that one reported in sea bream cultured in RAS in Israel and caused by *Mycobacterium marinum* (Davidovich et al., 2020). Movement of live fish could have been the main source of the infection, especially in the RAS farm where different fish species are farmed (sea bream, sea bass, red drum). Compared to FT, environmental condition in RAS could have further favoured the permanence of pathogens, enhancing fish exposure to mycobacteria and thus resulting in a more severe infection. Blood chemistry analysis revealed a lowering of energy status in symptomatic fish and a worsening of their physiological status probably due to the persistence of chronic stress conditions and systemic inflammation. Since there are still no commercial vaccines and effective treatments against fish mycobacteriosis, epidemiological surveillance, application of strict quarantine, biosecurity protocols and best management practices shall be implemented, also to minimize the risk of zoonosis.

References

Davidovich N. et al. (2020). Cutaneous appearance of mycobacteriosis caused by *Mycobacterium marinum*, affecting gilthead seabream (*Sparus aurata*) cultured in recirculating aquaculture systems. Aquaculture, 528, 735507.

Di Marco P. et al. (2017). Insights into organic farming of European sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata* through the assessment of environmental impact, growth performance, fish welfare and product quality. Aquaculture, 471, 92-105.

Mataragka A. et al. (2021). Diagnostic investigation for the detection of mycobacteria in samples of fish feeds and tissue from sea bream and sea bass with severe granulomatous lesions. Aquaculture, 546, 737283

Mugetti, D. et al. (2020). *Mycobacterium pseudoshottsii* in Mediterranean Fish Farms: New Trouble for European Aquaculture? Pathogens 2020, 9, 610.

Mugetti D. et al. (2021). Investigation of potential reservoirs of non-tuberculous mycobacteria in a European sea sass (*Dicentrarchus labrax*) farm. Pathogens, 10, 1014.

ZINC AND COPPER NUTRITION AT FIRST FEEDING AFFECTS GROWTH AND HEPATIC LIPID METABOLISM IN JUVENILE *Oncorhynchus mykiss*

P. Antony Jesu Prabhu*[1], M. Bidon[2], L. Larroquet[2], S. Fontagné-Dicharry[2]

[1]Feed and Nutrition group, Institute of Marine Research, Bergen 5817, Norway
[2]INRAE, Univ. Pau & Pays Adour, E2S UPPA, NUMEA, 64310 Saint-Pée-sur-Nivelle, France
*E-mail: antony.philip@hi.no

Introduction
Fish meal has been progressively replaced by plant ingredients in salmonid feeds to good effect with respect to macro-nutrients. However, metabolic consequences of altered micro-nutrient supply is less studied. It is known that Zn and Cu are important essential trace elements and involved in several metabolic reactions. Although the role of dietary Zn and/or Cu in influencing lipid metabolism is beginning to be understood in teleost (Zheng et al., 2015; Meng et al., 2016), their practical implications for aquaculture are less clear. It was shown that LC-PUFA bioconversion in fish is improved by a multi-micronutrient supplement including Zn (Lewis et al., 2013; Giri et al., 2016). The requirement for Zn and Cu to rainbow trout is 37 mg Zn/kg feed and 5 mg Cu/kg feed, respectively. Plant based salmonid feeds are limited in Zn supply, while provide excess Cu. These dietary changes warrant investigations on the functional importance of Zn and Cu nutrition at critical windows of early life stages on their impact during later stages. In this context, we hypothesized that the metabolic capacity of fish towards utilization of dietary lipids might be altered by early nutritional history of dietary Zn or Cu.

Material and methods
The feeding trial lasted 24 weeks and had three phases. Growth was recorded every 3 weeks, mortality and feed intake were monitored daily. In Phase I, 200 first feeding rainbow trout fry (52 ± 3 mg) were fed for 6 weeks with one of the four experimental feeds with two levels of Zn (Zn-, 70 or Zn+, 130 mg/kg) or Cu (Cu-, 10 or Cu+, 20 mg/kg) in a 2 x 2 factorial design, in triplicate. At the end of phase I, ninety fish per tank were sampled for growth and body composition analysis. In Phase II, all the groups were fed with a commercial feed for the next 12 weeks. In Phase III, all the groups were challenged with the Zn-Cu- diet for another 6 weeks. At the end of Phase II and III, eight fish per tank were sampled for plasma metabolite, liver fatty acid profile and gene expression, and whole-body proximate composition analysis.

Results
Growth: Dietary Zn supplementation significantly increased mean body weight and whole-body Zn levels, while body lipid and gross energy level was reduced by Cu supplementation at the end of Phase I. In Phase II, when all groups were fed the commercial feed, no differential effect was observed on growth or body composition. In phase III, when the fish were re-introduced to the Zn-Cu- diet, growth differences became significant. Rainbow trout fed Zn supplemented diet during first feeding (Zn+ and Zn+Cu+) showed significantly higher weight gain and final weight at the end of phase III. On the contrary, they also had significantly low whole-body Zn levels.

Body trace element status: Fish fed Zn+ diets had significantly increased body Zn status at the end of phase I, which was no more apparent at the end of phase II and the contrary was significant at the end of phase III. Dietary Cu did not affect body Cu status at any stage of the experiment. Dietary Mn concentrations were reduced in fish fed Zn+ and Cu+ until end of phase II, but the effect disappeared in phase III when all the groups were fed Zn-Cu- diet.

Plasma lipid metabolites: Circulating levels of total cholesterol and phospholipids in the plasma at the end of phase III were significantly lower in fish with Zn+ early history. No effect was seen on plasma glucose, triglycerides or non-essential fatty acid concentrations.

Hepatic lipid metabolism: Although total lipid content was the same, the fatty acid profile in the polar lipids was affected by early stage Zn or Cu nutrition. Notably, 18:3n-6, 18:4n-3 and 22:5n-3 were lower in fish with Zn+ history. Whereas, 20:2n-6 and 20:3n-3 were lower with Cu+ history. Expression of genes involved in LC-PUFA biosynthesis especially those involved in the fatty acid chain elongation (*elovl2 and elovl5)* and desaturation *(d6d and d9d*) were downregulated by Zn+ early history.

Discussion
In plant-based feeds, increased dietary Zn at first feeding not only improved the growth of rainbow trout fry, but also enhanced growth when sub-optimal Zn supply was encountered later in the juvenile stage. Growth dilution due to enhanced growth in Zn+ fed fish reduced whole body Zn levels at the end of phase III. Impact of Zn or Cu restriction at first feeding on hepatic fatty acid profile and expression of genes in intermediary metabolism at juvenile stage indicate of a possible programming effect, warranting further investigation.

(Continued on next page)

Acknowledgements
This work was financially supported by AQUAEXCEL2020 research infrastructure project funded under the EU's Horizon 2020 programme.

References

Zheng, J.-L., Luo, Z., Hu, W., Liu, C.-X., Chen, Q.-L., Zhu, Q.-L. & Gong, Y. (2015) Different effects of dietary Zn deficiency and excess on lipid metabolism in yellow catfish *Pelteobagrus fulvidraco*. Aquaculture, 435, 10-17.

Meng, F., Li, M., Tao, Z., Yuan, L., Song, M., Ren, Q., Xin, X., Meng, Q. & Wang, R. (2016) Effect of high dietary copper on growth, antioxidant and lipid metabolism enzymes of juvenile larger yellow croaker *Larimichthys croceus*. Aquaculture Reports, 3, 131-135.

Giri, S.S., Graham, J., Hamid, N.K.A., Donald, J.A. & Turchini, G.M. (2016) Dietary micronutrients and in vivo n – 3 LC-PUFA biosynthesis in Atlantic salmon. Aquaculture, 452, 416-425.

Lewis, M.J., Hamid, N.K.A., Alhazzaa, R., Hermon, K., Donald, J.A., Sinclair, A.J. & Turchini, G.M. (2013) Targeted dietary micronutrient fortification modulates n – 3 LC-PUFA pathway activity in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 412–413, 215-222.

DIET AND HOST GENETIC BACKGROUND DRIVE DIFFERENCES IN THE TRANSCRIPTOMIC PROFILE OF INTESTINAL MICROBIOTA IN GILTHEAD SEA BREAM

M.C. Piazzon*[1], F. Naya-Català[2], A. Sitjà-Bobadilla[1], J. Pérez-Sánchez[2]

[1]Fish Pathology Group and [2]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, CSIC, Spain
E-mail: carla.piazzon@csic.es.

Introduction

In the animal production sector, there is an increasing interest in manipulating intestinal microbiota due to their undeniable key effects on host health and welfare. To that aim, numerous studies have been conducted in order to define microbial populations and changes in fish intestine under different conditions. However, most of the studies conducted so far are focused on describing the presence and abundance of bacterial populations based on DNA sequencing technologies. In the current study, we aimed to define the actual metabolic potential of fish intestinal resident microbiota, by studying the gene expression of all gut microbes instead of just defining presence/absence of bacteria. To that aim, we used two families of gilthead sea bream (*Sparus aurata*) selected for heritable growth (fast- and slow-growth families) and fed control or plant-based diets. In previous studies, the fast-growth family showed a more continuous growth across seasons, genetically regulated intestinal plasticity to maximize nutrient absorption when fed plant-based diets, improved resilience to intestinal parasites, and a plastic microbiota that efficiently adapted to the metabolic challenges induced by diet changes (Perera *et al*., 2019; Piazzon *et al*., 2020). Upon diet changes, only a small percentage of the adherent bacterial populations changed in the fast-growth family, but these small changes seemed to account for higher metabolic changes (inferred metagenome and pathway analysis) when compared to the slow-growth family. Here, we compare the transcriptional profile of the total microbial populations in fast- and slow-growth families in order to validate and have further details on the previous observations.

Methods

Two gilthead sea bream families, selected for fast- (e6e2) and slow-growth (c4c3) were kept together in the same open-flow tanks and fed a control or a well-balanced plant-based diet during nine months. Eight animals per group were sacrificed and the adherent microbiota from the anterior intestinal portion was collected and used for RNA extraction. RNA samples were pooled in groups of two to yield a total of four samples per group. After rRNA removal, RNA was sequenced (Illumina, 150PE). Reads were quality filtered and the transcriptome was reconstructed using Trinity. For annotation, the obtained unigenes were aligned with Bacteria, Fungi, Archaea and Viruses sequences from the NCBI's NR database using Diamond. Partial least squares discriminant analyses (PLS-DA) and DESeq2 were used to determine group differences and differentially expressed genes among groups. Gene ontology analysis was performed using GOSeq.

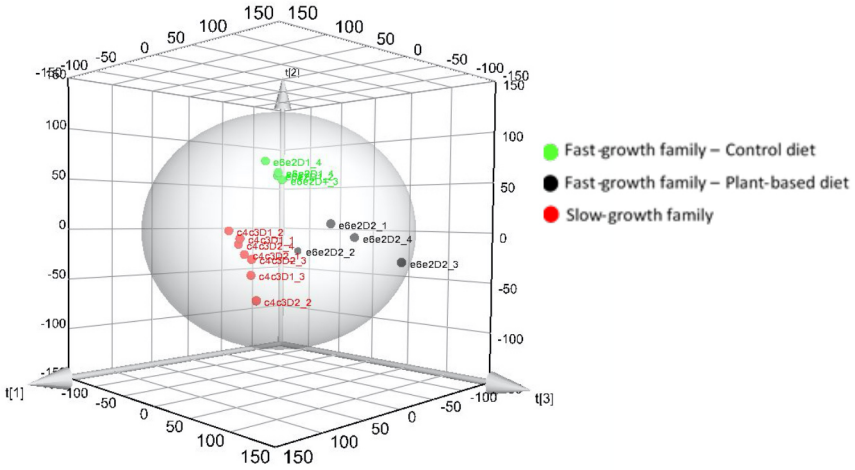


Figure 1: Three-dimensional PLS-DA score plot of the model constructed using all transcripts expressed by the microbiota of the four groups. Note that the model significantly separated fast-growth individuals fed the control (green) and plant-based (black) diets, but failed to separate the slow-growth group (red) by diet.

(Continued on next page)

Results

The total number of annotated transcripts found in this study was 35,144, from which 232 (0.7%) were Archaea, 17,516 (49.8%) Bacteria, 15,703 (44.7%) Fungi, and 1,693 (4.8%) Virus. Regarding the level of expression of the transcripts per group and organism, no differences were detected between families, but differences were found between dietary groups. Plant-based diets increased the number of Fungi transcripts and decreased bacteria transcripts. Diet and genetic background were responsible for changing the expression of 425 and 329 transcripts, respectively. PLS-DA analysis showed a significant separation between fast- and slow-growth families, and within the fast-growth family the different diets showed a significantly different profile. However, diet driven differences were not detected in the slow growth family (Fig. 1). This was supported by the DESeq2 analysis which showed a change in 271 transcripts in the fast-growth family when fed different diets, whereas only 40 differentially expressed transcripts were found in the slow-growth group. GOSeq analyses revealed that plant-based diets were upregulating genes involved in anatomic structure morphogenesis and development, and downregulating genes involved in several metabolic pathways, lipid localization, cytolysis and killing of cells of other organisms, and response to stress in the fast-growth group. Downregulation of vitamin metabolism and upregulation of carbohydrate metabolism, cytokine production, movement and entry into host, and cell proliferation was found in the slow growth group.

Conclusions

Fungi and Bacteria transcripts constitute almost 95% of the transcriptome of gilthead sea bream intestinal microbiota and Fungi transcripts are found roughly in the same abundance as bacterial, highlighting the importance of these two groups of microorganisms in the metabolism of intestinal microbiota. Microbial expression of genes related to tissue remodelling in fast-growth families when fed plant-based diets indicate a possible role of the microbiota in intestinal reshaping (Perera *et al*., 2019). Gene expression patterns found in the slow-growth family indicate a shift towards inflammation and bacterial invasion when these animals are fed plant-based diets. The current results support the previous findings describing fast-growth families' microbiota as plastic communities able to achieve large metabolic changes with fewer changes in the bacterial composition (Piazzon *et al*., 2020) allowing the animals to efficiently adapt to diet changes. These results highlight the relationship between selection for heritable growth and metagenome and the impact on animal health and ability to adapt to changes without detrimental effects.

References

Perera E., Simó-Mirabet P., Shin H.S., Rosell-Moll E., Naya-Català F., De las Heras V., *et al*. 2019. Selection for growth is associated in gilthead sea bream (*Sparus aurata*) with diet flexibility, changes in growth patterns and higher intestine plasticity. *Aquaculture*, 507:349–360.

Piazzon M.C., Naya-Català F., Perera E., Palenzuela O., Sitjà-Bobadilla A., Pérez-Sánchez J. 2020. Genetic selection for growth drives differences in intestinal microbiota composition and parasite disease resistance in gilthead sea bream. *Microbiome*, 8:168.

Acknowledgements: AquaIMPACT (H2020 #818367); Bream-AquaINTECH (RTI2018–094128-B-I00); RYC2018-024049-I/AEI/10.13039/501100011033.

CAN COMMON CARP (*Cyprinus carpio*) BE ACUTELY STRESSED ?

Constanze Pietsch*

School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, 3052 Zollikofen, Switzerland
constanze.pietsch@bfh.ch

The rearing of fish in aquaculture often includes exposure of the fish to acute stress as well as chronic stress. Distress interferes with appetite and well-being of an animal, but also eustress leads to increased activity and therefore is classified as a stressor. Although carp are often assumed to be robust fish showing a high stress tolerance, the question was if there are markers that indicate that carp have previously been exposed to acute stressors. In addition, fish obviously are thought of being capable of distinguishing different stressors in order to react appropriately to environmental changes. For persons working with fish, it is essential to identify the stress levels of the reared fish and especially negative effects of stress effects should be avoided. Consequently, it is a pre-requisite to better understand the stress responses of fish. The presentation will therefore summarize the factors influencing carp welfare and will reveal effects of different acute stressors on the subsequent stress responses that can be observed within the fish body.

IMPROVING MICRODIET FORMULATIONS FOR LARVAE OF TWO FLATFISH SPECIES: SENEGALESE SOLE AND TURBOT

Wilson Pinto[a*], Maria Morais[a], Inês Quintino[a], Sofia Engrola[b] and Luís E.C. Conceição[a]

[a]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[b]CCMAR, Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal

*E-mail: wilsonpinto@sparos.pt

Introduction

Flatfish species, such as Senegalese sole (*Solea senegalensis*) and turbot (*Scophthalmus maximus*) are successful niche species in European aquaculture, due to their high quality white flesh and high market value. However, these species face different scenarios in husbandry during the early developmental stages: while in Senegalese sole a significant R&D effort performed in weaning procedures during the last two decades has translated into an evolution in feeding regimes, microdiet quality and optimization of larval zootechnical procedures (Pinto et al., 2018), published effort on turbot early life-stages feeding and nutrition is very limited. Currently, under normal rearing conditions, Senegalese sole larval survival at the end of two months of development may reach 60-70 %, whereas in turbot lower values are commonly reached (20–30%). One of the hypotheses for this limitation in turbot survival during the early development stages is the lack of microdiets tailored to its nutritional requirements and feeding behaviour. To this end, this study aims at demonstrating the impact of microdiet formulation evolution in Senegalese sole larval husbandry, as well as demonstrating the first steps in the creation of tailored diets for turbot larvae.

Materials and methods

In Senegalese sole, a trial for specific microdiet development aimed at establishing its dietary preference for a main protein source. To this end, four microdiets were formulated and produced by cold-extrusion, comprising of the following main protein sources: fishmeal (Fish), squidmeal (Squid), mixture of plant-proteins (Veg) and mixture of marine and plant-based ingredients (Mix). Sole were reared under standard procedures in triplicate tanks and fed on these experimental diets from 28 to 53 DAH. Diets were supplied to the tanks using automatic feeders, set to supply 8 meals in a 24 hour period (two hour feeding and one hour of stoppage time). In turbot, a trial aiming at determining its dietary preference for a main protein source was also conducted. In this case, four microdiets were also produced by cold-extrusion and the following dietary treatments were considered: Fish, Squid, krillmeal (Krill) and Mix. Turbot post-larvae were reared under similar zootechnical procedures described for Senegalese sole from 23 to 49 DAH. At the end of both trials fish were analysed for dry weight, survival, relative growth rate (RGR) and feed conversion ratio (turbot only).

Results

At the end of the sole experiment, no significant differences were observed in larval dry weight, RGR (varying from 6.3 to 8.6 % per day) and survival (Figure 1) when post-larvae were fed diets Fish, Squid, Veg and Mix. Conversely, diet Squid negatively affected turbot dry weight, survival (Figure 1) and feed conversion ratio (0.7-1.1), with post-larvae dealing positively with diets Fish, Krill and Mix. No significant differences were found between treatments for larval relative growth rate during the course of the experiment (varying between treatments between 14.5 and 15.1 % day^{-1}).

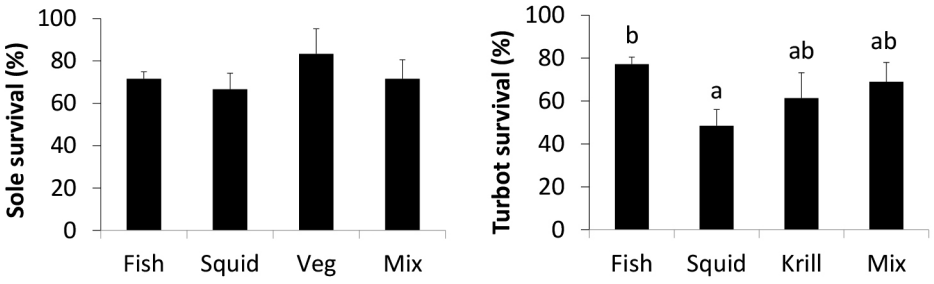


Figure 1. Survival of Senegalese sole (left) and turbot (right) fed microdiets containing different main protein sources. Fish: fishmeal; Squid: squidmeal; Veg: Mixture of plant protein sources; Mix: mixture of marine and plant-based proteins; Krill: krill meal.

(Continued on next page)

Discussion

In general terms, Senegalese sole and turbot post-larvae displayed different responses to microdiets containing different protein sources. Senegalese sole post-larvae were more eclectic, not showing a clear preference between diets containing marine or plant-based proteins. The relative growth rate (RGR; up to 8.6 % day^{-1}) reached for the different treatments was satisfactory, but continuous research focusing on the impact of different nutritional compositions, including protein complexity (Canada et al., 2017), indispensable amino acid profile (Canada et al., 2016), dietary lipid levels and essential fatty acid composition (Pinto et al., 2016), have boosted Senegalese sole growth performance, nowadays reaching RGR values of 12-13 % day^{-1} during weaning. On the other hand, turbot larvae performed best on diets containing fishmeal, krill and a mixture of marine and plant-based proteins, with a clear detrimental effect on larval performance being observed in fish fed a high quality squid-meal based diet.

Interestingly, both flatfish species also present opposite feeding behaviours: while Senegalese sole is more passive and feeds in the bottom of the tanks, turbot is more active and feeding occurs at water surface (Bruno et al., 2018). Such comparison of feeding behaviour, together with diet preference results herein obtained, suggest that microdiets suitable for Senegalese sole may not be optimised for turbot, both in terms of nutrition and, particularly, in terms of microdiet physical properties in water (dispersion at surface and sinking behaviour). Overall, results support that diets should be optimised in terms of nutritional composition and physical behaviour in water to boost turbot growth and survival at early developmental stages. Moreover, this work supports the concept that dietary customisation for marine fish larvae of different species will contribute to the production of high quality juveniles in aquaculture.

References

Bruno, E., Højgaard, J.K., Hansen, B.W., Munk, P., Støttrup, J.G., 2018. Influence of swimming behavior of copepod nauplii on feeding of larval turbot (*Scophthalmus maximus*). Aquaculture International 26, 225-236.

Canada, P., Engrola, S., Richard, N., Lopes, A.F., Pinto, W., Valente, L.M.P., Conceição, L.E.C., 2016. Dietary indispensable amino acids profile affects protein utilization and growth of Senegalese sole larvae. Fish Physiology and Biochemistry 42, 1493-1508.

Canada, P., Conceição, L.E.C., Mira, S., Teodósio, R., Fernandes, J.M.O., Barrios, C., Millán, F., Pedroche, J., Valente, L.M.P., Engrola, S., 2017. Dietary protein complexity modulates growth, protein utilisation and the expression of protein digestion-related genes in Senegalese sole larvae. Aquaculture 479, 273-284.

Pinto, W., Engrola, S., Santos, A., Bandarra, N.M., Dias, J., Conceição, L.E.C., 2016. Can Senegalese sole post-larvae effectively grow on low dietary DHA and lipid levels during weaning? Aquaculture 463, 234-240.

Pinto, W., Engrola, S., Conceição, L.E.C., 2018. Towards an early weaning in Senegalese sole: A historical review. Aquaculture 496, 1-9.

Acknowledgements

This project has received funding from the Ministry of the Sea, Portuguese Republic, through the program Blue Growth, Innovation and SME, under reference PT-INNOVATION-0025.

FEEDING FISH THROUGH CIRCULAR BIOECONOMY: MICROALGAE AS AQUAFEED INGREDIENT CULTURED IN HYDROPONIC EFFLUENTS

João Pires[1*], Inês B. Maia [1,2,3], Patrícia Diogo[1], Alexandre Rodrigues[1], Peter Schulze[4], Victória del Pino[1], Hugo Pereira[4] and João Navalho[1]

[1] Necton SA, Olhão, Portugal
[2] Centre of Marine Sciences, University of Algarve, Faro, Portugal
[3] Faculty of Sciences and Technology, University of Algarve, Faro, Portugal
[4] GreenCoLab, University of Algarve, Faro, Portugal
* Correspondence: joao.pires@necton.pt

Microalgae present a high potential for application as an ingredient for aquafeeds, due to their high-quality biomass, rich in proteins and long chain fatty acids. In addition, microalgae are also a more sustainable source than other traditional feed ingredients obtained by unsustainable sources, such as fisheries. However, improvement of industrial microalgae production is crucial in the upcoming years towards to increase sustainability and reduce production costs. This is essential for the application of microalgae as a whole aquafeed ingredient[1]. An interesting approach to reduce microalgae production costs is the use of effluents instead of fresh or seawater, which complements wastewater treatment procedures. Hydroponic cultures have nitrate contents that are generally above the legislation permits and are difficult to dispose of safely in the environment, resulting in a public health issue. The ALGACYCLE project aims to re-apply the nutrients from greenhouse hydroponic effluents, improving water quality, and to develop two microalgal products, thus promoting circular bioeconomy. The resulting microalgal biomass will be tested for application in the aquaculture and agriculture sector, in the form of biostimulants and aquafeeds, the latter to be used in the feeding of salmon *(Salmo salar*). In 15 European countries, the percentage of the population exposed to nitrate levels in drinking-water above 50 mg/L that promote nitrate toxicity problems, is estimated to be consumed by 10 million people, being a public health issue. Hydroponic cultures effluents have nitrate contents that are generally above the legislation permits and are difficult to dispose safely in the environment. These nutrients are being wasted and can be re-used to produce high quality biomass and facilitating the agricultural waste disposal. The vectorization of the nitrates for microalgae production would support the sequestration of a health and environmental harmful component (nitrates) in a valuable biomass, to be further applied in the production of aquaculture fish. Two species with tolerance to high nutrient concentrations and a high potential for aquaculture nutrition are being tested, namely *Scenedesmus* sp. and *Koliella antarctica*. Altogether, this strategy will contribute to the sustainability of microalgae industrial production while reducing production costs and contributing to the Blue Growth sector.

References

1. Yarnold, J., Karan, H., Oey, M. & Hankamer, B. Microalgal Aquafeeds As Part of a Circular Bioeconomy. *Trends Plant Sci*. **24**, 959–970 (2019).

Acknowledgements

Work funded by ALGACYCLE - PT-INNOVATION-0023, EEA GRANT

EFFECT OF TILAPIA *Oreochromis niloticus* ON SUSPENDED SOLIDS CONSUMPTION WHEN INTEGRATED WITH *Litopenaeus vannamei* IN BIOFLOC SYSTEM

L. Poersch, M. Holanda, A. Cardoso, G. Lara, P. Furtado, G. Santana and W. Wasielesky

Federal University of Rio Grande – FURG – Institute of Oceanography – Rua do Hotel, 02. CEP 96210-030, Rio Grande, RS, Brazil
E-mail: lpoersch@gmail.com

Introduction

Total suspended solids (TSS) increase during the shrimp production in BFT system, which can affect water quality and animal performance. To control the excess of TSS during the shrimp production in the BFT system can be used mechanical filters (settling tanks) or the alternative integration with species that act in different trophic levels – Integrated Multitrophic Aquaculture (IMTA). The present study aimed to evaluate two stocking densities of tilapia in integrated culture with the shrimp *Litopenaeus vannamei* in biofloc system on a pilot scale.

Material and Methods

Two stocking densities of tilapia were tested, 35 and 65 fish m^{-3} in a recirculating system with 18 m^3 tanks for shrimp culture and 4 m^3 for tilapia culture with recirculation of 965.66 ± 92.83 L h^{-1} during 78 days. The initial weight of the shrimp was 0.9±0.1 g and of the tilapia was 7.1±3.2 g. The shrimp received the amount of feed according to the feeding table and the fish were underfed to stimulate them to consume the bioflocs.

Results

Tilapia densities did not affect shrimp growth (11.5±1.9 g for treatment with 35 fish m^{-3} and 10.1±0.7 g for treatment with 65 fish m^{-3}). The tilapia presented a FCR less than 1, proving the consumption of the bioflocs by the fish. The clarification time was shorter when compared to other studies with shrimp monoculture and among the treatments, where there was lower fish stocking density, there was a reduction of 10 hours in the system clarification when compared to the treatment with higher fish stocking density. The results demonstrate the feasibility of integrated shrimp and tilapia culture on a pilot scale, without compromising shrimp productivity.

RECENT CARP EDEMA VIRUS ISOLATES FROM CZECHIA ARE HIGHLY SIMILAR TO ISOLATES FROM CONTINENTAL EUROPE

L. Pojezdal[1]*, M. Palikova[2,3], H. Minarova[1,2], J. Motlova[1], K. Matejickova[1], V. Piackova[4]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]University of Veterinary Sciences Brno, Czech Republic
[3]Mendel University in Brno, Czech Republic
[4]University of South Bohemia in České Budějovice, Czech Republic
E-mail: pojezdal@vri.cz

Introduction

With a stable annual production of around 20 000 tonnes, common carp *(Cyprinus carpio)* is the most valuable aquaculture species in the Czech Republic. Emerging diseases, such as the carp edema virus disease (CEVD) detected in the country since 2013 (Matejickova et al. 2020) pose a threat to the industry. Molecular epidemiology, in the form of sequencing of isolates, can help understand the distribution of the virus in the country.

Material and Methods

In total, 637 samples representing 124 locations (individual farms or ponds) were examined in the year 2020 for the presence of the virus using conventional and real-time PCR (Way et al, 2017). Partial P4a gene sequences (357 bp) of detected CEV isolates were obtained via Sanger sequencing and compared to isolated uploaded to the GenBank database.

Results

CEV was diagnosed in six locations, namely four common carp farms and two koi carp ponds. Clinical signs in farmed common carp varied from asymptomatic to a 100% mortality rate in one of the facilities, meanwhile both positive koi in ponds showed sleepy-disease-like symptoms. Phylogenetically, two of the established CEV genogroups were detected: Genogroup I in common carp and genogroup IIa in koi. The isolates from common carp showed up to 100% identity with previously published sequences from Czechia (Matejickova et al. 2020), Germany (Adamek et al. 2017), Hungary (Adamek et al. 2018) and Great Britain (Way et al. 2017).

Conclusions

High levels of similarity in the routinely sequenced partial P4a gene of carp edema virus suggest wide dissemination of the pathogen and possible transmission of specific isolates, most probably via the transport of live fish, ornamental or farmed.

Funding

This research was supported by the Ministry of Agriculture of the Czech Republic project NAZV QK1710114 and by the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869. The project is financed by the European Regional Development Fund in the operational programme VVV MŠMT.

References

Adamek M, Matras M, Jung-Schroers V, Teitge F, Heling M, Bergmann SM, Reichert M, Way K, Stone DM, Steinhagen D. 2017. Comparison of PCR methods for the detection of genetic variants of carp edema virus. Dis Aquat Organ. (2017) 126(1):75-81.

Adamek M, Baska F, Vincze B, Steinhagen D. 2018. Carp edema virus from three genogroups is present in common carp in Hungary. J Fish Dis. (2018) 41:463–8.

Matejickova K, Pojezdal L, Pokorova D, Reschova S, Piacková V, Palíkova M, et al. Carp oedema virus disease outbreaks in Czech and Slovak aquaculture. J Fish Dis. (2020) 43:971–8.

Matras M, Borzym E, Stone D, Way K, Stachnik M, Maj-Paluch J, et al. Carp edema virus in Polish aquaculture - evidence of significant sequence divergence and a new lineage in common carp Cyprinus carpio (L.). J Fish Dis. (2017) 40:319–25.

Way K, Haenen O, Stone D, Adamek M Bergmann SM, Bigarre L, et al. 2017. Emergence of carp edema virus (CEV) and its significance to European common carp and koi Cyprinus carpio. Dis Aquat Org. (2017) 126:155–66.

PROMOTING THE USE OF MICROALGAE BIOMASSES IN GILTHEAD SEABREAM FEEDS

T.V. Poletto*[1]; M. Sardinha[1 β·], A. Fernandes,[1,4], L.E.C. Conceição[1], R. Colen[2], S. Engrola[2], P. Pestana[3], J. Dias[1]

[1]SPAROS Lda., Olhão (Portugal)
[2]Centre of Marine Sciences (CCMAR), 8005-139 Faro (Portugal)
[3]NatureXtracts S.A. Caniçal, Madeira (Portugal)
[4]Faculty of Biosciences and Aquaculture, Nord University, Bodø (Norway)
[β]Current affiliation: Biomar AS, Global RD department, Havnegata 9 Trondheim, Norway
Email: tatianapoletto@sparos.pt

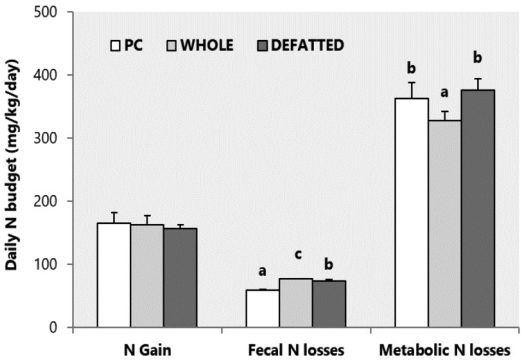
Introduction

Being an emergent industry, microalgae biomasses cannot yet be used as commodity ingredients in current aquafeeds. However, the future presents new consumer-driven challenges that are shifting the focus away from cost minimization production strategies, towards enhanced consumer confidence and product value. Microalgae, being a rich source of protein, n-3 LC-PUFAs, such as EPA and DHA, vitamins, trace minerals, carotenoids and antioxidants, may contribute towards a more sustainable and nature-like image of aquafeeds. Major criteria driving the industrial use of such novel and alternative ingredients are their nutritional profile, cost-effectiveness and market availability. In a foreseeable microalgae biorefinery scenario, the downstream processing entailing the extraction of specialty high value products, such as LC-PUFA rich oils and other liposoluble compounds, will generate large quantities of defatted biomasses, which need to be assessed as raw materials in aquafeeds. A study was undertaken to assess the potential of whole and defatted *Nannochloropsis oceanica* biomass in alternative feeds for gilthead seabream.

Methods

The trial comprised 3 dietary treatments: a control diet (CTRL), mimicking a commercial formulation containing 17.5% fishmeal, 2.5% fish protein hydrolysate, 10% poultry meal and several vegetable ingredients (wheat gluten, corn gluten, soybean meal) as major protein sources; and two alternative diets with lower levels of fishmeal (10%), higher levels of PAPs (poultry meal, feather meal hydrolysate, blood meal), soy-free and EU-derived rapeseed and sunflower meals, as major protein sources. These two diets comprised also the incorporation of microalgae (*Nannochloropsis oceanica*) at either 2% for the whole biomass (WHOLE) or 4% for the defatted biomass (DEFATTED). The defatting process of the algae biomass comprised cell disruption by bead milling followed by a supercritical fluid extraction (SFE). A blend of fish and rapeseed oils was used as main lipid source. In the algae-containing diets, due to the reduction of fishmeal, a minor addition of algae oil (derived from *Schizochytrium spp*.) was done to guarantee an identical dietary level of EPA+DHA among the feeds. Diets were isonitrogenous (CP: 49% DM), isolipidic (CF: 19% lipid) and isoenergetic (GE: 19.4 MJ/kg DM).

Homogenous groups of 26 seabream (IBW: 194 g) were stocked in 1000 L tanks. Each experimental treatment was tested in quadruplicate tanks over 89 days. Rearing tanks were supplied with flow-through gravel-filtered, aerated seawater (salinity: 36 ppt; temperature: 22.7 ± 2.5°C; dissolved oxygen > 5.8 mg·L^{-1}) and subjected to natural photoperiod during summer conditions. Additionally, at the end of the trial the apparent digestibility of protein and energy of the various diets was measured by the indirect method in tanks equipped with a fecal decantation column.



(Continued on next page)

Results and discussion

After 89 days of experimental feeding, no significant differences in survival, weight gain (FBW, SGR) or feed utilization (FCR, PER) were observed among treatments ($P>0.05$). No differences were found on the whole-body composition of fish in terms of moisture, ash, protein and energy ($P>0.05$), whereas fish fed the DEFATTED diet showed a significantly lower fat content than those fed the CTRL diet ($P<0.05$). In line, fish fed the DEFATTED diet showed a significantly lower whole-body lipid retention than those fed the CTRL diet ($P<0.05$). In comparison to the CTRL diet, both WHOLE and DEFFATED diets resulted in a significantly lower digestibility of protein and energy ($P<0.05$).

Dietary treatments had no impact on the daily N gain ($P>0.05$). However, in association to a lower protein digestibility, fish fed diets WHOLE and DEFATTED showed significantly higher daily fecal N losses than those fed the CTRL diet ($P<0.05$). Although associated to the highest fecal N losses, the WHOLE diet showed also a significant reduction of daily metabolic N losses ($P<0.05$)

Fish derived from the various treatments were assessed by a consumer panel (n=100). This consumer acceptance test, performed by SenseTest Lda (Portugal), evaluated not only the external appearance of fish, but also sensory properties (odour, flavour, texture) in cooked fillets. In a scale of 1 to 9, with 9 representing a highly liked attribute, average scores for the various criteria ranged between 7.7 and 7.8, without a statistical differentiation among treatments ($P>0.05$).

Conclusions

Data from this study indicates that microalgae (*Nannochloropsis oceanica*) presented as a whole or defatted biomass can be useful ingredients in alternative feeds for gilthead seabream. However, further attention should be paid to aspects related to the digestibility of the whole algae biomass to avoid a potential increase of fecal nutrient losses. Additionally, the evaluation of microalgae in aquafeeds should also assess its potential benefits on the immune status of fish and comprise an estimation of environmental impact using Life Cycle Assessment (LCA) criteria.

Acknowledgements

This work has received funding from the Bio Based Industries Joint Undertaking (BBI JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 745754 (project MAGNIFICENT). This output reflects the views only of the author(s), and the European Union and BBI JU cannot be held responsible for any use which may be made of the information contained therein. This project has also received funding from the Portuguese Foundation for Science and Technology through project UIDB/04326/2020 to CCMAR.

EFFECT OF A DIETARY BACTERIA AND YEAST BASED PARAPROBIOTICS AND THEIR COMBINATION UPON THE MUCOSAL BARRIER DEFENCES AND IMMUNITY OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

Pontefract, N.[a]*, Rawling, M.[a], Leclercq, E.[b], Castex M.[b], Merrifield, D.[a]

[a] Plymouth University, Drake Circus, PL4 8AA, UK
[b] Lallemand SAS, 19 rue des Briquetiers, 31700, Blagnac, France
*nicola.pontefract@plymouth.ac.uk

Introduction
The importance of the mucosal-associated lymphoid tissues (MALTs) present in the gut, gills and skin (GALT, GIALT and SALT respectively) of fish is well established. Due to their direct contact with the external environment, MALTs are one of the primary barriers to pathogens. Recently, the potential interconnectivity of the MALTs is receiving increased attention, particularly with regard to how feed additives may exert a beneficial influence upon the skin and gill mucosa. This study assessed the effect of two Paraprobiotics on rainbow trout mucosal responses: a multi-strain yeast fraction product (MsYF; Rawling et al., 2021) and a bacteria paraprobiotic compound (Para; heat-killed bacteria), tested alone or in combination.

Materials and methods
An 8-week feeding trial was conducted on rainbow trout (BW_i= 36.7 ± 0.5g) at the Aquaculture and Fish Nutrition Research Facilities of the University of Plymouth (UK). Four iso-nitrogenous and iso-lipidic diets were manufactured and randomly assigned to tanks in triplicate: 1] Control, 2] MsYF (1.5 kg/t), 3] Para (0.3 kg/t), 4] Combination (Comb: MsYF: 1.5kg/t + Para: 0.3kg/t). The functional ingredients were provided by Lallemand SAS (France). Skin, gill and distal intestine were sampled for histological analysis and qPCR gene expression at two successive time-points (5 and 8 weeks).

Results
Compared to the control group, fish fed the supplemented diets exhibited significantly increased goblet cell area fractions (GCAF) in the skin epidermis at both 5 and 8 weeks (Fig 1). No diet effect on the goblet cell population was observed in the gill (not shown).

Upregulation of a number of genes associated with mucin production and tight junction permeability were observed in the GIALT (*Mucin-17-like, Claudin 10d, 10e* and *12*; Fig 1A) and the SALT (*Calreticulin, claudin 30* and *Mucin-17-like;* Fig 1B). Further, several genes associated with Th2-type cytokines (*IL4/13a, TGFβ*) were affected in the GIALT (Fig 1A) and GALT (Fig 1C) at week 5 and 8, suggesting that the presentation of different microbial associated molecular patterns within the MsYF and bacterial parabiotic show the potential to initiate specific cell-mediated immunity in these tissues.

Discussion
This study indicates the potential of the dietary additives to distinctly influence the mucosal barrier defences of the gill, skin and gut at the inclusion levels tested. Significant increases in the expression of several immune-related genes were observed in the GIALT and GALT, along-with significantly elevated expression of tight junction (TJ)- and mucin-related genes within the GIALT, SALT and GALT. These were associated with increased goblet cell area fraction in the skin epidermis.

Conclusion
These results support the proposed interconnectivity of the MALT and suggest distinct transcriptomic responses associated to the dietary application of different Paraprobiotics and their combination. Further research is required to investigate implications in terms of fish phenotypic responses under different situations.

References
Rawling, M.; Leclercq, E.; Foey, A., Castex, M., Merrifield, D., 2021. A novel dietary multi-strain yeast fraction modulates intestinal toll-like-receptor signalling and mucosal responses of rainbow trout (*Oncorhynchus mykiss*). PLoS One 16, e0245021.

(Continued on next page)

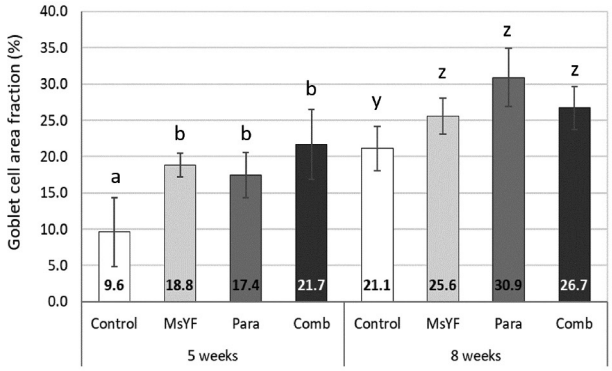
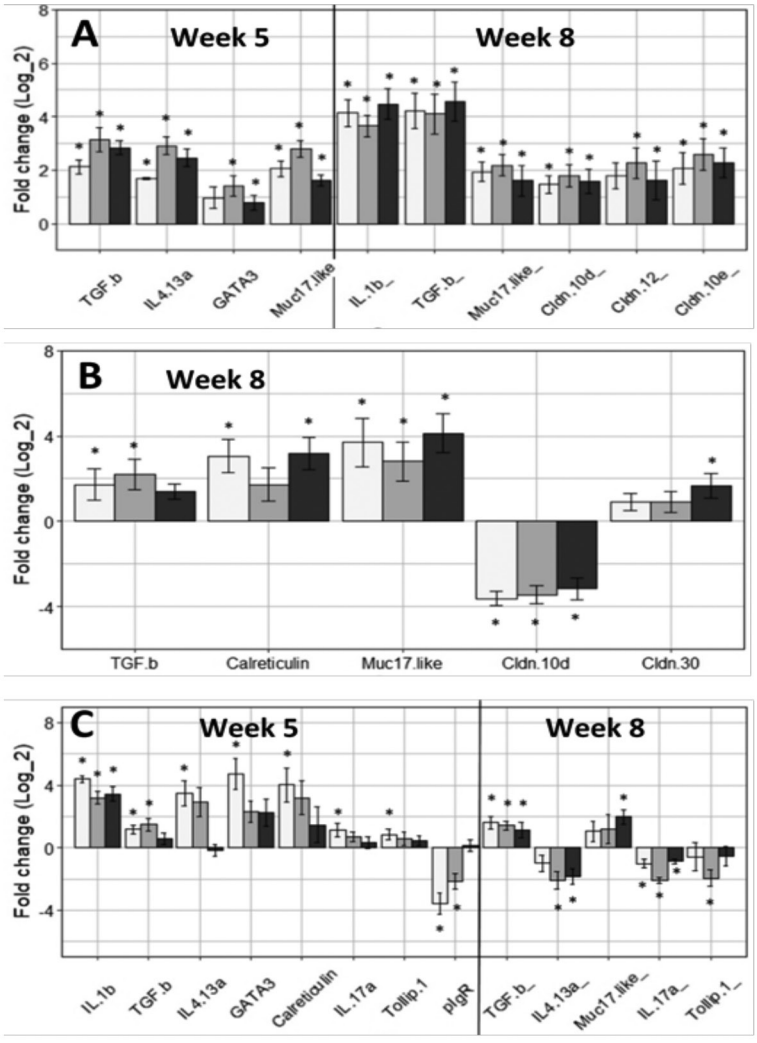


Figure 1. GCAF in the skin after 5 (n=6) and 8 weeks (n=12). Mean ± SD. Different letters within the same time-point denote significant differences between treatments (p<0.05).

Figure 2. Targeted gene expression in A) the gill, B) skin and C) gut. Mean ± SEM. * denote a significant difference between the test and control groups (p<0.05).



EXTREME EVENTS AND LAGOON FARMS: THE INFLUENCE OF DRASTIC CHANGES IN SALINITY ON OYSTER GROWTH

E.M.D. Porporato[1*], D. Brigolin[2], C. Bertolini[3], R. Pastres[3], M. Baroli[1], P. Graham[1], G. Brundu[1]

[1]IMC—International Marine Centre, Loc. Sa Mardini, 09170 Oristano, Italy
[2]Dipartimento di Culture del Progetto, Università IUAV di Venezia, 30135 Venezia, Italy
[3]Dipartimento di Scienze Ambientali, Informatica e Statistica, Università Ca' Foscari di Venezia, 30172 Venezia, Italy
* e.porporato@fondazioneimc.it; erika.porporato@gmail.com

Introduction

Coastal ponds and lagoons are among the most productive ecosystems in the world and play a fundamental role in providing different ecosystem services, including food provision. In Italy most of the oyster farms are located in Sardinia, where *Crassostrea gigas* is mainly farmed in lagoons and coastal ponds (Graham et al., 2019). These areas are subjected to extreme and rapid perturbations, such as freshwater intrusion, leading to marked seasonal salinity fluctuations.

The present study was carried out in S'Ena Arrubia, a small and shallow lagoon located in the centre-western coast of Sardinia, which borders the Arborea plain, a natural depression which is part of the coastal flood plain, reclaimed at the beginning of the 1900s. In this area, freshwater input is supplied from two affluent rivers, "Rio Sant'Anna" and "Canale delle acque basse", the latter is a channel below the sea level, where a dewatering pump is located. From September to December 2018 an experimental oyster farm was positioned in the sea-mouth of S'Ena Arrubia lagoon to evaluate the effects of two different gears (*i.e.*: Ortac and floating bag) on the oyster's quality aspect (see Brundu et al., 2020). In November 2018, during this experimental rearing farm an exceptional rainy event took place causing a marked drop in salinity in the lagoon.

In this preliminary study we evaluated the potential oyster growth in S'Ena Arrubia lagoon applying a Dynamic Energy Budget (DEB) model, secondly we implemented the model including salinity as a forcing variable. The aim of this study is to: (i) evaluate the potential oyster growth in a Sardinian lagoon through a DEB model application; (ii) include salinity in the DEB model; (iii) compare the model outputs considering and not considering the salinity.

Methods

We collected data on *C. gigas* growth through an experimental field study, in order to parameterise, and subsequently, validate the DEB model. Contextually, temperature, salinity, and chlorophyll-a data were collected in the experimental area and used as environmental forcing variables for the model. The individual DEB model implemented in this study was originally developed by Pouvreau et al. (2006) and further improved by Lavaud et al. (2017) to account for the salinity effects. The parameters used in the model are based on previously published papers (Pouvreau et al., 2006; Bertolini et al., 2021).

Based on the results obtained for *C. virginica* (Lavaud et al., 2017), we considered the main impact of salinity modification on the filtration rate, due to a reduced or stopped oyster feeding at low salinity. In order to simulate this effect, a linear correction factor on filtration rate was applied (Lavaud et al., 2017):

$$C_S = \begin{cases} 1, at\ S \geq S_H \\ \dfrac{S - S_L}{S_H - S_L}, at\ S_L < S < S_H \\ 0, \quad at\ S \leq S_L \end{cases}$$

where, based on literature values, SL and SH (low and high salinity threshold) are fixed at 13 and 25 respectively (Graham et al. 2020), and S is the registered salinity value. We ran two DEB models, considering and not considering the salinity correction on filtration rate. Finally, we evaluated the model outputs comparing the predicted and observed values.

(Continued on next page)

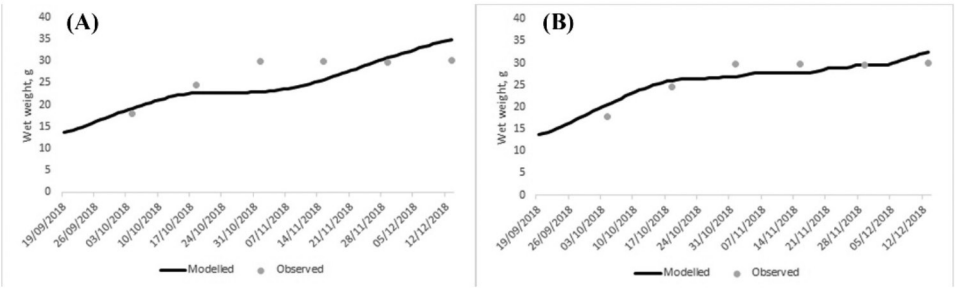


Figure 1. Model validation: predicted and observed values for the total mean wet weight of oysters without (A) and with (B) salinity correction.

Results and discussion

The two DEB model outputs, without and with the salinity correction, are represented in Figure 1 (A and B respectively). In general, the prediction for oyster growth in terms of wet weight provided a good agreement for both models, with a more accurate prediction for the model considering the salinity correction. The visual impression provided is confirmed by the results obtained from the comparison of predicted and observed values. Indeed, the linear regression of predicted versus observed values of oyster mean wet weights gave a determination coefficient $R^2 = 0.83$ considering the salinity correction, and $R^2 = 0.49$ without considering the salinity effect.

This preliminary application highlights how the implementation of salinity within a DEB model can improve the growth prediction. In recent years intense rain events have become more frequent, and are expected to increase due to climate change, inducing consequent pressures on lagoons and coastal ponds. In this context, being able to predict oyster growth applying well established models including the salinity as an environmental forcing could drive to more reliable results, which can contribute to assess the suitability of the different lagoons for aquaculture use. Hence, these results could also be meaningful for the site selection process within the Allocated Zone for Aquaculture identification process, currently ongoing in the Sardinia region.

References

Bertolini, C., Brigolin, D., Porporato, E.M.D., Hattab, J., Pastres, R., & Tiscar, P. G. (2021). Testing a Model of Pacific Oysters' (*Crassostrea gigas*) Growth in the Adriatic Sea: Implications for Aquaculture Spatial Planning. Sustainability, 13(6), 3309.

Brundu, G., Loi, B., Chindris, A., Graham, P., Bernabè, D., Giménez Papiol, G. (2020). Rearing equipment on the quality aspects and growth performance of *Crassostrea gigas* (Thunberg, 1793): Implications for marketability. Aquaculture Research, 51(7), 2936–2947.

Graham, P., Brundu, G., Scolamacchia, M., Giglioli, A., Addis, P., Artioli, Y., Telfer, T., Carboni, S. (2019). Improving pacific oyster (*Crassostrea gigas*, Thunberg, 1793) production in Mediterranean coastal lagoons: validation of the growth model "ShellSIM" on traditional and novel farming methods. Aquaculture, 516, 734612.

Lavaud, R., La Peyre, M. K., Casas, S. M., Bacher, C., La Peyre, J. F. (2017). Integrating the effects of salinity on the physiology of the eastern oyster, *Crassostrea virginica*, in the northern Gulf of Mexico through a Dynamic Energy Budget model. Ecological Modelling, 363, 221-233.

Pouvreau, S., Bourles, Y., Lefebvre, S., Gangnery, A., Alunno-Bruscia, M., 2006.Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. Journal of Sea Research, 56 (2), 156–167.

GALT LEUCOCYTES AS A HEALTH SCREEN FOR FUNCTIONAL FEEDS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

D. Porter[1], R. Heavyside[2], D. Peggs[2], C. McGurk[2], S.A.M. Martin[1]

[1]Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Aberdeen AB24, 2TZ
[2]Skretting ARC, Sjøhagen 15, 4016 Stavanger, Norway

Email: r01dp19@abdn.ac.uk

Introduction

The advances of functional feeds for farmed fish are now regarded as a key factor in improving fish health and performance. Although there has been significant research on the impact of functional feeds on the immune system, the mechanisms by which these occur is still poorly understood, as such there is a need for further research into the nutrition health interface for aquaculture species. As part of our research to examine direct immune responses to functional feed ingredients, cell lines and primary cell cultures are being used as an alternative method which also addresses 3Rs in reducing whole animal experiments. Cell cultures offer a quick and non-invasive method of identifying immune responses, however, the number of cell lines available is limited and the phenotype of these cells is likely to have changed from their original *in-vivo* state, especially in the intestine with the complex nature and multiple cell types present. Gut associated lymphoid tissues (GALT) leucocytes have been extracted and have shown to be a good screen to PAMPs in previous studies by Attaya et al, (2018) but their role as a viable alternative to traditional feeding trial methods is yet to be seen.

The present study was undertaken to identify the suitability of both cell culture and primary GALT leucocyte models as potential alternative methods to test functional feed ingredients on. In addition to the primary culture cells, a rainbow trout macrophage cell line (RTS11) and a rainbow trout gut epithelial cell line (RTgutGC), were used as the cell line models (Ganassin and Bols, 1998) (Kawano et al., 2011). The cells were challenged with a variety of PAMPs and multiple types of the prebiotic β glucan to test their suitability as a health screen for functional feeds.

Materials and Methods

RTgutGC cells were cultured in flasks at 20oC in growth media (Leibovitz L-15 media + 10% FBS + 1% Penicillin/Streptomycin). RTS11 were cultured in flasks at 20oC in growth media (Leibovitz L-15 media + 30% FBS + 1% Penicillin/Streptomycin). Fish for GALT leucocytes extraction were maintained in 1 m-diameter fiberglass tanks with recirculating freshwater at 14 °C and fed twice a day with a commercial diet at 1.5% bodyweight. Fish were sampled at the same time of day on each occasion used. GALT leucocytes were extracted from the distal intestine using the method previously described by Attaya et al, 2018 with some alterations: cells were washed in PBS containing 1% Penicillin/Streptomycin, cells were digested for a singular two-hour digestion. GALT leucocytes, RTgutGC and RTS11 cells were added to a 24 well plate at a concentration of 2.5x105 cells/well and resuspended in 1ml total of stimulation media (Leibovitz L-15 media + 1% FBS + 1% Penicilin/Streptomycin). Cells were stimulated with 100μg/ml Poly IC, 10μg/ml PHA, 20μg/ml rIL-1β (Provided by Dr. Tiehui Wang (Scottish Fish Immunology Research Centre)), and β-glucans 1 and 3 100μg/ml (provided by Skretting Ltd) for 4 hours before RNA extraction. Following cell stimulation cells were lysed directly in TRIzol and stored at 80oC. RNA was then extracted following the manufacturer's instructions Reverse Transcription was completed using the Quantitect® Reverse Transcription Kit (Qiagen) following the manufacturer's instructions. Primers for each gene were designed using NCBI primer blast or were identified in published papers and then checked using Primer-Blast. Primers were designed with at least one primer across an exon-exon junction to ensure only the cDNA was being amplified. qPCR on the cDNA was carried out using the Lightcycler 480 machine (Roche). Relative gene expression was calculated using GenEx which included the use of three housekeeping genes for normalization.

Results

Stimulations with Poly IC (Figure 1) show a significant upregulation in both MX and IFN1α for RTS11 at all time points, RTgut and GALT leucocytes. The results also show that RTgut are more sensitive to Poly IC stimulation at the 4hr time point compared to the GALT leucocytes and RTS11 cell lines. The RTS11 and GALT Leucocytes show similar responses to Poly IC.

(Continued on next page)

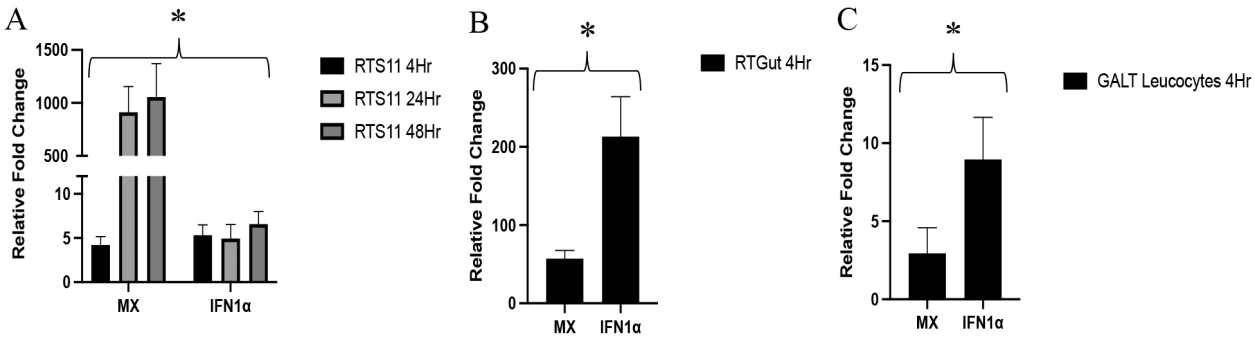


*Figure 1 - RTS11, RTguGCt and GALT leucocyte response to Poly IC. A – RTS11 response to Poly IC at 4hr, 24hr and 48hr time points. B – RTgutGC Immune response to Poly IC at 4hrs. C – GALT leucocyte Immune Response to Poly IC at 4hrs. * = P<0.05*

Discussion

The results of this study suggest that both cell lines and GALT leucocytes provide a novel insight into the immune response to functional feeds. The results show that whilst both the RTS11 and RTgutGC cell lines do respond to both PAMPs and β-glucans their immune responses are similar but not the same as primary cell cultures, primarily since there is only one cell type rather than multiple cell types that would be seen in-vivo. This suggests that the cell lines are a beneficial precursor to feeding trials if they are used to screen novel feed additives prior to the feeding trial. The GALT leucocytes are a good alternative to identify the immune response of the gut directly rather than looking at the systemic effects. The results from the GALT leucocyte stimulations show that stimulation with the PAMPs; Poly IC, PHA, rIL-1β show expected responses to target genes. Poly IC showed significant upregulation in MX and IFN1α compared to the control in RTS11, RTgut and GALT leucocytes. MX encodes for a guanosine triphosphate (GTP)-metabolizing protein that participates in the cellular antiviral response which is induced by Type I and II interferons such as IFN1α; an antiviral cytokine that is released as a key part of the innate immune response to viral infections. Other stimulants including the T cell mitogen PHA and proinflammatory cytokine Il-1β were also examined in the model systems which also showed expected immune modulation to PAMPs. B-glucan stimulated results show similar responses to published data (Douxfils et al, 2017, Ji et al, 2019) with β-glucans causing an upregulation in il-1β, il-8, il-4 and tnfα. These results suggest further research into the direct mechanisms of actions of both β-glucans will help define the direct immunological effects of these functional feed components, enabling focussed future designs of diets.

Funding Acknowledgements:
DP is funded by Skretting and University of Aberdeen for a PhD studentship.

References

Attaya, A., Wang, T., Zou, J., Herath, T., Adams, A., Secombes, C. and Yoon, S., 2018. Gene expression analysis of isolated salmonid GALT leucocytes in response to PAMPs and recombinant cytokines. Fish & Shellfish Immunology, 80, pp.426-436.

Douxfils, J., Fierro-Castro, C., Mandiki, S., Emile, W., Tort, L. and Kestemont, P., 2017. Dietary β-glucans differentially modulate immune and stress-related gene expression in lymphoid organs from healthy and Aeromonas hydrophila -infected rainbow trout (Oncorhynchus mykiss). Fish & Shellfish Immunology, 63, pp.285-296.

Ji, L., Fu, S., Sun, G., Li, X. and Liu, Y., 2019. Dietary β-0glucan modulate haematological parameters, cytokines and gene expression in TLR and ERK pathways of rainbow trout (Oncorhynchus mykiss) during infection by Aeromonas salmonicida. Aquaculture Research, 51(3), pp.906-917.

Kawano, A., Haiduk, C., Schirmer, K., Hanner, R., Lee, L., Dixon, B. and Bols, N., 2011. Development of a rainbow trout intestinal epithelial cell line and its response to lipopolysaccharide. Aquaculture Nutrition, 17(2), pp.e241-e252.

Ganassin, R. and Bols, N., 1998. Development of a monocyte/macrophage-like cell line, RTS11, from rainbow trout spleen. Fish & Shellfish Immunology, 8(6), pp.457-476.

OSTREID HERPES VIRUS INFECTION IN PACIFIC OYSTER TISSUE EXPLANTS

Robert Potts*, Tim Bean, Ross Houston

The Roslin Institute, University of Edinburgh, Midlothian, EH25 9RG
Email: r.potts-1@sms.ed.ac.uk

Introduction

Pacific oysters (*Crassostrea gigas*) are one of the most important aquaculture species globally. Sustainable production of oysters is hampered by outbreaks of mass mortality caused by Oyster Herpes Virus (OsHV). Better understanding of the genetics underlying resistance to OsHV is essential to preventing future outbreaks, as vaccination or treatments are not feasible in the field, leaving biosecurity as the main defence. Furthermore, it is important to consider the full range of life stages of the oyster as they are exposed to OsHV throughout their life.

The aim of this study was to use whole-tissue explants challenged with OsHV to determine gene expression changes in different tissues and at different life stages. This data can then be used to inform selective breeding or gene editing approaches to enhancing OsHV resistance.

Materials and methods

Live pacific oysters from three key life stages (spat <2cm, juvenile 5-6cm, and adult >15cm) are obtained from a UK oyster farm. Heart, mantle, muscle and gill tissues are dissected and sterilized using antibiotic and antifungal treatments under sterile conditions. Tissues were cultured in custom media. OsHV was added to challenge groups. RNA and DNA were extracted from samples across 96 hours. RNAseq and qPCR were used to quantify gene expression.

Discussion and future work

The development of a system for maintaining whole tissues from pacific oysters is an exciting development that has been investigated for the first time (Potts et al. 2020). This system facilitates investigation into the host genetic response to infection at different life stages and backgrounds of disease resistance (Degremont et al 2021). Elucidation via qPCR and RNAseq will reveal genes of interest as potential targets for genome editing. Furthermore, tissue specific responses will be valuable for better understanding OsHV virology. Key questions to address are the main site of OsHV replication within the oyster and potential sites for virus latency or senescence. Additionally, this approach may enable the complete OsHV lifecycle in the laboratory, which has not been achieved to date.

References

Potts, R. W. A., Gutierrez, A. P., Cortés-Araya, Y., Houston, R. D., Bean, T. P. 2020 Developments in marine invertebrate primary culture reveal novel cell morphologies in the model bivalve Crassostrea gigas. PeerJ. 8, e9180. (doi:10.7717/peerj.9180)

LionelDégremont, BenjaminMorga, EliseMaurouard, Marie-AgnèsTravers. 2021 Susceptibility variation to the main pathogens of Crassostrea gigas at the larval, spat and juvenile stages using unselected and selected oysters to OsHV-1 and/or V. aestuarianus. Journal of Invertebrate Pathology Volume 183, July 2021, 107601

PROTECTIVE EFFECT OF ANTIOXIDANT SUPPLEMENTED MICRODIETS AGAINST DOXORUBICIN-INDUCED SKELETAL DEFORMITIES IN *Sparus aurata* LARVAE

Sunil Poudel* [1,2,3], Gil Martins[1,2,3], Marisol Izquierdo[5], M. Leonor Cancela[1,2,4], Paulo J. Gavaia[1,2]

[1]Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2]Faculty of Medicine and Biomedical Sciences (FMCB)
[3] PhD Program in Biomedical Sciences, FMCB
[4]Algarve Biomedical Center, University of Algarve, Faro, Portugal
[5]Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria
Email: spoudel@ualg.pt

Introduction

Oxidative stress has been related to various skeletal pathologies altering the activity of osteoclasts and osteoblasts, affecting bone remodelling. Reactive oxygen species are key components to increase oxidative stress, increase bone resorption and inhibit bone formation. A large number of studies suggests the importance of the antioxidant system to reduce the effect of bone pathologies.

Materials and Methods

Microdiets were prepared manually mixing squid powder first with water-soluble components, then with fat and lipid-soluble vitamins, and finally with gelation dissolved warm water. Resveratrol (34mg/kg) and Doxorubicin (30mg/kg) were dissolved on polar molecule were as MitoTempo was dissolved in water. *S. aurata* of 30 days were randomly stocked into 18 experimental tanks at a density of 2100 larvae/tank. The larvae were fed with experimental microdiets with antioxidant and pro-oxidants alone or in combination. The antioxidants microdiets were fed every hour from 8:00 to 20:00 whereas, prooxidant was fed only once a week and was continued with a respective combination of control or antioxidant diets. The microdiets combinations were Control, antioxidants (Resveratrol and MitoTempo) prooxidant (Doxorubicin), and in combination (Doxorubicin with Resveratrol and MitoTempo). The effect of antioxidant and pro-oxidant supplemented diet on skeletal formation, the incidence of skeletal deformities and the stages of bone mineralization, mineral content were examined.

Results

At 45 dah, pro-oxidant supplementation increased the incidence of bone deformities and affected areas and decreased bone mineralization, effects that were significantly reversed with combination with antioxidants. Doxorubicin significantly reduced the total length of larvae which was significantly reversed with the co-treatment with Resveratrol. Deformities charge were significantly high on Doxorubicin supplemented group and were significantly reduced on combining with antioxidants. Cluster analysis shows a distinct difference between the groups on the distribution of skeleton anomalies. The mineralization of the skeleton elements were significantly affected by Doxorubicin. However, while combining with Resveratrol the effect was rescued. The differences were also observed on Meristic count between the groups supplemented with antioxidants and pro-oxidants supplemented microdiets. Calcium and Phosphorus content were also decreased on Doxorubicin supplemented groups.

Conclusion

In conclusion, our results showed that antioxidant supplements effectively prevent incidence of bone anomalies and mineralization defects. Resveratrol and MitoTempo supplementation can reverse pro-oxidant induced effects on bone anomalies, mineralization and oxidative stress.

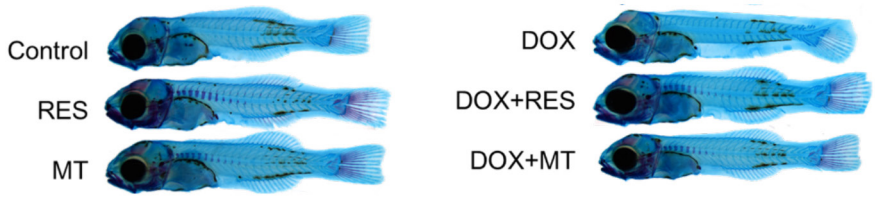


Figure: *S. aurata* larvae at 45 dah fed with microdiets supplemented with antioxidants and pro-oxidants stained with an Acid-free double staining protocol for bone and cartilage.

Acknowledgements: 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, and by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020.

REVERSAL OF DOXORUBICIN-INDUCED BONE LOSS BY ANTIOXIDANT SUPPLEMENTED MICRODIETS ON ZEBRAFISH

Sunil Poudel* [1,2,3], Gil Martins[1,2,3], Marisol Izquierdo[5], M. Leonor Cancela[1,2,4], Paulo J. Gavaia[1,2]

[1]Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2]Faculty of Medicine and Biomedical Sciences (FMCB)
[3] PhD Program in Biomedical Sciences, FMCB
[4]Algarve Biomedical Center, University of Algarve, Faro, Portugal
[5]Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria

Email: spoudel@ualg.pt

Introduction

Various skeletal disorders have been linked to oxidative stress, which affects bone remodelling by modifying the activity of osteoclasts and osteoblasts. Increased oxidative stress, increased bone resorption, and inhibited bone formation are all caused by reactive oxygen species. Antioxidants that can counteract the toxic effect of pro-oxidants on bone would be helpful for the prevention of secondary osteoporosis in humans and for promoting a better skeletal health in fish produced in aquaculture.

Materials and Methods

We used two antioxidants, Resveratrol, a natural antioxidant, and MitoTempo, a mitochondria-targeted antioxidant, to counteract the negative effects of doxorubicin on bone using zebrafish. Microdiets were supplemented with Resveratrol (34mg/kg), MitoTempo (5mg/kg), and Doxorubicin (30mg/kg). Zebrafish (5 dpf) were randomly stocked into 24 experimental tanks at a density of 100 larvae/tank for 30 days. The larvae were fed with experimental microdiets with antioxidant and pro-oxidants alone or in combination. The antioxidant supplemented microdiets were fed by standardized spatula 15mg/day and increased by 5mg/day every week. Antioxidant supplemented microdiets were fed 4 times a day, whereas pro-oxidant diet was fed only once a week. The microdiet combinations were Control, Resveratrol, MitoTempo, Doxorubicin, Doxorubicin + Resveratrol and Doxorubicin + MitoTempo. The effect of antioxidant and pro-oxidant supplemented diet on skeletal formation, the incidence and distribution of skeletal deformities and the stages of bone mineralization and mineral content were determined.

Results

At 30 days, survival of zebrafish larvae was significantly decreased under doxorubicin treatment but significantly reversed upon co-treatment with antioxidants. Doxorubicin supplementation also delayed larvae development and significantly decreased total length, however this effect was significantly reversed by co-treatment with antioxidants. The effect of microdiets on standard length was compared with the standard Zebrafeed diet (Sparos Lda, Olhão, Portugal). Resveratrol supplementation significantly increased the length as compared to Zebrafeed and control microdiet. Doxorubicin also induced an increase on the incidence of skeletal deformities and affected areas, and decreased bone mineralization. These effects were significantly reversed with an antioxidant co-treatment. Calcium, Phosphorus, Sodium, Potassium and Magnesium contents were also decreased on Doxorubicin supplemented groups whereas, while in co-feeding with antioxidants the loss of mineral content were significantly rescued.

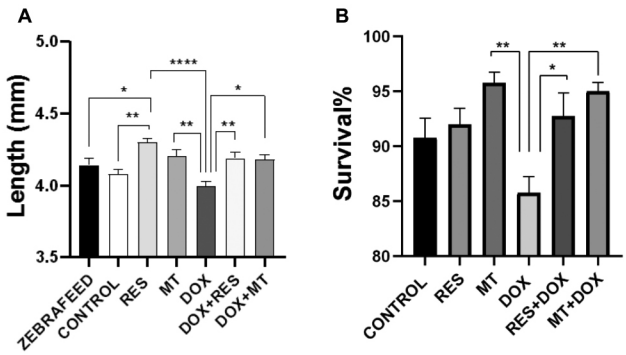


Figure: *Zebrafish* larvae fed with microdiets supplemented with antioxidants and pro-oxidants. Total length at 15 days (A), Final Survival (B).

(Continued on next page)

Conclusion

Our results showed that microdiets supplemented with antioxidant effectively prevent the incidence of bone anomalies and mineralization defects in Zebrafish. The antioxidants supplementation also protects against pro-oxidants induced bone anomalies, delay in development, defective mineralization, and oxidative stress. The use of antioxidants in fish diets can be beneficial for improving the health and quality of aquaculture produced fish.

Acknowledgements: 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, and by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020.

EFFECT OF INCREASED DIETARY PROTEIN LEVELS IN *Sardina pilchardus* ON GROWTH PERFORMANCE AND PROTEIN TURNOVER

Ana C. Matias[a*], Jorge Dias[b], Marisa Barata[a], Bárbara Requeijo[a], Florbela Soares[a], Cátia L. Marques[a], Laura Ribeiro[a] and Pedro Pousão-Ferreira[a]

[a]IPMA - Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station; Av. Parque Natural da Ria Formosa, s/n, 8700-194 Olhão, Portugal
[b]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
E-mail: ana.matias@ipma.pt

Introduction

Atlantic sardine (*Sardina pilchardus*) is one of the most important, social and economic, species found throughout the eastern Atlantic Ocean and its associated seas, mainly due to their precious nutritional characteristics such as richness in omega-3 fatty acids (Bandarra et al., 2018). However, in the beginning of 2015 the sardine biomass reached worrying lowest historical levels being the stock considered at risk of reduced reproductive capacity (Silva et al., 2015). For this reason, Atlantic sardine production in aquaculture is an opportunity to circumvent this stock risk situation with concomitant contribution to the species diversification in this sector. One main target of aquaculture sardines could be the canning industry, a Portuguese tradition with more than one century of existence. The Aquaculture research station in Olhão from the Portuguese Institute for the Ocean and Atmosphere (EPPO-IPMA) invested on sardine production and has now second-generation sardines. Animal production implies the fulfillment of a bunch of conditions such as their nutritional needs to insure an optimal growth and health status. In fish diets, protein usually represents the most expensive dietary component, therefore it is important to understand and determine fish protein requirements not only from an economical perspective but also from zootechnical and environmental ones. With this study, we intend to investigate the protein requirements of *S. pilchardus* juveniles based on protein degradation analysis and growth performance.

Materials and methods

Second generation sardine juveniles (19.9 ± 2.8 g) produced at EPPO-IPMA were fed with five diets containing different crude protein concentrations (CP 15%, 25%, 35%, 45% and 55%) for 97 days at 16.2 ± 0.5 ºC. The resulting growth parameters, protein expression and proteolytic activity enzymes in hepatic and muscle tissues were analyzed to evaluate the effect of dietary protein concentration in protein turnover and growth. Techniques like protein extraction, enzymatic activity and semi-quantitative protein expression were employed.

Results

Fish growth increased proportionally to the crude protein content in the diets, being 15% protein concentration responsible for growth arrest (Figure 1). At the cellular level, this growth impairment was reflected in significant high levels of protein degradation in the muscle of sardine juveniles but not in the liver. Also, this study allowed us to identify a novel tool to analyze protein degradation in *S. pilchardus*.

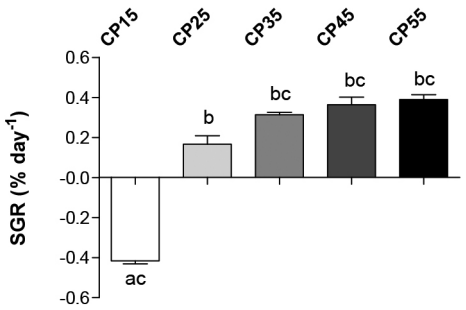


Figure 1 - Specific growth rate (SGR) of juvenile sardine (*Sardina pilchardus*) fed diets containing different protein concentrations (CP 15%, 25%, 35%, 45% and 55%). Values presented as mean ± SD. Significant groups are indicated by different letters for $p \leq 0.05$.

(Continued on next page)

Discussion and conclusion

The balance between protein synthesis and breakdown (protein turnover) regulates whole-body protein mass. A slightly increase in protein synthesis over protein degradation results in somatic growth. Low levels of crude protein in fish diets implies less amount of available amino acids for metabolism. Since the deficiency of amino acids may change protein turnover (Higuera et al., 1998), we hypothesized that the amount of these molecules could affect protein degradation and consequently juvenile sardine growth. In our study, fish growth seems to be related to the increasing crude protein concentration, which can be explained by a decrease of protein degradation in muscle. Our results based on protein turnover analysis, suggested that sardine juveniles can be fed with a diet containing 35% CP without compromising growth performance.

References

Bandarra, N. M., Marçalo, A., Cordeiro, A. R., Pousão-Ferreira, P., 2018. Sardine (*Sardina pilchardus*) lipid composition: Does it change after one year in captivity? Food Chemistry 244, 408-413.

Higuera, M., Garzón, A., Hidalgo, M. C., Peragón, J., Cardenete, G., Lupiáñez, J. A., 1998. Influence of temperature and dietary-protein supplementation either with free or coated lysine on the fractional protein-turnover rates in the white muscle of carp. Fish Physiol Biochem 18, 85-95.

Silva, A., Moreno, A., Riveiro, I., Santos, B., Pita, C., Rodrigues, J. G., Villasante, S., 2015. Sardine fisheries: resource assessment and social and economic situation. EU Parliament. European Parliament-Directorate-General for Internal Policies Directorate B-Structural and Cohesion Policy, Brussels, 55 p.

Acknowledgments

This work has been financed through the DIVERSIAQUA II (Mar2020-P02M01-0656P) project.

INITIAL TRIALS ON *Plocamium cartilagineum* AND *Sphaerococcus coronopifolius* CULTIVATION AND CHARACTERIZATION

Inês Freitas[1], Carlos Cardoso[2], João Francisco[2], Narcisa Bandarra[2], Pedro Pousão-Ferreira[1], Raquel Quintã[1]

[1]Instituto Português do Mar e Atmosfera, EPPO – Estação Piloto de Piscicultura de Olhão, Av. 5 de Outubro, 8700-305 Olhão
[2]Instituto Português do Mar e Atmosfera, Divisão de Aquacultura, Valorização e Bioprospecção, Av. Alfredo Magalhães Ramalho, 6, 1495-165 Algés
Email: pedro.pousao@ipma.pt

Introduction

Seaweeds show potential for climate change mitigation, biodiversity increase, and waste nutrients removal[1,2]. Therefore, their cultivation is attractive as it shows ecosystem services potential, while the biomass can be used in a wide range of industries. The red seaweed species *Plocamium cartilagineum* and *Sphaerococcus coronopifolius* are present in Portugal and thrive on our coasts, namely in Algarve. These two species have been considered for many industries, like pharmaceutical, medical, and cosmetics, due to their properties and the fact that they can produce compounds that are not usually found in terrestrial plants, like unique bioactive, complex, exotic acyl lipids and fatty acids[3], but despite these properties, there is still little knowledge on their cultivation potential. The objectives of this work were the cultivation of *P. cartilagineum* and *S. coronopifolius* in inland IMTA system, assessment of their bioremediation potential, and produced biomass' characterization.

Materials and methods

Seaweed cultivation: Trial 1- *Plocamium cartilagineum (6 weeks: June* 9th - July 21st); Trial 2- *Sphaerococcus coronopifolius* (6 weeks: July 27th - September 7th). Different densities (2, 4, 6, and 8 g DW L^{-1}) were tested in duplicated, the water flow was 68 L h^{-1} and there was a nutrient supply of nitrogen (NH_4CL, $NaNO_3$) and phosphorus (NaH_2PO_4). The productivity of the tanks was calculated by the equation: (g DW m^{-2}) = (DW / FW) x [($F_{FW} - I_{IW}$) / A]. The produced biomass was analysed in terms of carbon and nitrogen tissue content (% DW) carried out in an Elementar Analyzer coupled to an Isotopic Ratio Mass Spectrometer, protein content (% DW) by the Dumas method, total lipids content (% DW) by the Bligh and Dyer method, ash content (%DW) by the AOAC method (AOAC 920.153), total polyphenolic content (mg GAE/100 g DW) using a spectrophotometric method, antioxidant activity using the ABTS method (µmol Trolox/100 g), FRAP and DPPH methods, and fatty acid profile (mg/g) by acid catalysis and GC.

Results and discussion

On the two trials, there were no significant differences on the productivity between the different densities tested ($p < 0.05$). The productivity of the two species was low when compared to other species[4,5], and the productivity of *P. cartilagineum* was higher than the productivity of *S. coronopifolius*. The nutrient removal capacity of the two species was analysed in terms of the N removed in the seaweed biomass produced and calculated from N content and seaweed yield. Because of the lower productivity, these results were also low, but when comparing only the N content, *P. cartilagineum* and *S. coronopifolius* showed to have bioremediation potential if the ideal conditions for their growth are found[4,5].

Table 1: Total polyphenolic content of the analysed seaweed, initial and final of the different densities tested. Values are expressed as the mean ± SD (n = 4). Values in a column without a common lowercase letter and values in a row without a common uppercase letter are significantly different ($p < 0.05$).

	Total polyphenols (mg GAE/100 g)			
	P. cartilagineum		*S. coronopifolius*	
	Water	Ethanol 96%	Water	Ethanol 96%
Initial biomass	$82.5 \pm 3.34^{a\,A}$	$42.9 \pm 1.74^{a\,A}$	$83.3 \pm 2.51^{a\,A}$	$95.5 \pm 10.11^{a,b\,B}$
Density 4 g L^{-1}	207.9 ± 10.30^{b}	100.4 ± 7.22^{b}	154.7 ± 5.52^{b}	109.1 ± 13.72^{b}
Density 8 g L^{-1}	189.7 ± 5.02^{c}	63.0 ± 6.98^{c}	158.6 ± 27.01^{b}	79.4 ± 6.38^{a}

Table 2: Results of the evaluation of the antioxidant activity of the analysed seaweed, initial and final of the different densities tested. Values are expressed as mean ± SD (n = 4). Values in a column without a common lowercase letter and values in a row without a common uppercase letter are significantly different ($p < 0.05$).

	Antioxidant activity ABTS method (µmol Trolox/100 g)			
	P. cartilagineum		*S. coronopifolius*	
	Water	Ethanol 96%	Water	Ethanol 96%
Initial biomass	$1617.0 \pm 177.76^{a\,A}$	$1687.7 \pm 561.11^{a\,A}$	$2829.8 \pm 128.09^{a\,B}$	$1738.1 \pm 539.44^{a\,B}$
Density 4 g L^{-1}	4434.8 ± 262.04^{b}	3336.2 ± 169.89^{b}	4488.8 ± 243.57^{b}	3778.1 ± 579.95^{b}
Density 8 g L^{-1}	4296.3 ± 531.97^{b}	2310.5 ± 305.50^{a}	4137.0 ± 250.60^{b}	3274.2 ± 493.96^{b}

(Continued on next page)

In phenol content and antioxidant activity there was an increase at the end of the trials and *S. coronopifolius* had higher values than *P. cartilagineum*. The results obtained were in the range previously obtained in different studies for other species, being variability dependent on species, environmental conditions, and time of harvesting[6,7].

At the end of the two trials, there was an increase in the levels of polyunsaturated fatty acids, as seen before, there was also an increase in antioxidant activity that will serve to reduce fatty acid oxidation, since polyunsaturated fatty acids have a greater tendency to oxidize. On the two tested species the main saturated and unsaturated fatty acids were C16:0 and C20:5 (n-3), respectively. These results are in accordance with others obtained for other red seaweed, and had lower values than brown species[3].

Conclusion

Although *P. cartilagineum* and *S. coronopifolius* did not have high productivity values, they showed a high content of nitrogen in their tissues, which can show that both species have a bioremediation potential, dependent on an improvement of the biomass production. The results also show that EPA concentrations are in the range of values reported for red species, and these two species are a rich source of n-3 PUFA (18 – 29 %). At the end of the trials, the composition of the two species changed, total polyphenolic compounds, antioxidant activity and fatty acids composition increased, which indicate that the cultivation conditions had an impact on their composition, and can bring benefits to industrial applications, so further experimental work is required.

References

1. Froehlich HE, Afflerbach JC, Frazier M, Halpern BS. Blue Growth Potential to Mitigate Climate Change through Seaweed Offsetting. *Curr Biol*. 2019;29(18):3087-3093.e3. doi:10.1016/j.cub.2019.07.041
2. Neveux N, Bolton JJ, Bruhn A, Ras M. The Bioremediation Potential of Seaweeds: Recycling Nitrogen, Phosphorus, and Other Waste Products. *Wiley-VCH Verlag GmbH Co KGaA*. 2018:217-239. doi:10.1002/9783527801718.ch7
3. Dawczynski C, Schubert R, Jahreis G. Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem*. 2007;103(3):891-899. doi:10.1016/J.FOODCHEM.2006.09.041
4. Abreu MH, Pereira R, Yarish C, Buschmann AH, Sousa-pinto I. IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture*. 2011;312(1-4):77-87. doi:10.1016/j.aquaculture.2010.12.036
5. Mata LFR Da. Integrated aquaculture of Bonnemaisoniaceae: physiological and nutritional controls of biomass production and of halogenated metabolite content. *Thesis - Fac Ciencias do Mar e do Ambient*. 2008:175.
6. Quirós AR-B, Lage-Yusty MA, López-Hernández J. Determination of phenolic compounds in macroalgae for human consumption. *Food Chem*. 2010;121(2):634-638. doi:10.1016/j.foodchem.2009.12.078
7. Rajurkar N, Hande SM. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian J Pharm Sci*. 2011;73(2):146-151. doi:10.4103/0250-474X.91574

WHERE AQUACULTURE MEETS RESEARCH: AQUACULTURE RESEARCH STATION, FACILITIES AND RESEARCH LINES

Cátia L. Marques, Florbela Soares, Laura Ribeiro, Catarina Matias, Raquel Quintã, Ana Candeias-Mendes, Marisa Barata, Sara Castanho, Hugo Ferreira, João Araújo, Ana Gamboa, Ivo Monteiro, Ravi Araújo, Márcio Moreira, Pedro Pousão-Ferreira*

IPMA - Portuguese Institute for the Ocean and Atmosphere/EPPO - Aquaculture Research Station, Avenida do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal

*pedro.pousao@ipma.pt

The Portuguese Institute for the Ocean and Atmosphere (IPMA, I.P.) is a public research institute and act as a counselor to the national authorities on the sea and atmosphere. IPMA, I.P., possesses a strong cluster of competences for the ocean and marine resources related to research, carried out by different groups, particularly dedicated to aquaculture and fisheries.

The Aquaculture Research Station of Olhão (EPPO, figure 1) stands out for the unique experimental conditions on aquaculture at the national and international levels. This marine core facility is equipped to carry out production studies at every scale from bench-top laboratory work to a much larger semi-industrial level. EPPO has an area of about 7ha with more than 200 tanks, including an hatchery fully equipped for research and experimental production with different rearing circuits (for broodstock, larvae, juvenile production and research with live animals), a support building (with rooms for trophic chain production, daily routines and biological sampling), several analytical laboratories (biochemical, histological, molecular, microbiological and fish pathology), an unit for seafood packing, an area for pre-fattening (for earthen ponds and sea cages production) and 17 earthen ponds. It holds breeders of several marine fish species (e.g. meagre, gilthead seabream, seabass, Senegalese sole and sardine among others), microalgae and invertebrates as well as the know-how on the production of these species.

Production of new species, nutrition, welfare, environmentally friendly production systems and assessment of onshore and offshore and production systems for fish grow-out are some of research lines developed at EPPO (figure 2).

Acknowledgments: The research was funded by DIVERSIAQUA II (Mar2020-P02M01-0656P) project.

Figure 1 - Aerial view of the EPPO

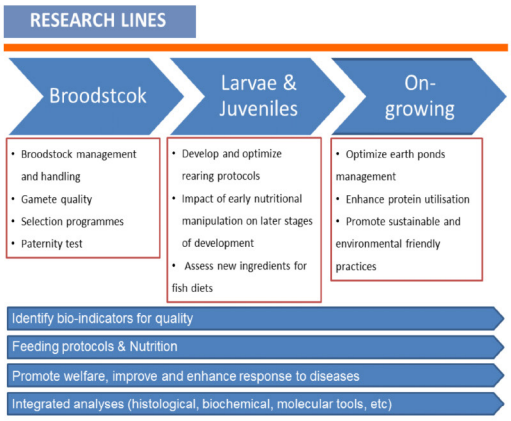


Figure 2 - Research lines developed at EPPO

, microalgae and invertebrates as well as

NUTRITIONAL VALUE OF *Holothuria poli:i* A NEW RESOURCE FOR AQUACULTURE EUROPEAN

Ermelinda Prato*, Isabella Parlapiano, Giovanni Fanelli, Francesca Biandolino

CNR-IRSA, Water Research Institute, Via Roma 3, Taranto, Italy
*E-mail: linda.prato@iamc.cnr.it

Sea cucumber (Echinodermata: Holothuroidea) represents an important seafood whose consumption is becoming popular around the world because of their bioactive compounds which have many human health benefits.

The increasing market demand and retail prices of up to USD 300–500 kg-1 (dried), has led to an exploitation often indiscriminant and excessive with a reduction of sea cucumber stocks. Although information on food composition is essential for market exchange and for consumer protection, only a few studies refer to the nutritional profile of sea cucumbers. In this study, the nutritional properties of body wall and gonad of *Holothuria polii* male and female collected from central Mediterranean Sea, were analysed. Specimens of *H. polii* were collected from an area of Mar Grande in Taranto (Ionian Sea, southern Italy) by scuba diving, during October-November 2019.

Table 1. Proximate composition of *Holothuria polii*

	Body wall		Gonad	
	Female	Male	Female	Male
Moisture (%)	82.20 ± 0.4	81.85 ± 0.6	80.51 ± 0.5	83.32 ± 1.0
Ash (%)	4.32 ± 0.3	4.27 ± 0.4	5.17 ± 0.3	4.15 ± 0.5
Lipid (g/100g dw)	3.68 ± 0.0	3.45 ± 0.0	18.53 ± 0.3	6.57 ± 0.1
Protein (g/100g dw)	32.35 ± 1.3	33.77 ± 1.1	62.42 ± 2.6	54.56 ± 1.5

Table 2. Main fatty acids of *H. polii*

Fatty acids mg/100g	Sex	Body wall	Gonad
C16:0	female	266.06 ± 65.3	2462.25 ± 165.3
	male	241.57 ± 27.1	285.59 ± 58.8
C18:0	female	231.31 ± 45.7	1201.93 ± 115.1
	male	223.82 ± 15.4	693.97 ± 45.6
C16:1	female	185.14 ± 20.2	2889.60 ± 38.1
	male	220.13 ± 24.5	217.08 ± 10.5
C18:1n9c	female	102.91 ± 12.3	1012.39 ± 76.0
	male	119.84 ± 12.1	453.03 ± 78.0
C18:3n3	female	296.54 ± 59.4	736.46 ± 96.8
	male	253.87 ± 34.7	329.35 ± 25.0
C20:4n6	female	588.41 ± 53.1	1337.74 ± 71.4
	male	538.39 ± 42.3	850.19 ± 10.0
C20:5n3	female	323.49 ± 38.8	1656.90 ± 154.6
	male	273.94 ± 33.1	847.98 ± 20.1
C22:6n3	female	47.19 ± 6.6	133.36 ± 16.7
	male	41.55 ± 8.0	100.91 ± 25.9

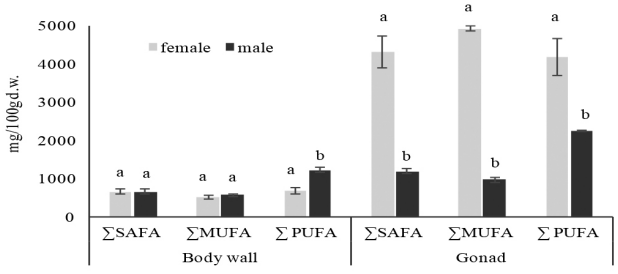


Fig. 1. Sum of SAFA, MUFA and PUFA of *H. polii*

Table 3. Amino acid profile of *H.polii*

Aminoacids	Sex	Body wall	Gonad
Isoleucine (Ile)	female	7.08 ± 0.48	5.14 ± 0.41
	male	6.68 ± 0.87	10.72 ± 0.64
Leucine (Leu)	female	17.17 ± 0.62	22.72 ± 1.75
	male	11.78 ± 0.74	15.66 ± 0.87
Lysine (Lys)	female	16.22 ± 0.48	9.51 ± 0.62
	male	13.47 ± 0.77	37.69 ± 3.21
Methionine (Met)	female	5.91 ± 0.56	2.81 ± 0.35
	male	5.92 ± 0.56	5.14 ± 0.40
Cysteine (Cys)	female	5.04 ± 0.76	1.41 ± 0.26
	male	3.99 ± 0.43	0.91 ± 0.05
Phenylalanine (Phe)	female	7.91 ± 0.68	5.07 ± 0.65
	male	6.47 ± 0.60	8.25 ± 0.43
Tyrosine (Tyr)	female	7.40 ± 0.62	7.76 ± 0.69
	male	7.06 ± 0.36	7.85 ± 0.84
Threonine (Thr)	female	11.15 ± 0.57	6.83 ± 0.54
	male	10.36 ± 0.75	11.92 ± 0.65
Valine (Val)	female	7.97 ± 0.84	6.27 ± 0.64
	male	6.86 ± 0.44	11.17 ± 0.78
Histidine (His)	female	3.49 ± 0.57	2.80 ± 0.50
	male	3.16 ± 0.25	5.12 ± 0.47
Aspartic acid (Asp)	female	5.17 ± 0.44	6.07 ± 0.83
	male	3.78 ± 0.25	15.63 ± 1.09
Arginine (Arg)	female	15.75 ± 0.69	8.72 ± 0.89
	male	13.28 ± 0.83	21.21 ± 1.42
Serine (Ser)	female	9.55 ± 0.87	5.68 ± 0.98
	male	8.26 ± 1.00	10.07 ± 0.45
Glutamic acid (Glu)	female	16.45 ± 1.25	9.80 ± 1.01
	male	10.41 ± 0.90	29.88 ± 1.76
Proline (Pro)	female	15.79 ± 0.98	6.40 ± 0.31
	male	12.84 ± 0.89	22.63 ± 1.90
Glycine (Gly)	female	20.69 ± 1.19	6.00 ± 0.24
	male	16.49 ± 0.92	28.30 ± 1.19
Alanine (Ala)	female	11.50 ± 1.00	4.58 ± 0.39
	male	11.31 ± 0.61	9.51 ± 0.07

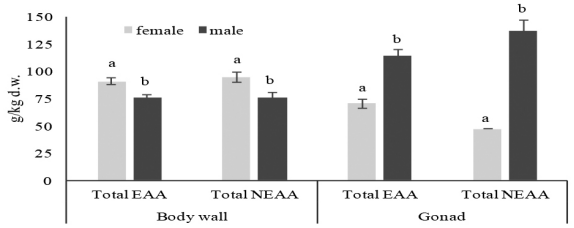


Fig. 2. Sum of Essential and Non essential AAs of *H. polii*

(Continued on next page)

In general, *Holothuria polii* was found to have low fat and high protein contents so, can be considered as a dietetic food. The *H. polii* species was rich in terms of PUFA, especially the gonad of female. Arachidonic acid (20:4 n6) and eicosapentaenoic acid (20:5 n3), which are significant for nutrition are present in highest concentration. This sea cucumber species was a good source of Essential Aminoacids (EAA) and an ideal nutritional seafood for human consumption. The EAA/Total AA ratio of *H. polii* was in the range 45.4-59.8%, with the highest value in the gonad of female.

Therefore, *H. polii* could be take into account for its potential culture. Our results indicate that *H. polii* has a balanced nutritional quality and contains compounds that can contribute for a healthy and well-balanced diet.

GENOME-WIDE ASSOCIATION STUDY OF HYPOXIA STRESS TOLERANCE IN RAINBOW TROUT

M. Prchal[1,2], D. Lallias[2], H. Lagarde[2], J. D'Ambrosio[2], P. Patrice[3], Y. François[3], C. Poncet[4], A. Desgranges[5], P. Haffray[3], M. Dupont-Nivet[2], F. Phocas[2]

[1,2] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[3] SYSAAF, French Poultry and Aquaculture Breeders Association, 35042 Rennes, France
[4] GDEC, INRAE, Université Clermont-Auvergne, 63039 Clermont-Ferrand, France
[5] SARL Milin Nevez, 29610 Plouigneau, France

Email: mprchal@frov.jcu.cz

Introduction

Hypoxia is one of the most critical threats for future aquaculture sector due to the global climate changes. Global warming decreases dissolved oxygen leading to decline of health and welfare of cultured aquatic organisms and has also deleterious impacts on growth, reproduction, immunity and other energy demanding activities (Farrel and Richards, 2009; Gallage et al., 2016). Although many studies have revealed that there is an adaption strategy in low oxygen tolerance for fish species (Zhu et al., 2013), Genome-Wide Association Studies (GWAS) of hypoxia stress tolerance in aquaculture species are rare (Li et al., 2017; Wang et al., 2017).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most commonly farmed salmonid species that requires high level of dissolved oxygen in comparison to other fish species. However, GWAS of hypoxia stress tolerance in rainbow trout is still missing. Therefore, objectives of this study were to estimate heritability and to detect quantitative trait loci (QTL) associated with hypoxia tolerance.

Materials and methods

The experimental stock was established from 190 dams and 98 sires of a commercial selected diploid population of Milin Nevez breeding company (Bretagne Truite Group, France). The stock was reared under commercial conditions until the experiment. Fish were then transported to the ANSES-SYSAAF Fortior Genetics platform and acclimatized before acute hypoxia challenge test. 1,320 individuals were P.I.T. tagged and fin-clipped for later DNA extraction and genotyping. The challenge to hypoxia was sub-divided into seven batches (one per day) and in each batch a random sample of fish (app 188 fish per batch) was challenged. At the beginning of each batch the initial oxygen level was recorded. Gradual decline of oxygen was conducted by bubbling nitrogen. When fish lost their equilibrium, they were removed from the tank, identified (PIT-tag reading), weighed (mean weight 50.8 g) and euthanized in Eugenol. The corresponding time and oxygenation level were recorded. The challenge ended when the last fish lost its equilibrium and was removed of the tank.

All individuals were genotyped for 57,501 SNPs with the Axiom™ Trout Genotyping array at the INRAE genotyping Platform Gentyane. After quality controls and imputation of missing genotypes approximately 28,875 SNPs and 1,297 individuals were used in the statistical analysis. Tolerance to hypoxia was analysed as time to loss equilibrium (TLE) with the day of trial as a fixed effect in the final model. Using BLUPF90 package (Misztal et al., 2014) GBLUP analysis was performed to estimate heritability of the trait with AIREMLF90 program and to detect QTL with POSTGSF90 program (Aguilar et al., 2014).

Results

Genomic heritability of hypoxia tolerance was moderate (0.37 ± **0.04). We also identified** a few QTLs with the most significant one on chromosome 31 with possible candidate genes in the region spanning approximatively from 20 Mb to 23 Mb on the Arlee genome reference assembly (USDA_OmykA_1.1.).

Discussion and conclusion

Preliminary results revealed that the tolerance to hypoxia is a heritable trait. In addition, a significant QTL was identified on chromosome 31 with several putative QTLs detected on other chromosomes. However, further statistical analyses including more dense genotypes and Bayesian approach need to be conducted to precise our preliminary results.

(Continued on next page)

Acknowledgements
This study was supported by the European Maritime and Fisheries Fund and FranceAgrimer (Hypotemp project, n° P FEA470019FA1000016) and project CZ.02.2.69/0.0/0.0/18_053/0016975 - Development of the USB – International Mobilities II.

References

Aguilar, I., Misztal, I., Tsuruta, S., Legarra, A., Wang, H., 2014. PREGSF90 – POSTGSF90: Computational tools for the implementation of single-step genomic selection and genome-wide association with ungenotyped individuals in BLUPF90 programs. In: Proceedings of the 10th WCGALP. Vancouver, Canada.

Farrell, A. P., Richards, J. G. 2009. Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. Fish Physiology, 27: 487–503.

Gallage, S., Katagiri, T., Endo, M., Futami, K., Endo, M., Maita, M. 2016. Influence of moderate hypoxia on vaccine efficacy against Vibrio anguillarum in Oreochromis niloticus (Nile tilapia). Fish and Shellfish Immunology, 51: 271–281.

Li, H. L., Gu, X. H., Li, B. J., Chen, C. H., Lin, H. R., Xia, J. H., 2017. Genome-wide QTL analysis identified significant associations between hypoxia tolerance and mutations in the GPR132 and ABCG4 genes in Nile tilapia. Marine Biotechnology, 19(5): 441-453.

Miszta,l I., Tsuruta, S., Lourenco, D., Aguilar, I., Legarra, A., Vitezica, Z., 2014. Manual for BLUPF90 family of programs. Athens Univ Georg.

Wang, X., Liu, S., Jiang, C., Geng, X., Zhou, T., Li, N., Liu, Z., 2017. Multiple across-strain and within-strain QTLs suggest highly complex genetic architecture for hypoxia tolerance in channel catfish. Molecular Genetics and Genomics, 292(1): 63-76.

Zhu, C. D., Wang, Z. H., Yan, B.,.. 2013. Strategies for hypoxia adaptation in fish species: a review. Journal of Comparative Physiology B, 183 (8): 1005-1013.

IMPROVING RAINBOW TROUT *Oncorhynchus mykiss* FEEDING EFFICIENCY USING ARTIFICIAL INTELLIGENCE: VIDEO OBSERVATION OF BEHAVIOUR AND X-RAY IMAGING OF STOMACH FULLNESS TO SUPPORT A MODEL SIMULATION OF FISH FEEDING (FISHMET)

Steven Prescott*[1], Joseph De Prisco[1], Ivar Rønnestad[2], Sergey Budaev[2], Natalie Panasiak[1], Charlotte Dupont[3], Dimitri Trotignon[3], Dominique Durand[4], Lars Ebbesson[4], Franck Le Gall[5] and Tamás Bardócz[1].

[1]AquaBioTech Group, MST 1761, Mosta (Malta)
[2]Department of Biological Sciences, University of Bergen, Bergen (Norway).
[3]Bioceanor, 06560 Valbonne (France)
[4]NORCE Norwegian Research Centre AS, Bergen, (Norway)
[5]Easy Global Market, 06560 Valbonne (France)
Email: sgp@aquabt.com

Introduction

Innovation has played a major role in shaping European aquaculture, and it remains essential for the sector's continued survival. Advances in digital technology offer new opportunities for innovation that might contribute to the industry's continued success, stimulating initiatives for the development of artificial intelligence, automation, and Internet of Things based solutions. The iFishIENCi project aims to optimise feeding in fish production systems through combined application of machine learning algorithms, video observation of fish behaviour, and water quality digital sensors and software. The approach uses FishMET, a model simulation of fish feeding behaviour and related physiological and metabolic processes. The ability of FishMET to accurately simulate these processes is limited by a lack of data describing their functional relationships for different species within varying conditions. Obtaining the required data is challenging as these relationships have a dynamic and apparently complex nature. To address these issues, an ongoing series of experiments are being performed to a) validate a combination of methods for obtaining data that describe rainbow trout (*Oncorhynchus mykiss*) feeding behaviour, feed intake, and stomach fullness, to b) identify and describe relationships between these processes and c) generate data that is useful for the development and training of machine learning algorithms. If distinct levels of stomach fullness and hunger are associated with different behaviours, it may be possible to use a combination of machine learning and real-time video monitoring of fish behaviour to assist with the optimisation of feeding regimes beyond what is currently possible.

Materials and methods

A recirculation aquaculture system facility was stocked with rainbow trout of varying size and held at densities of approximately 35kg/m^3. The fish were subject to varied meal regimes delivered by automated feeders, and feed consumption, stomach fullness, behaviour and water quality data were assessed. Behaviour was recorded by video camera, and feed consumption was quantified using a labelled diet and X-ray imaging, as described by McCarthy et al. (1992). Digital sensors and cloud integrated monitoring were used to record temperature and dissolved oxygen. Machine learning algorithms based on the Sciit-learn python library were trained and used for target acquisition and fish tracking, and *k*-means clustering techniques were used to evaluate inter-fish distances and grouping.

Results

Fish in video images were successfully targeted and tracked using algorithms. Aggregations of fish below feeders were observed before feeding events and were effectively characterised by *k*-means clustering algorithms. Strong schooling is observed post feeding but increased clustering pre-feeding was not consistently observed. In feed markers are visible in X-ray images and calculated feed consumption quantities compare well to known quantities of feed consumption.

(Continued on next page)

Discussion

The ability of algorithms to identify, track, and characterise clustering is promising, although more exploration is required. *k*-means clustering scores were not consistently higher before mealtimes despite the absence of aggregations post-feeding. The incidence of aggressive behaviours, which may disrupt clustering, appear to increase before meals, and singles behaviour types may not be reliable as standalone cues. Aggregation may be an anticipatory response in fish conditioned to receiving feed at set times and from the same location, and so its usefulness as an indicator of hunger is yet to be determined. Behaviour such as aggression or higher swimming velocity may be more appropriate indicators of hunger state, although behavioural expression may differ across cultivation conditions and size classes. The McCarthy et al. (1992) method was successfully used to quantity feed consumption. Its suitability for quantifying relative fullness across separate regions of the gastrointestinal track is now being investigated. This information can be useful for understanding hunger, satiety, anticipation, and associated behaviours. Ongoing experimentation continues to assist the development of machine learning algorithms that may reveal relationships between stomach fullness, hunger state and behaviour.

References

McCarthy, I. D., Houlihan, D. F., Carter, C. G., and Moutou, K.,1993. Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. Proceedings of the Nutrition Society, 52, 427–436.

OYSTER LONGLINE DESIGN OPTIMIZATION: AQUACULTURE PILOT STUDY IN THE BELGIAN PART OF THE NORTH SEA

A. B. K. Pribadi[1]*, G. Verao Fernandez[1], A.M. Declercq[2], B. Stechele[2], N. Nevejan[2], B. Groenendaal[3], S. Debels[3], D. Delbare[4], D. Vandercammen[5], S. Petit[6], T. Kerkhove[7], J. Vanaverbeke[7], S. Degraer[7], E. Lataire[1]

[1]Maritime Technology Division, Ghent University, Ghent (Belgium)
[2]Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Ghent (Belgium). [3]Brevisco, Hendrik Baelskaai 38, 8400 Oostende (Belgium)
[4]Research Institute for Agriculture, Fisheries and Food, Ankerstraat 1, 8400 Oostende (Belgium). [5]Parkwind, Sint Maartenstraat 5, 8400 Oostende (Belgium)
[6]Jan De Nul NV, Tragel 60, 9308 Hofstade-Aalst (Belgium)
[7]Marine Ecology and Management, Operational Directory Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussel (Belgium)
E-mail: ajiebramakrishna.pribadi@UGent.be

Introduction

As part of the European Horizon 2020 project UNITED (UNITED, 2021), an offshore longline system (Morse and Rice, 2010) will be installed to assess the feasibility of cultivating European flat oysters (*Ostrea edulis*) in the Belgian part of the North Sea 25 nautical miles off shore. Five different culture systems are studied: SEAPA baskets stacked on a ladder frame; oysters glued on dropper lines; frames consisting of horizontal and vertical grow-out sticks; frames as spat collectors; trays on dropper lines. To accommodate these culture systems, a mooring configuration suitable for the harsh offshore environment is designed. The main cultivation line is 57 m long, making up the total length of 263 m of mooring and backbone ropes from South-West screw anchor to the North-East screw anchor. The distance between both anchors is 250 m. The water depth at the test location is at -30.1 m Mean Lower Low Water Spring.

Materials and methods

A set of boundary conditions are defined based on the operational limitations, space available and guidelines' recommendations. Fig.1 shows the flowchart of the mooring design iterative process. The longline system will be installed with anchors within a zone of four wind turbines. Taking this into account, during the operational conditions, the area taken by the mooring system has to be less than 0.12 km^2. This governs the longline design characteristics as initial input. Then, a hydrostatic calculation is performed to ensure that the cultivation line can be lifted 5 m above still water level and the lifting capacity of the vessel is adequate to perform maintenance/installation operations. Lastly, dynamic simulations incorporating waves and current induced load are performed for various scenarios to determine the material properties of the mooring components. Numerical simulations are performed utilising an in-house mooring dynamic solver based on the lumped-mass approach to discretize the lines (Hall and Goupee, 2015; Pribadi et.al, 2019) and the Morison Equation (Morison, 1950) to model the hydrodynamic forces. The Morison coefficients are taken from the guidelines set in the DNVGL OS301 (DNVGL, 2018). The sea states used for the Ultimate Limit States (ULS) calculation and safety factors for the line elements are taken from the recommendations provided in the NS9415 document (Norwegian Standard, 2010).

Results

The ULS simulation that is taking the 50-year return period of waves () and current (parallel to the longline, is giving the maximum predicted tension of 125 kN on the mooring lines. The state of the mooring system during the ULS simulation at = 63.8 s is shown in Fig. 2. A maximum load of 71 kN is calculated for the 1-year return period of waves () and current parallel (to the system. The load time-series of the two sea states are shown in Fig. 3.

Discussion and conclusion

An iterative design process has been performed to define the technical requirements needed to install a mooring system for the cultivation of flat oyster in an offshore wind farm. The mooring system is designed with a "set and forget" principle, meaning that the system will start with an excess buoyancy on day-1 of installation. By the winter season when the worst storm occurs, the backbone would be fully submerged up to 10 m of depth, due to the increase of biomass (oysters and biofouling), making it less exposed to the wave actions. Then, after one year of installation, a maintenance operation will be needed to adjust the buoyancy.

(Continued on next page)

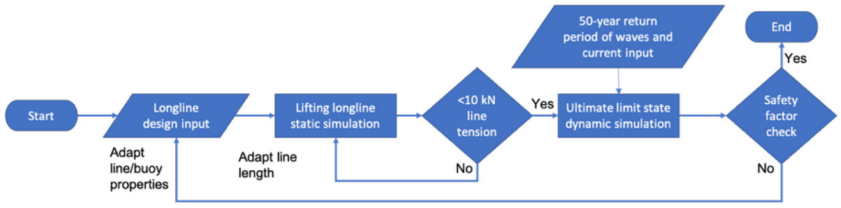


Fig. 1. Flowchart describing the oyster longline mooring design process

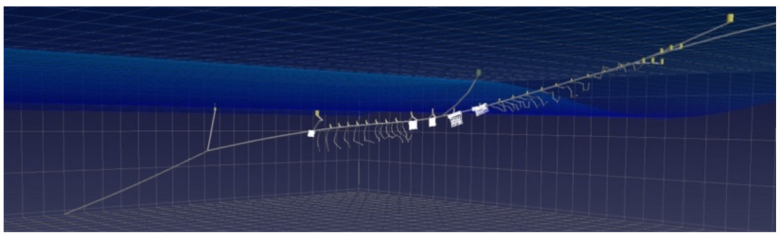


Fig. 2. Snapshot of the oyster longline system during ULS numerical simulation

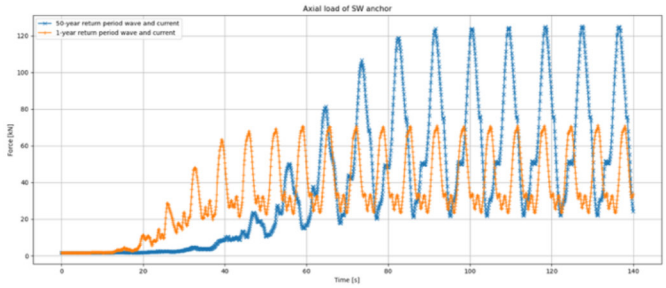


Fig. 3. Axial load comparison between the sea states of 50-year and 1-year return period

This Project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no 862915

References

UNITED: Multi-Use offshore platforms demoNstrators for boostIng cost-effecTive and Eco-friendly proDuction in sustainable marine activities. Retrieved April 1, 2021, from https://www.h2020united.eu/

Morse, D. and Rice, M. A., 2010. Mussel aquaculture in the Northeast. NRAC Publication, 211.

Hall, M. and Goupee, A., 2015. Validation of a lumped-mass mooring line model with DeepCwind semisubmersible model test data. Ocean Engineering, 104: 590-603. DOI: 10.1016/j.oceaneng.2015.05.035.

Pribadi, A.B.K., Donatini, L., Lataire, E., 2019. Numerical modelling of a mussel line system by means of lumped-mass approach. Journal of Marine Science and Engineering, vol. 9. DOI: 10.3390/jmse7090309.

Morison, J.R., O'Brien, M. P., Johnson, J. W., and Schaaf, S. A., 1950. The force exerted by surface waves on piles. J. Pet. Technol., vol. 2.

Det Norske Veritas Germanischer Lloyd (DNV-GL), 2018. OS-301 Position mooring.

Norwegian Standard, 2010. NS 9415.E:2009 Marine fish farms requirements for site survey, risk analyses, design, dimensioning, production, installation and operation.

RECOVERY OF HAEMAL LORDOSIS IN JUVENILE ZEBRAFISH (*Danio rerio*)

A. Printzi[1,2]*, D. Mazurais[2], P.E. Witten[3], J-L. Zambonino-Infante[2], G. Koumoundouros[1]

[1]Biology Department, University of Crete, Crete, Greece
[2] IFREMER, University of Brest, CNRS, IRD, LEMAR, F-29280, Plouzané, France
[3] Department of Biology, Gent University, Gent, Belgium
Email: alikipr95@gmail.com

Introduction

Skeletal responses of vertebrates to increased mechanical stimuli overrule genetic determination, ranging among shifts on bone volume, size, shape, composition and mineralization (Suniaga *et al*. 2018). Through exercise, an effective means of imposing increased mechanical loads to the fish skeleton, a V-shaped curvature in the haemal part of the vertebral column can be induced (Printzi *et al*. 2021). Haemal lordosis, a common skeletal abnormality reported in several aquaculture species, has been recently observed to be reversible in Gilthead seabream and European sea bass during on-growing period (Fragkoulis *et al*. 2019, 2021). Given the fact that the most important bone cell remodeling procedures (Witten and Hall 2015) and underlying signaling molecules have been identified, the aim of the present study is to examine the recovery potential of haemal lordosis and the underlying mechanisms in zebrafish, a model vertebrate species.

Materials and methods

Early juveniles of 12 mm TL (total length) and normal skeletal phenotype were subjected to continuous exercise against water velocity of 8.0 TL·s^{-1} (Printzi *et al*. 2021) for a four-day period. Lordotic juveniles after the swimming challenge test (SCT) were monitored individually for a week under common husbandry conditions (0.0 TL·s^{-1}). Feeding regime and abiotic conditions remained constant throughout the experiment. During the trial, lordosis development was followed by means of in vivo examination, whole mount staining for bone and cartilage, histology and gene expression analysis. Two control groups consisting of normal fish after SCT, as well as non-exercised juveniles of the same size were acquired. Three replicates out of a single spawning event were performed.

Results and Discussion

Macroscopic examination of the fish revealed that haemal lordosis has a great recovery potential in zebrafish juveniles. Recovery of the lordotic phenotype was first detected at 4 days post-SCT, with the 72.5% of the originally abnormal fish (Fig. 1b) presenting a completely normal phenotype (Fig. 1c). Likewise, the progress of the recovery process reached 89.5% at the end of the recovery week (Fig. 1a).

The present study demonstrates that complete recovery of haemal lordosis in zebrafish is achieved within one week of fish swimming in "static" water. Our results obtained from histological and transcriptomics analyses (data will be presented on the poster) present evidence for the involvement of extensive bone formation through remodeling in the recovery process. These results agree with the observation that bone remodeling is involved in the partial recovery of vertebrae centra fusion in Atlantic salmon (Witten *et al*. 2006). Obtaining control of skeletal abnormalities that effect the external phenotype, either through prevention or recovery, can enhance the quality of final aquaculture products.

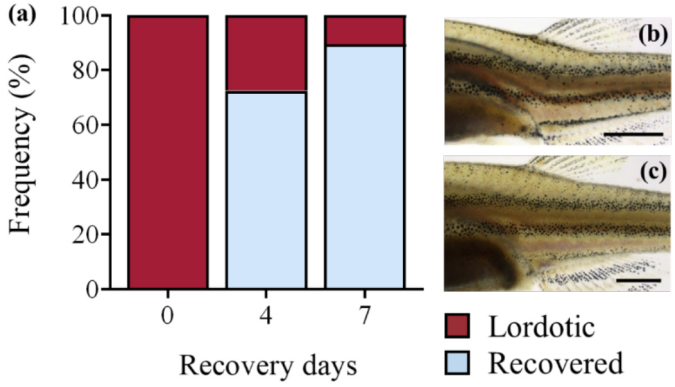


Figure 1. Development of the lordotic phenotype after swimming challenge test (recovery period, 0.0 TL s^{-1}). Fish categorization into lordotic (b) or recovered (c) is based on external morphology. Scale bars equal 1mm.

(Continued on next page)

References

Fragkoulis S, Printzi A, Geladakis G, Katribouzas N, Koumoundouros G (2019) Recovery of haemal lordosis in gilthead seabream (*Sparus aurata* L.). Sci. Rep. 9(1), pp.1-11.

Fragkoulis S, Kourkouta Ch, Printzi A, Geladakis G, Kerasovitis D, Koumoundouros G (2021, October 4th-8th) Recovery of haemal lordosis in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758), Aquaculture Europe, Madeira, Portugal.

Printzi A, Fragkoulis S, Dimitriadi A, Keklikoglou K, Arvanitidis C, Witten PE, Koumoundouros G (2021) Exercise induced lordosis in zebrafish *Danio rerio* (Hamilton, 1822). J. Fish Biol. 98:987–994.

Suniaga S, Rolvien T, vom Scheidt A, Fiedler IAK, Bale HA, Huysseune A, P. Eckhard Witten PE, Amling M, Busse B (2018) Increased mechanical loading through controlled swimming exercise induces bone formation and mineralization in adult zebrafish. Sci. Rep. 8:3646.

Witten PE and Hall BK (2015) Teleost skeletal plasticity: modulation, adaptation, and remodelling. Copeia, 103, 727–739.

Witten PE, Obach A, Huysseune A, Baeverfjord G (2006) Vertebrae fusion in Atlantic salmon (*Salmo salar*): Development, aggravation and pathways of containment. Aquaculture 258, 164–172.

EMBRYONIC TEMPERATURE AFFECTS SKELETAL DEVELOPMENT IN ZEBRAFISH

A. Printzi[1,2*], A. Kekelou[1*], G. Koumoundouros[1]

[1] Biology Department, University of Crete, Heraklion, Crete, Greece
[2] IFREMER, University of Brest, CNRS, IRD, LEMAR, F-29280, Plouzané, France
Email: alikipr95@gmail.com, grad1002@edu.biology.uoc.gr

Introduction
Teleost skeleton is subjected to continuous changes as a response to internal (e.g. allometric growth) or external stimuli (Witten & Hall 2015, Balbuena-Pecino *et al*. 2019). Water temperature during the early life stages is an important driving factor of fish phenotypic plasticity, with significant effects on the ontogenetic pattern and the meristic counts of skeleton (e.g. Sfakianakis *et al*. 2004, Christou *et al*. 2018). In the present study zebrafish was used as a model to examine whether a short temperature exposure during the embryonic stage affects a) skeletal development (ossification rate, vertebrae counts) during the following larval period, and b) the response of vertebral column against intense swimming activity.

Materials and methods
Experimental temperatures (24, 28, 32 °C) were applied in duplicate, during the embryonic period, whereas after hatching all groups were reared under the same conditions (Fig. 1). The ossification rate of different skeletal elements was studied on random samples of larvae, taken from each experimental population every 2-3 days. Larvae were subjected to a calcein staining protocol following Du *et al*. (2001), anesthetized, photographed under a fluorescence stereoscope (Olympus SZX16) and measured for total length (TL). In total, ca 180-220 larvae per developmental temperature (TD) were examined.

Swimming challenge test (SCT) followed the protocol of Printzi *et al*. (2021) and was applied for four days (at 8 TL s^{-1}), on 20 fish per thermal group and replicate. SCTs were performed at the end of metamorphosis (ca 13 mm TL). After the test all fish were stained for bone and cartilage (Walker & Kimmel 2007) and examined for the presence of haemal lordosis.

Results and discussion
During the early larval stage (<6.5 mm TL), fish having experienced a higher embryonic temperature (32°C) presented a delayed ossification rate against those of 24 and 28 °C (Fig. 2A). In the following developmental period (>6.5 mm TL), no significant effects of temperature on ossification rate were observed. Furthermore, embryonic temperature significantly affected the number of haemal vertebrae and the response vertebral column to SCT. As embryonic temperature increased the number of haemal vertebrae decreased ($p<0.05$, Kruskal-Wallis test) and the incidence of lordosis increased ($p<0.05$, G-test).

Our results demonstrate that a short temperature raise (withing the species tolerance zone) during the critical embryonic period may have significant, long lasting effects on zebrafish skeleton. In addition to their relevance to global climate change, our findings have relevance to the mitigation of lordosis development in reared fish.

(Continued on next page)

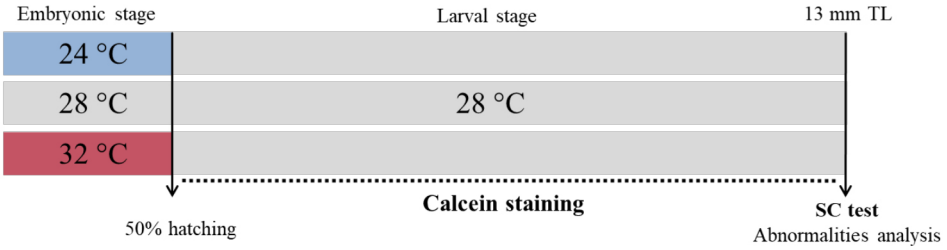


Fig. 1. Experimental design of the study. Fish were subjected to one of three developmental temperatures up to hatching and then at a common temperature till metamorphosis.

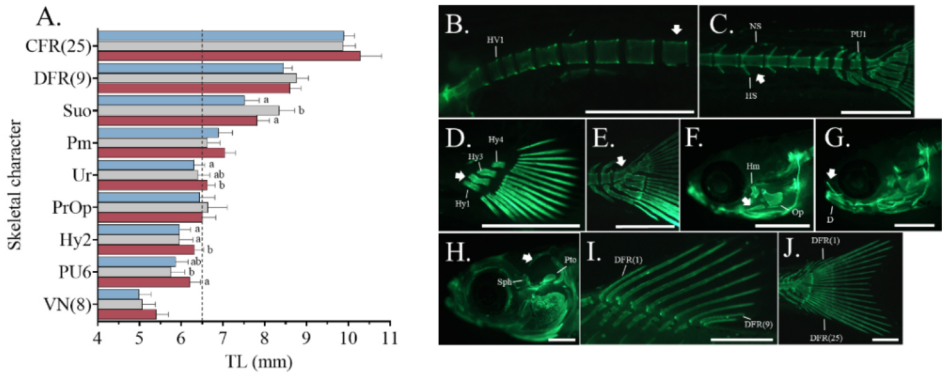


Fig. 2. Total Length (TL) at the ossification onset of selected skeletal elements. VN(8), 8th pre-haemal vertebral centrum (arrow in B). PU6, 6th pre-ural centrum (arrow in C). Hy2, hypural 2 (arrow in D). Ur, urostyle (arrow in E). PrOp, pre-operculum (arrow in F). Pm, pre-maxillary (arrow in G). Suo, supraoccipital bone (arrow in H). PFR(9), 9 dorsal rays present (I). CFR(25). 25 caudal rays present (J). Error bars equal to 1 SE. Scale bars equal to 0.5 mm.

References

Balbuena-Pecino S, Riera-Heredia N, Vélez EJ, Gutiérrez J, Navarro I, Riera-Codina M, Capilla E (2019) Temperature affects musculoskeletal development and muscle lipid metabolism of Gilthead sea bream (*Sparus aurata*). Front. Endocrinol., 10: 173.

Christou M, Iliopoulou M, Witten E, Koumoundouros G (2018) Segmentation pattern of zebrafish caudal fin is affected by developmental temperature and defined by multiple fusions between segments. J. Exp. Zool. (Mol. Dev. Evol.), 330: 330-340.

Du SJ, Frenkel V, Kindschi G, Zohar Y (2001) Visualizing normal and defective bone development in zebrafish embryos using the fluorescent chromophore calcein. Dev. Biol., 238: 239-246.

Printzi A, Fragkoulis S, Dimitriadi A, Keklikoglou K, Arvanitidis C, Witten PE, Koumoundouros G (2021) Exercise induced lordosis in zebrafish *Danio rerio* (Hamilton, 1822). J. Fish Biol. 98: 987-994.

Sfakianakis DG, Koumoundouros G, Divanach P, Kentouri M (2004) Osteological development of the vertebral column and of the fins in *Pagellus erythrinus* (L. 1758). Temperature effect on the developmental plasticity and morpho-anatomical abnormalities. Aquaculture, 232: 407-424.

Walker M, Kimmel C (2007) A two-color acid-free cartilage and bone stain for zebrafish larvae. Biotech. Histochem., 82: 23-28.

Witten PE and Hall BK (2015) Teleost skeletal plasticity: modulation, adaptation, and remodelling. Copeia, 103: 727-739.

THE LANDSCAPE OF TWO MAJOR HISTONE MODIFICATIONS IN ATLANTIC SALMON (*Salmo Salar*) FED A SURPLUS OF METHIONINE AND B VITAMINS

A.L.K. Putman*[1,2],A.C. Adam[3], T. Saito[3], M. Espe[3], K.H. Skjærven[3], and E.A. Miska[1,2]

[1] The Gurdon Institute, Wellcome Trust/Cancer Research UK, Cambridge (United Kingdom)
[2] Department of Genetics, University of Cambridge, Cambridge (United Kingdom)
[3] Institute of Marine Research, Bergen (Norway)
*E-mail: alkp2@cam.ac.uk

Histone post-translational modifications act as dynamic regulators of the genome. Despite their crucial role in genome regulation, the impact of dietary nutrients upon histone modification landscapes remain poorly understood, particularly in fish models. In this project, by supplying Atlantic salmon (*Salmo salar*) with a surplus nutrient package, we measure the impact of altered one-carbon (1C) metabolism on the enrichment of two histone modifications associated with activation: H3K27ac and H3K4me3.

Crucial to cell function, one-carbon (1C) metabolism provisions methyl groups for a range of biological processes. These particularly include the regulation of gene expression via DNA methylation and histone post-translational modifications. The activity of 1C metabolism is dependent upon the availability of select nutrients, particularly folic acid, pyridoxine (B6), cobalamin (B12), and methionine.

To measure the impact of an increased intake of 1C nutrients upon growth, epigenetic state, and gene expression, we raised groups of Atlantic salmon (*Salmo salar*) on two plant-based diets with varying levels of 1C nutrients: one control (Low1C) using nutrients at recommended levels, and one moderate+ diet (Med1C) with increased levels of 1C nutrients (NRC 2011). Respectively, the Low1C and Med1C diets contained 2.6 mg/kg and 4.8 mg/kg folate, 6.75 mg/kg and 9.31 mg/kg B6, 0.15 mg/kg and 0.18 mg/kg B12, and 6.7 g/kg and 9.5 g/kg methionine (Espe *et al*. 2020; Adam *et al*. 2021). Muscle tissues from the Low1C and Med1C treatment groups were collected for various sequencing applications during both freshwater and saltwater life stages.

To characterize the changes in chromatin landscape resulting from limitation of 1C nutrients, we mapped the enrichment of two histone post-translational marks associated with activation: H3K4me3 (a marker of active promoters) and H3K27ac (marking active enhancers and promoters). We performed Cut&Run of both marks in single muscle samples from the Low1C and Med1C treatment groups (n=2 per tank, 3 tanks/treatment). Sequencing was performed for both freshwater and saltwater life stages. Levels of gene expression (RNA-seq) and DNA methylation (RRBS) were measured in these same muscle samples, allowing for multi-omic comparisons across data types (Saito *et al*. 2020; Adam *et al*. 2021).

Comparative enrichment analysis between the diet treatments identified differentially bound regions for both marks (p-value<0.05). Annotation and functional enrichment analysis of these regions implicated multiple pathways involved in cell signaling and amino acid metabolism. These results reflect those obtained from DNA methylation and transcriptome sequencing of the same samples, suggesting that regulatory changes in response to altered 1C metabolism are coordinated across epigenetic mechanisms and transcription. In this poster, we characterize the impact of altered 1C metabolism on two major histone modifications in a teleost model, contributing to the growing area of investigation concerning the impact of nutrition on chromatin architecture.

References

Adam, A.C., Saito, T., Espe, M., Whatmore, P., Fernandes, J., Vikeså, V., & Skjærven, K. 2021. Metabolic and molecular signatures of improved growth in Atlantic salmon (Salmo salar) fed surplus levels of methionine, folic acid, vitamin B6 and B12 throughout smoltification. *British Journal of Nutrition,* 1-14.

Espe M., Vikeså V., Thomsen T.H., Adam A.C., Saito T. and Skjærven K.H. 2020. Atlantic salmon fed a nutrient package of surplus methionine, vitamin B12, folic acid and vitamin B6 improved growth and reduced the relative liver size, but when in excess growth reduced. *Aquacult Nutr* 26, 477-489.

National Research Council (NRC). 2011. Nutrient Requirements of Fish and Shrimp. *The National Academies Press*. Washington DC, USA. 392pp.

Saito, T., Whatmore, P., Taylor, J.F., Fernandes, J.M.O., Adam, A.C., Tocher, D.R., Espe, M., Skjærven, K.H. 2020. Micronutrient supplementation affects transcriptional and epigenetic regulation of lipid metabolism in a dose-dependent manner. *Epigenetics* 31, 1-18.

HIGH CONVERGENCE AREAS FOR AQUACULTURE IN RIA FORMOSA LAGOON IDENTIFIED UNDER AQUA&AMBI PROJECT: A FIRST ASSESSMENT OF WATER QUALITY

Hugo Quental-Ferreira[1], Carlos Sousa[2], Domitília Matias[1], Gabriela Oliveira[1], Maria Emília Cunha[1], Laura Ribeiro[1]*

[1] IPMA- Instituto Português do Mar e da Atmosfera, I.P. / Estação Piloto de Piscicultura de Olhão EPPO, Av. Parque Natural da Ria Formosa, s/n 8700 - Olhão, Portugal
[2] CCMAR - Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8 Faro, Portugal
lribeiro@ipma.pt

Introduction

The natural richness of Ria Formosa coastal wetlands (South Portugal) provides significant economic resources to the local community, with its impact visible since the late 19th century [1]. Several human activities exist along these areas (aquaculture, salt farming, fishing, etc.) and balancing the interests of the different economical activities and other from environmental protection and recreational use is challenging.

With the aim to better understand these spatial relations, project AQUA&AMBI (phase 1 and phase 2) aimed to identify and rank present-day economic activities in Ria Formosa, in terms of compatibility with aquaculture, by analysing existing legal framework, spatial constrains, and infrastructure development. The resulting ranking system reflects areas where many activities may be developed simultaneously with aquaculture and without major constraints (High convergence), and areas where compatibility is reduced but also no major constraints are identified (Medium convergence). Low convergence areas have significant constraints for economic activities development, with a corresponding low compatibility. The identification of these areas greatly contributes to the creation of sustainable management models for Ria Formosa that can be expanded with further data sources harnessing the flexibility Geographical Information Systems (GIS).

The characterization of the water physical-chemical properties in the proximity of high convergence areas can verify the real potential of these areas for aquaculture development. The present work outlines a short campaign focused on collecting observations during representative seasonal periods (Summer, Autumn, Winter, Spring), providing an indicative description of existing conditions.

Material and methods

Two sampling areas (Fig. 1) were selected based on proximity to high convergence areas related to salt farming ("Bela Mandil" section, 9 points), and earthen ponds aquaculture ("EPPO" section, 8 points). Points distribution was defined to cover the tidal channels in each section, with a maximum distance of 500 m between each point. Sample collection was performed at the peak of high tides (max. 3.6 m in august and min. 3.24 m in January). Physical, chemical, and biological parameters were acquired at different sampling points for the two areas. Samples and data were collected from August 2020 until May 2021 during five campaigns. The water quality parameters and indicators analysed were dissolved oxygen (DO), temperature (T), pH, turbidity, chlorophyll, nutrients (ammonia, nitrites, and nitrates), and suspended particulate matter (SPM). The first five parameters were measured *in situ* with oxygen meter (model HI9829, Hanna Instruments) and a multiparametric probe (model EXO2, YSI). For the remaining parameters, water samples were collected at each sampling station, for nutrients water was filtered on site with Whatman membrane filters (pore size 0.45 μm and 47 mm diameter) using a swing-lock support, and kept refrigerated for further laboratory analysis.

(Continued on next page)

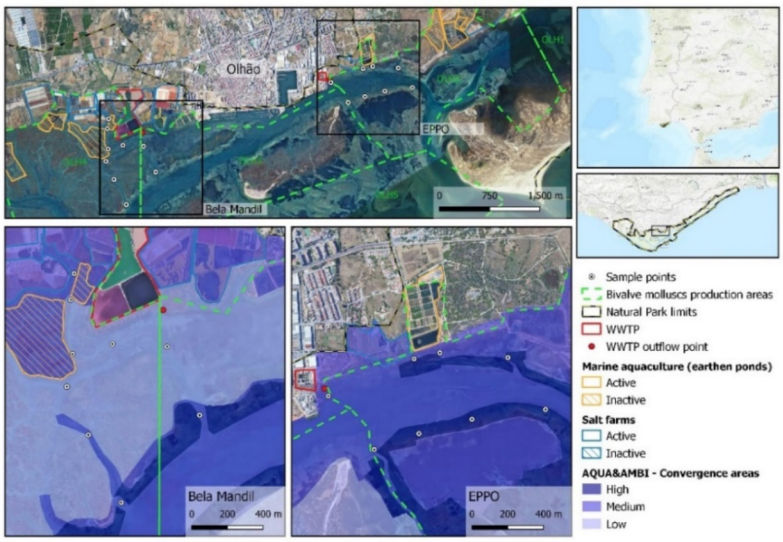


Figure 1 – Convergence areas in Ria Formosa, identified during phase 1 of AQUA&AMBI project, with potential for aquaculture development and other activities without major constraints within legal framework.

Results and Discussion

The studied parameters varied within similar range of values for the different sampling points at both convergence areas, suggesting a quite stable environment. Autumn affected the magnitude and variability between these areas, specially of chlorophyll, with values ranging between 1.60 ± 0.53 μg/L in EPPO area and 0.60 ± 0.30 μg/L at BELA-MANDIL area. Regardless of the convergence area, the lowest and the highest temperature measured were 12.3 in Winter and 22.9 ºC in Summer. Dissolved Oxygen values ranged between 5.28 and 11.4 mg/L, and pH from 7.90 and 9.38. The highest variations observed across both areas and seasons were, in decreasing order, for chlorophyll (from 0.00 to 2.44 μg/L), ammonia (from 0.11 to 1.84 μg/L), and nitrites values (from 0.62 to 49.35 μg/L). No major differences were evident between sampling points regarding the main channel proximity, except for dissolved oxygen in Summer, with lower values (≅ 6.0 mg/L) farthest and higher values (≅ 8.0 mg/L) in the proximity of the channel. The homogeneous water quality at both convergence areas seems to be related with the high hydrodynamic conditions of Ria Formosa Lagoon [2]. The preliminary results evidence the good water quality of the studied areas along the year, which varied within values adequate to rear marine fish and shellfish species common in this region [3]. Understanding either natural or anthropogenic processes around each convergence area will increase the capacity to predict how internal processes in Ria Formosa affect the water quality coming from the sea [2].

References

1.. DOI: 10.1016/j.landusepol.2020.104544
2.. DOI: 10.1016/j.scitotenv.2021.146311
3. DOI:10.1007/978-1-4615-5407-3

Acknowledgments

This study was supported by INTERREG programme through projects AQUA&AMBI's (0240-AQUAAMBI-5-P, 0750-AQUAAMBI 2 -5-P).

CLINICAL CASES OF FISH PATIENTS IN PRIVATE PRACTICE

Presented by Jena Questen DVM CertAqV

World Aquatic Veterinary Medical Association and Aspen Park Veterinary Hospital

fish@drquesten.com

Introduction

Private clinical aquatic animal practice provides the opportunity for the veterinary practitioner familiar with aquatic species to treat a variety of species and diseases. This talk is a selection of interesting cases seen by this aquatic practitioner over the past few years. We will discuss species as varied as Koi, Goldfish varieties, Oscars, and Axolotls. Diseases diagnosed in this practice include husbandry concerns, parasites, and sand impactions. Most cases have radiographs which are useful for learning. This presentation is good for anyone interested in the common diseases and presentations of a variety of fish species.

Materials and Methods

This is a case review of the medical records of multiple fish patients.

Conclusion

There is ample opportunity for learning in the private practice area of aquatic animal medicine. A variety of species and diseases can be encountered at any time which will be of interest to anyone involved in the health and care of aquatic animal species.

FIRST ATTEMPTS OF ONTOGENY DEVELOPMENT IN THE GOLDEN MULLET (*Liza aurata*)

Raquel Quirós-Pozo[1*], Javier Roo-Filgueira[1], Juan Antonio Calderón[2], Sara Ramírez-Bolaños[1], Anais Ventura-Castellano[1], Lidia Robaina[1]

[1]Grupo de Investigación en Acuicultura, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Spain
[2]CIFP Marítimo Zaporito, San Fernando, Spain
*Corresponding author: raquel.quiros101@alu.ulpgc.es (R. Quirós-Pozo)

Introduction

The Mugilidae family presents a great potential for the sustainable diversification of aquaculture, due to their eurythermal, euryhaline, and low trophic nature (Crosetti & Blaber, 2015). Among them, the golden mullet (*Liza aurata*), is an interesting species due to its fast growth in the early stages (Hotos et al., 2020) and good filet quality (Quirós-Pozo et al., 2021). However, the culture of these species continues depending on fry collection from the wild, which produces high mortalities and potential impact in wild populations, so as international interest in mullet's rises, the control of broodstock management and larval rearing increase in importance. In this respect, the knowledge of the sequential timing of appearance and functionality of the different organs and tissues is crucial to develop adequate rearing protocols and feed formulas. In this regard, histological studies are among the major tools to identify the larval critical periods and growth patterns (Sarasquete et al., 2014).

Material and Methods

Broodstock management

125 golden mullets (*Liza aurata*) were captured from a semi-natural estuary in San Fernando (Cádiz, Spain) and stocked in 10 m3 tanks in an open-water system in the facilities of the CIFP Zaporito (San Fernando, Cádiz). Fish were initially sampled to determine the medium size and weight, sex ratio, and the rate of sexual maturation in mature animals. Fish had been fed just from natural feed present in the estuary, being the natural conditions determined at the moment of sampling between 20.9ºC and 23.4Cº (sunrise and sunset respectively) for the temperature, and 7.99 mg/l and 30 ppm for the oxygen level and salinity respectively; photoperiod was approximately 12 hours light and 12 hours darkness.

Larval rearing and samplings

After being acclimated indoor in an open-water system with similar conditions to that of the estuary, natural spawnings were obtained from September to late November. The broodstock was fed for all the period with a commercial extruded feed (R5 Europe, Skretting). Eggs were daily collected and incubated in a 350l tank with soft aeration from the bottom, and seed in the final tanks (1 m3) at 3 dph (days post-hatching). Green water culture was carried out with a 1:1 mix of *Nannochloropsis gaditana* and *Isochrysis galbana*. Rotifers (*Brachionus plicatilis*) were added every day to achieve a total amount of 6-10 rotifers/ml in the tank. Microdiet (Gemma wean, Skretting) was offered from 20 dph, and Artemia sp. nauplii (1-0.5 nauplii/ml) was used when rotifers were retired from 24 to 28 dph. From 28 dph the larvae were fed just with the microdiet. Photoperiod was natural, the temperature ranged from 15.1 to 18.5ºC, and medium values for salinity and oxygen were 30.9±1.9 ppm and 7.3±0.5 mg/l, respectively. Two different spawnings were used to carry out all the samples, the first one (S1), from 0 dph to 9 dph, and the second one (S2) from 5 dph to 16 dph. Samples of larvae were taken every day at the first hour in the morning (before larval feeding) and preserved in 4% formalin for histological analyses (n=10 for S1 and n=20-30 for S2).

Results

Total weight and length of mature fish ranged from 398-150g, 38-26cm, and 564-274g, 43-35cm, for males and females, respectively, being fish able to spawn naturally after one year of stocking at cultured conditions. At hatching, the larvae presented unpigmented eye spots, and the digestive system consisted in a closed tube of cubical simple epithelium. Melanofores covered the different layers along the body. At 1 dph, olfactory vesicles were visible. At 2 dph, retinal cells and eye pigments started to differentiate. Also, the primordial liver and exocrine pancreas could be identified caudoventral to the yolk sac. At 3 dph, the mouth was opened while the yolk sac was mostly reabsorbed, being present the oil globule. Pseudo-stratified epithelium started to differentiate in the primordial stomach and gut. From 6 dph, the digestive system increased in complexity, with intestinal villi well developed and the ileocecal valve well differentiated. The spleen was visible from 7 dph onwards. Liver increased cell organization and lipid deposition, and progressively reabsorbed oil globule until days 11-16. Present results may help to better understand *Liza aurata* physiology and development, and thus to obtain a higher insight to adjust this species culture conditions and feeding pattern.

(Continued on next page)

References

Crosetti, D., & Blaber, S. J. (Eds.). (2015). Biology, ecology and culture of grey mullets (Mugilidae). CRC Press.X

Hotos, G.N., Avramidou, D., 2020. Growth of fry fish of 4 species of family mugilidae in experimental recirculation water system. Eur. J. Biol. Biotechnol. 1 (4). https://doi.org/10.24018/ejbio.2020.1.4.46.

Quirós-Pozo, R., Ventura-Castellano, A., Ramírez-Bolaños, S., Roo-Filgueira, J., & Robaina, L. (2021). Evaluation of Aloe vera by-product against cereals in feeds for golden mullet (Liza aurata). Aquaculture Reports, 20, 100659. https://doi.org/10.1016/j.aqrep.2021.100659

Sarasquete, C., Ortiz-Delgado, J. B., Martos-Sitcha, J. A., Heras, V. D. L., Yúfera, M., & Martínez-Rodríguez, G. (2014). Ontogeny and functional histochemistry of the digestive and visual systems and other organs during the larval development of the thick-lipped grey mullet, Chelon labrosus.

IN VIVO BIOACTIVITY TEST TOWARDS BIOPROSPECTION IN AQUACULTURE INGREDIENTS AND ADDITIVES

S. Ramírez-Bolaños[1]*, R. Urbatzka[2], A. Ventura-Castellano[1], R. Quirós-Pozo[1], A.M.P.B.V. Moreno[3], L. Robaina.[1]

[1]Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, Telde 35214, Spain
[2]CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos s/n, 4450-208, Matosinhos, Portugal
[3]Instituto Nacional de Investigação e Desenvolvimento Agrário (INIDA). CP nº 84 São Jorge dos Órgãos, ilha de Santiago. República de Cabo Verde
E-mail: sara.ramirez@ulpgc.es

Introduction

In the global context of climate change, the reduction and reuse of waste are necessary to reduce the carbon footprint of human industrial processes (FAO, 2018). Aquaculture, and its expected growth in the next decades, should play a fundamental role beyond circular economy by highly promoting the use of more sustainable ingredients and additives from the recirculation of matter. In the Macaronesian region, islands context, several projects are being developed to point out the value of different scalable agricultural by-products for their possible introduction in the aquaculture industry, like Islandap Advance (MAC2/1.1a/299) and Aquacircular (Bioasis/1.1b/086), where this study has been developed. To better understand novel by-products benefits before running large in vivo assays, a in vivo test of the bioactivity present in residues have been developed, to complement their previous characteristics. From the preliminary results obtained with different materials, establishing 3 steps for the bio-prospection & characterization of agricultural by-products result more useful to pre-determine their potential in feeds for aquaculture.

Material and Methods

The characterization of the aloe vera (*Aloe barbadiensis)* by-product (ABP), carob pod (*Ceratonia siliqua*) (CBP) and acacia pod (*Prosopis julifera*) (JBP) were performed following the next steps:

1. Proximate composition was determined following the protocols of AOAC (1995) for moisture, ash and crude protein, and lipids were determined by Folch *et al*. (1957). The fatty acids composition was obtained by transmethylation of total lipids (Christie, 1982) and separated and quantified by liquid chromatography following the protocol of Izquierdo *et al*. (1989).
2. The polyphenol content was obtained following three different protocols (Arranz *et al*., 2009; Hartzfeld *et al*., 2002; Pérez-Jiménez *et al*., 2008; Pérez-Jiménez *et al*., 2009).
3. The in vivo bioactivity was determined in zebrafish larvae (5 dpf). The extractable and hydrolysable fractions were freeze-3.dried and resuspended in DMSO to a final concentration of 50ug/mL. Lipids reducing activity were quantified by Nile Red Assay (Noinart *et al*., 2017), and lipase 6 and protease activity were measured using PED6 kits (Thermo Fisher, USA).

Results and Discussion

All the by-products under these assays had been tested in previous nutrition trials, ABP and PBP were tested in feeds for rohu fingerlings and golden mullets, as an alternative source of carbohydrates (Chovatiya *et al*., 2018; Quirós-Pozo *et al*., 2021); furthermore, CBP use is widely extended in human nutrition (Arribas *et al*., 2019). The results of this experiment are in agreement with previously reported ones; thus, the three by-products showed a high amount of carbohydrates (from 78 to 88%) and a low quantity of lipids (0.98 to 2.93%); ABP has the highest amount of ashes and, PBP and CBP display 8.9% and 6.5% of proteins. Also, they all 3 are rich in palmitic acid, oleic acid and linoleic acid, and ABP moreover presents 14% of DHA. All by-products were demonstrated to be rich in Non-Extractable Polyphenols (NEPP); CBP presented the highest amount of NEPP and, also total polyphenols. These compounds can be responsible for the antioxidant capacity described in CBP (Ibrahim *et al*., 2020), and they also can explain the activity of lipid reduction that was observed in zebrafish in the present trial exposed to the extractable polyphenol fraction of ABP; CBP and JPB presented antagonist effects on protease activity in zebrafish treated with the extractable fractions. In the light of the results obtained, the three by-products are promising for aquafeeds and further and comprehensive in vivo research studies should be performed. On the other hand, the in vivo bioactivity test developed was a useful extra test to better support biochemical analysis and is being better implemented in our labs.

Bibliography

Arranz, S. et al. (2009). High contents of nonextractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. Journal of Agricultural and Food Chemistry, 57, 7298–7303.

Arribas, C. et al. (2019). Extrusion effect on proximate composition, starch and dietary fibre of ready-to-eat products based on rice fortified with carob fruit and bean. LWT, 111, 387-393.

Association of Analytical Communities (1995). Official methods of analysis of AOAC International. Association of Analytical Communities.

Chovatiya, S. et al. (2018). An investigation on the use of Prosopis juliflora pods as a carbohydrate source supplemented with probiotics in the diet of Labeo rohita fingerlings.

Christie, W.W. (1982). Lipid Analysis, vol. 207. Pp 207. Oxford: Pergamon Press.

FAO (2018). Transforming Food and Agriculture to Achieve the SDGs: 20 interconnected actions to guide decision-makers. Technical Reference Document. Pp. 132. Rome, Italy.

Folch, J. et al. (1957). A simple method for total lipid extraction and purification. Journal of Biological Chemistry, 226(497–509), 18.

Hartzfeld, P.W. et al. (2002). Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. Journal of Agricultural and Food Chemistry, 50, 1785–1790.

Ibrahim, R. M. et al. (2020). Utilization of Carob (Ceratonia siliqua L.) Extract as Functional Ingredient in Some Confectionery Products. Food and Nutrition Sciences, 11(8), 757-772.

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T. & Kitajima, C. (1989). Requirement of larval red seabream Pagrus major for essential fatty acids. Nippon Suisan Gakk., 55, 859–867.

Noinart, J. et al. (2017). A new ergosterol analog, a new bis-anthraquinone and anti-obesity activity of anthraquinones from the marine sponge-associated fungus Talaromyces stipitatus KUFA 0207. Marine drugs, 15(5), 139.

Pérez-Jiménez, J. et al. (2009). Proanthocyanidin content in foods is largely underestimated in the literature data: an approach to quantification of the missing proanthocyanidins. Food Research International, 42, 1381–1388.

Pérez-Jiménez, J. et al. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Research International, 41, 274–285.

Quirós-Pozo, R. et al. (2021). Evaluation of Aloe vera by-product against cereals in feeds for golden mullet (Liza aurata). Aquaculture Reports, 20, 100659.

NEW TOOL FOR ENVIRONMENTAL FOOTPRINT CALCULATION OF THE MEDITERRANEAN AQUACULTURE PRODUCTS

Saioa Ramos[1*], Maite Cidad[1], Lohitzune Larrinaga[2], Miguel Angel Cuevas[2]

1 AZTI. Parque Tecnológico de Bizkaia. Astondo Bidea, Edificio 609. E-48160 Derio, Spain
2 INGENET Polígono Industrial Torrezar, Edificio 3, Nave 2, 48410 Orozko, Spain

Email: sramos@azti.es

Introduction

Aquaculture is playing, and will continue to play, a significant role in boosting global fish production and in meeting rising demand for fishery products. A recent session of the FAO Committee on Fisheries stressed the increasingly key role of aquaculture in fish production for human nutrition and poverty alleviation in many rural areas. The huge growth expected for aquaculture products makes necessary a more sustainable aquaculture development to mitigate the environmental impacts linked to this growth. Aquaculture can contribute to the objective of filling the gap between EU consumption and production of seafood in a way that is environmentally, socially and economically sustainable (COM (2013)229). Indeed, when practicing responsibly, fish farming can help provide livelihoods and feed a global population that will reach nine billion by 2050.

In 2013 European Commission proposes the Product Environmental Footprint (PEF) and Organization Environmental Footprint (OEF) methods as a common way of measuring environmental performance (COM2013/179/EU). To validate the methodology, a series of pilots for different products are developing, and Marine Fish was selected as one of the 11 food products. The package establishes the Life Cycle Assessment (LCA) methodology to measure environmental performance of European products and organizations.

Within this framework the LIFE+ AQUAPEF project was launched in 2018. The aim of the project is to provide the first software tool specifically designed for Mediterranean aquaculture sector that enables the calculation in an easy way of a harmonized environmental impact, complying PEF methodology.

Methodology

Based on the method described in Marine Fish PEF category rules, where main steps for a common use of LCA in marine fish are established, a webtool to facilitate the calculation of environmental footprint of Mediterranean aquaculture products has been developed.

The webtool gather the information of the main steps of the Mediterranean offshore aquaculture sector: feed production, hatchery, growing, preparation and distribution. Moreover, it also considers consumption and end-of-life based on average data.

AQUAPEF tool provides a common framework in which users from different stages of the supply chain introduce a series of production data. To facilitate the data gathering, the tool offers the possibility to send the questionnaires to the main suppliers of the chain. This data is confidential, and it will be visible to the user just if the suppliers give the authorization for that.

The collected inventory data is then transformed into the 16 environmental impact categories recommended by the International reference Life Cycle Data system (Fazio et al., 2018) based on the PEF-compliant public datasets. As far as the aim of the tool is to provide a system for the SME's, it has been designed as user friendly and very intuitive. The effectiveness and usefulness of the tool has been validated with the companies of the AQUAPEF project.

(Continued on next page)

Results and discussion

The webtool has been developed during 2020. Three main section has been distinguished:

- User profile: The first section of the tool comprises the creation of company-profile where the company interested in the assessment of the environmental footprint should include the information required for the evaluation of the environmental footprint, such as location of facilities or product/species produced.

- Questionnaire: Afterwards a second section is enabled, where an easy-to-understand questionnaire is presented for each type of stakeholder. As such, feed producers should include data regarding plant and animal-based ingredients (quantities and origin), energy and water consumption and packaging requirements. Hatcheries should include inventory data regarding feed, energy and water consumption, while Growing Farms should introduce data related to feed, energy, packaging and net maintenance material consumption, together with waste and biowaste management data. Finally processing facilities should include data regarding the energy, water and packaging requirements, together with the waste and wastewater quantities.

  A model based on Bringolin et al., 2014 has been implemented in the tool in order to account for the N, P and C emissions of the fish metabolism and faeces.

- Results: In the result section, users could obtain the full Environmental Footprint of their products. A specific table with hot-spot assessment is also including.

Finally, they will be able to compare their results with average values for their sector.

The tool has been validated in 3 case studies by comparing the outcome of the AQUAPEF tool with the calculations performed with SimaPro 9.1 commercial software. Results confirms that the tool calculates the EF correctly. Additionally, based on a qualitative questionnaire, potential improvements to increase the user-friendliness of the tool have been identified. For instance, automatic data gathering from ERP and benchmark possibilities will be added to ensure the usability.

Conclusion

The AQUAPEF webtool is currently in beta-testing stage and is expected to be commercialized by 2023. We have validated that this tool could promote the calculation of the environmental footprint linked to the aquaculture products. Moreover, the obtained results could be also used to improve the environmental performance of the aquaculture products. Main barriers for a large-scale implementation are, on the one hand, the amount and the quality of data required to feed the tool and, on the other hand, the lack of certification schemes which guaranteed that obtained results reflects the real consumption and waste ratios.

Acknowledge

This project is co-funded by LIFE European Environment Programme (LIFE17 ENV/ES/000193), which is the EU's financial instrument supporting environmental, nature conservation and climate action projects throughout the EU.

References

Brigolin, D., Meccia, V. L., Venier, C., Tomassetti, P., Porrello, S., & Pastres, R. (2014). Modelling biogeochemical fluxes across a Mediterranean fish cage farm. Aquaculture Environment Interactions, 5(1), 71-88.

Fazio, S., Castellani, V., Sala, S., Schau, EM., Secchi, M. and Zampori, L. (2018) Supporting information to the characterisation factors of recommended EF Life Cycle Impact Assessment methods, EUR 28888 EN, European Commission, Ispra, 2018, ISBN 978-92-79-76742-5, doi:10.2760/671368, JRC109369.

DIETARY INCLUSION OF *Chlorella vulgaris* AND *Acutodesmus obliquus* MODULATES THE RAINBOW TROUT (*Oncorhynchus mykiss*) IMMUNE STATUS

L. Ramos-Pinto[1*], C. Teixeira[1,2,4], D. Peixoto[1,4], A. C. M. Rodrigues[5], R. Rocha[5,6], P. Rema[1,3], J. Henriques[2], J. Dias[2], L.E.C. Conceição[2], B. Costas[1,4]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal
[2] SPAROS Lda., Área Empresarial de Marim, Lote C, Olhão, Portugal
[3] UTAD - Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Vila Real, Portugal
[4] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[5] CESAM – Centro de Estudos do Ambiente e do Mar, Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal
[6] Riasearch Unipessoal Lda., Murtosa, Portugal

*E-mail: lourenco.pinto@ciimar.up.pt

Introduction

In recent decades, research on species-specific and tailormade feeds that potentiate not only fish growth but also their welfare condition and immune status, has been recognized by the aquaculture industry. Reduced reliance on fish meal and oil in the formulations of diets for farmed fish is a priority to sustain aquaculture growth, which led the feed industry to explore alternative sources. In this regard, owing to their chemical composition, microalgae appear as a promising alternative aimed at enhancing the nutritive value of new feed formulations. Microalgae have gained considerable importance in recent decades due to potential exploitation in several markets (Odjadjare et al, 2017). Hence, some waste can be used for feed production following the idea of circular bio-economy. Microalgae have antioxidant properties, high-quality dietary protein and are a source of bioactive compounds (Shields and Lupatsch, 2012). Nonetheless, information about their use in diets for salmonids species, particularly in rainbow trout (*Oncorhynchus mykiss*) is still scarce. Hence, this work aimed to evaluate whether *Acutodesmus obliquus* and *Chlorella vulgaris* inclusion in aquafeeds could lead to optimal growth and healthy fish.

Materials and methods

Rainbow trout (32.6 ± 0.11 g) juveniles were obtained from a commercial hatchery (Trutalcôa Viveiros Lda) and maintained under a quarantine period of 2 weeks at the experimental research station of the University of Trás-os-Montes e Alto Douro (UTAD, Portugal). Triplicate groups of 30 fish were fed one of the four experimental diets for 60 days, three times a day. Four diets were tested in triplicates: a good quality commercial formula, positive control (PC), a circular economy driven formula without microalgae (NC) and a similar NC formula with 5% inclusion of *Acutodesmus obliquus* (ACUT5) or *Chlorella vulgaris* (NCHLO5). All these diets (2.5mm) were produced at SPAROS facilities and are isoenergetic, isonitrogenous and isolipidic. At 15 days (early) and 60 days (final sampling) of feeding the experimental diets, 18 fish from each experimental group, 6 per replicate tank were euthanized by an overdose of anaesthetic and weighed. Thereafter, liver was collected to assess hepatic oxidative stress status, whereas head-kidney (HK) was suited for gene expression analysis (both at 15 days and 60 days feeding period). Plasma samples were only collected after 60 days of feeding to evaluate humoral immune parameters.

Results

No mortalities were observed throught the feeding trial. Regarding plasma humoral parameters at the final sampling point (60 days), no differences were observed in plasma bactericidal activity and anti-proteases activity. However, IgM values increased in fish fed ACUT5 compared to those fed the PC diet. Regarding hepatic oxidative stress responses, rainbow trout fed ACUT5 for 15 days had higher catalase (CAT) activity than those fed other dietary treatments. While also during this short feeding period, fish fed ACUT5 presented lower total glutathione (tGSH) content compared to those fed PC and NC. After 60 days of feeding, no differences were observed on CAT activity and tGSH content. Though, glutathione S-transferase (GST) and lipid peroxidation (LPO) increased in fish fed NCHLO5 and PC diets, respectively, compared to those fed all other dietary treatments. LPO also increased in fish fed ACUT5 compared to those fed PC and NC. Regarding HK gene expression data, most differences were observed after 60 days of feeding. Fish fed NCHLO5 presented an upregulation of *il-8, pcb* and *cath* compared to NC diet. Also, fish fed NCHLO5 increased *il1b* expression compared to PC diet. Trout fed the ACUT5 diet increased *cd3* and *cath* transcripts compared to those fed NC diet.

(Continued on next page)

Discussion and conclusion

Several studies have already reported data regarding the effects of microalgae inclusion in diets of farmed fish species. However, few studies investigated their effects on health status and oxidative stress of rainbow trout. The results obtained in this trial suggest that the inclusion of both *A. obliquus* and *C. vulgaris* at 5% of feed does not negatively affect the rainbow trout survival and modulates immune status. In the present study, tGSH decreased in fish fed ACUT5 compared to PC and NC dietary treatments after 15 days. Peixoto et al., 2021 suggested that natural compounds present in microalgae can have advantages, decreasing the dependency on glutathione metabolism. Additionally, the antioxidant enzyme catalase, involved in key oxidative defence mechanisms, increased in trout fed ACUT5 after 15 days. Plasma IgM increased in fish fed diets with 5% inclusion of *A. obliquus* for 60 days, suggesting that this dietary treatment could boost the acquired arm of the immune system. Likewise, fish fed ACUT5 and NCHLO5 for 60 days displayed an immunostimulant state observed by the upregulation of the pro-inflammatory cytokines *il1b* and *il-8*. Also, the T cell marker, *cd3* was upregulated in fish fed ACUT5 compared to those fed control diets. This study suggests that inclusions of 5% of *A. obliquus* or *C. vulgaris* in diets for rainbow trout can represent a viable alternative to substitute other more recurring protein sources, ultimately leading to satisfactory performance and health status.

Acknowledgements

This work is part of project E!12463 MOONSHINE_40812, supported by EUROSTARS-2 programme, and by Portugal and the European Union through FEDER/ERDF and CRESC Algarve 2020, in the framework of Portugal 2020. This work is also a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). BC and CT were supported by FCT - Foundation for Science and Technology (IF/00197/2015 and PD/BDE/135541/2018, respectively).

References

Odjadjare EC, Mutanda T, Olaniran AO. Potential biotechnological application of microalgae: a critical review. Crit Rev Biotechnol. (2017) Feb;37(1):37-52.

Shields RJ, Lupatsch I. Algae for aquaculture and animal feeds. Technikfolgenabschätzung – Theorie und Praxis (2012) 21:23–37

Peixoto, D., Pinto, W., Gonçalves, A.T. *et al*. Microalgal biomasses have potential as ingredients in microdiets for Senegalese sole (Solea senegalensis) post-larvae. J Appl Phycol (2021).

INCLUSION OF *Salicornia ramosissima* BIOMASS IN DIETS FOR JUVENILE PACIFIC WHITE SHRIMP (*Penaeus vannamei*) SEEMS TO MODULATE THE IMMUNE RESPONSE

L. Ramos-Pinto[1*], M. Machado[1], S. Fernández-Boo[1], C. Teixeira[1,3,4], J. Dias[3], A. Barreto[2], R. Serradeiro[2], R. Rocha[2], B. Costas[1,4]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal
[2] Riasearch Unipessoal Lda, Murtosa, Portugal
[3] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[4] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
*E-mail: lourenco.pinto@ciimar.up.pt

Introduction

The Pacific white shrimp (*Penaeus vannamei*) is one of the principal crustacean species cultured worldwide, particularly in coastal areas of Pacific Ocean (Huang et al., 2016). This species has been affected by disease outbreaks worldwide, mostly caused by virus and bacteria, due to poor water quality and stressful rearing conditions, with important economic losses and serious mortality outbreaks (Kalaimani et al., 2013). Halophyte plants such as *Salicornia ramosissima* have the ability to grow in saline soils (marginal land) and/or be irrigated with seawater. The green tips of Salicornia are sold as food, while the woody part is considered a residue. However, this residue biomass is rich in valuable bio-active molecules that can be extracted using simple and affordable processing. These compounds include hydroxycinnamic acids (strong anti-inflammatory, and antioxidant), high quality protein, omega 3 and 6 fatty acids, carotenoids, and chlorophyll (Buhmann and Papenrock, 2013). The present study aimed to valorise the utilization of this residue in a circular economy concept by the inclusion of *S. ramosissima* biomass in diets for shrimp. For this purpose, the immunological profile, inflammatory response and disease resistance of shrimp fed diets rich in Salicornia were assessed.

Materials and methods

Pacific white shrimp (6.07 ± 0.05 g) were randomly distributed in 25 tanks (200L) and maintained in a RAS system (temperature was maintained at 28.1 ± 0.5 ºC, dissolved oxygen at 5.9 ± 0.3 mg L^{-1}, salinity at 19.4 ± 0.1 g L^{-1} and pH at 7.6 ± 0.1). Water parameters were measured daily. Five diets were tested in quintuplicates: a commercial like diet (CTRL) and 4 experimental diets including *S. ramosissima* stems (SS5 and SS10) or *S. ramosissima* leaves and seeds (SL5 and SL10) both in two different concentrations (5 and 10%, respectively). Shrimp were given 4 meals per day. In order to evaluate shrimp immune condition, haemolymph and hepatopancreas were collected from 3 shrimp per tank on days 31 (middle) and 55 (end of the experiment) to assess immune condition. After the 55 days feeding period, shrimps were bath challenged with *Vibrio parahaemolyticus*. 25 shrimp were immersed in tanks (20 L) in triplicates per diet with the inoculum adjusted to a final concentration of 2 x 10^8 cfu ml^{-1}, with strong aeration for 1 hour. Afterwards, shrimp were moved to new tanks with clean water and the recirculation system was re-established. After that, shrimp were fed according to the previous regime and 5 individuals from each tank (15 per group) were sampled for haemolymph collection at 4, 24 and 48 hours after the challenge period in order to assess cell proliferation and plasma immune parameters.

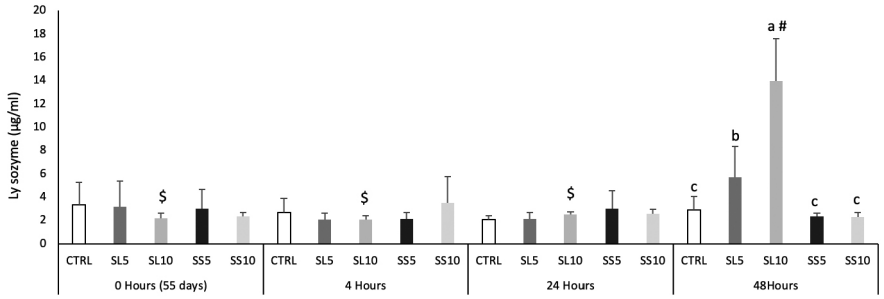


Figure 1. Lysozyme activity measured in plasma of Pacific white shrimp fed the dietary treatments at 55 days (0 hours) and at 4, 24 and 48 hours post-challenged with *Vibrio parahaemolyticus*. Values are expressed as means ± SD (n=15). Lower case letters indicate differences between diet within the same time. Symbols indicate differences between time within the same dietary treatment.

(Continued on next page)

Results

In terms of immune condition, no changes in haemocyte number among dietary treatments were observed after both 31 and 55 days of feeding. However, some modulation of the humoral parameters from haemolymph and gene expression of hepatopancreas were observed. A decrease of lysozyme and nitric oxide production was observed in diets containing an incorporation of *S. ramosissima* biomass while an increase of the antioxidant enzyme glutathione peroxidase expression in hepatopancreas was found up-regulated in shrimp fed SS10 after 31 days of feeding. Nonetheless at 55 days no clear changes were observed on the parameters analysed among dietary treatments. In response to the bath bacterial challenge with *V. parahaemolyticus*, haemocytes in circulation decreased following exposure to the pathogen regardless dietary treatment. Also, shrimp fed SL5 showed a higher production of nitric oxide than those fed SS10 at 24h. Lysozyme activity at 48h post infection increased in shrimp fed SL5 and SL10 when compared to those fed CTRL, SS5 and SL10 diets (Fig. 1). Moreover, shrimp fed SS5 dietary treatment displayed a significant higher cumulative mortality than the individuals fed the SL5 and CTRL diets after 5 days.

Discussion and conclusion

The present study suggests that the inclusion of *S. ramosissima* biomass in diets for juvenile shrimp does not clearly affect immune condition. Nonetheless, once immune mechanisms were activated by the presence of bacteria, the inclusion of 5% *S. ramosissima* seeds and leaves (SL5) seemed to modulate the inflammatory response resulting in a lower disease resistance to *V. parahaemolyticus* compared to the commercial like diet. Moreover, during the inflammatory response, an inclusion of 10% of leaves and seeds (SL10) was able to modulate shrimp humoral immune response by increasing lysozyme activity. Preliminary data from the present study point to health-promoting effects through the inclusion of *S. ramosissima* biomass in shrimp feeds, contributing to the principles of circular economy and avoiding waste of resources. Hepatopancreas gene expression is also being performed in fish sampled after the inflammatory stimulus.

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 86283 (project AQUACOMBINE). This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein. BC and CT were supported by FCT - Foundation for Science and Technology (IF/00197/2015 and PD/BDE/135541/2018, respectively).

References

Huang Z., Li X., Wang L., Shao Z. (2016). Changes in the intestinal bacterial community during the growth of white shrimp, *Litopenaeus vannamei*. Aquaculture Research, 47, 1737-1746;

Kalaimani N., Ravisankar T., Chakravarthy N., Raja S., Santiago TC., Ponniah AG. (2013). Economic losses due to disease incidences in shrimp farms of India. Fishery Technology, 50, 80-86;

Buhmann Anne, Papenbrock Jutta (2013) An economic point of view of secondary compounds in halophytes. *Functional Plant Biology* **40**, 952-967.

IMPLEMENTATION OF RECOMBINANT GONADOTROPIN THERAPIES TO CONTROL MATURATION FROM EARLY GAMETOGENESIS THROUGH TO SPAWNING OF COMMERCIALLY VALID NUMBERS OF VIABLE LARVAE OF FLATHEAD GREY MULLET (*Mugil cephalus*)

Sandra Ramos-Júdez[1], Ignacio Giménez[2]*, Josep Gumbau-Pous[1], Lucas Stephen Arnold-Cruañes[1], Alicia Estévez[1], François Chauvigné[3], Joan Cerdà[3], and Neil Duncan[1]*

[1]IRTA Sant Carles de Ràpita, 34540 Sant Carles de Ràpita, Tarragona, Spain
[2]Rara Avis Biotec, S. L., Valencia, Spain.
[3]IRTA-Institute of Biotechnology and Biomedicine (IBB), Universitat Autònoma de Barcelona, Parc de Recerca UAB, Mòdul B, E-08193 Bellaterra, Barcelona, Spain
*neil.duncan@irta.cat

Introduction

The flathead grey mullet represents a potential candidate in the diversification of European aquaculture products mainly in the Mediterranean area. However, flathead grey mullet broodstock held in intensive conditions show reproductive dysfunctions; females remain arrested at previtellogenesis (Ramos-Júdez et al., 2021) or early stages of vitellogenesis (Aizen et al., 2005) and males with fluent milt are not usually observed (Aizen et al., 2005; De Monbrison et al., 1997; Ramos-Júdez et al., 2021). In a previous study, the application of a long-term treatment with species-specific single-chain recombinant gonadotropins (rGths), recombinant follicle-stimulating (rFsh) and luteinizing (rLh) hormones, produced in CHO cells, and the use of rLh and Progesterone (P_4) at the later maturation stage was successful for obtaining eggs from females arrested at early stages of gametogenesis and sperm in males with no running milt that were used for *in vitro* fertilization. Nevertheless, low fertilization percentages (< 1%) were obtained (Ramos-Júdez et al., 2021). The present study aimed to demonstrate that long-term treatment with rFsh and rLh can result in eggs with higher fertilisation rates than the previously described.

Materials and Methods

The flathead grey mullet broodstock (mixed wild and from semi-extensive culture held for 1.5 to 3.5 years in intensive conditions) remained under natural light with 36 ‰ salinity water at 24ºC in one 10m^3 tank in recirculation (IRTAMar®) during the experiment, timed to be in the natural spawning season in summer. Maturity was determined from ovarian biopsies and ease to obtain sperm. A total 21 of females and 9 males received weekly treatment with species-specific single-chain recombinant gonadotropins (rGths) while 9 control females and 6 control males received weekly saline injections. The pattern of application of rFsh and rLh aimed to mimic the physiological fluctuations of Fsh and Lh; rFsh during first stages of gametogenesis followed by a subsequent decrease with an increase of rLh to regulate late gametogenesis (Lubzens et al., 2010; Schulz et al., 2010). Doses ranged from 6 to 12 μg kg^{-1} for rFsh and from 2.5 to 12 μg kg^{-1} for rLh. Females with completed vitellogenic growth (oocytes near to 600 μm in diameter) and males with running milt were selected for spawning induction. The individuals were stocked in a separate 10m^3 tank per treatment with the same conditions than the holding tank. To induce maturation, ovulation and spawning, females were treated with either (i) a priming dose of 30 μg kg^{-1} of rLh and a resolving dose of 40 mg kg^{-1} of P_4 as in Ramos-Júdez et al. 2021, (ii) priming and resolving doses of 30 μg kg^{-1} of rLh, or (iii) priming and resolving doses of 40 mg kg^{-1} of P_4 given 24:05 ± 0:40 h apart. One male from each spawning tank received a dose of 24 μg kg^{-1} of rLh while the others followed the weekly treatment. Spawning events had a sex ratio of 1:2 or 1:3 (female:male). Eggs were collected by surface out-flow egg collectors. Fertilization and hatching were examined and larvae survival was monitored in 96-well plates at 21 ºC until all hatched larvae had died.

Results

At the beginning of the experiment, 12 of the females (57%) belonging to the treated group were in previtellogenesis and 9 (43 %) in early vitellogenesis. All these females (100% of n = 21) completed vitellogenic growth with 603 ± 8 μm diameter of the most developed oocytes after 6 to 13 weeks of rGths treatment. Fertilized spawns were obtained after inducing with rLh + P_4 or rLh + rLh (priming and resolving injections) with a spawning success of the 85% (8 of 9 attempted) and 100% (n = 6), respectively. All treated males were spermiating. The eggs obtained after both treatments presented 64 ± 22 % embryo survival and 58 ± 25 % hatching percentages. The mean fecundity of the females with fertilized spawns was 1,245,600 ± 552,117 eggs kg^{-1} bw (~ 1,700,000 eggs female^{-1}). The percentage survival of larvae in 96-well plates was 67 ± 18 % at 9 days post hatching. The treatment P_4 + P_4 for maturation induction had a lower ovulation success (50%) and spawning success (17%) with no fertilized eggs. Success was independent of the initial gonadal stage of females (previtellogenesis or early vitellogenesis). In comparison, control females did not show any advance in gonadal development from initial stages (remained at previtellogenesis or developed atresia in some of the early vitellogenic oocytes) and control males did not produce fluent sperm.

(Continued on next page)

Discussion and Conclusion

The present study indicates that oogenesis from previtellogenesis until spawning can be driven by the application of exogenous species-specific rFsh and rLh in a teleost species, as 100% of flathead grey mullet females completed vitellogenic growth, and maturation, ovulation and spawning were successfully achieved with the application of rLh as priming and resolving injections. The use of single-chain recombinant gonadotropins produced in CHO cells as a treatment to induce oogenesis from previtellogenesis or stimulate vitellogenesis to the completion of oocyte growth with high spawning success and egg quality as described in the present study offers reliability and replicability to control reproduction of the flathead grey mullet. The obtention of tank spawning contrasts with the study by Ramos-Júdez et al. (2021) in which eggs were hand stripped as no spawning was observed. The presence of good quality males with very fluent sperm in the tank in the present study may have played an important role in the stimulation of spawning and the spawning success. The fecundities and larval survivals obtained in the present study from flathead grey mullet females, indicate that the induction of 6 - 7 females (~1 kg) per season could enable a hatchery production of ~ 1 million fry. In addition, it would be possible to apply the present protocol to develop out-of-season breeding programs in this species.

Acknowledgements: Thanks to Cristian Martínez Rodríguez and the IRTA staff for technical help. This study was funded by the Spanish Government, MINECO, project: RTI2018-094710-R-I00

References

Aizen, J., Meiri-Ashkenazi, I., Tzchori, I., Sivan, B., Rosenfeld, H., 2005. Enhancing spawning in the grey mullet (Mugil cephalu) by removal of dopaminergic inhibition. Gen. Comp. Endocrinol. 142, 212–221. https://doi.org/10.1016/j.ygcen.2005.01.002

De Monbrison, D., Tzchori, I., Holland, M., Zohar, Y., Yaron, Z., Elizur, A., 1997. Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, (Mugil cephalus): Preliminary studies. Bamidjeh 49, 214–221.

Lubzens, E., Young, G., Bobe, J., J, C., 2010. Oogenesis in teleosts: How fish eggs are formed. Gen Comp Endocrinol. 165, 367–389. https://doi.org/10.1016/j.ygcen.2009.05.022

Ramos-Júdez, S., Chauvigné, F., González-López, W.Á., Rosenfeld, H., Cerdà, J., Giménez, I., Duncan, N., 2021. Providing recombinant gonadotropin-based therapies that induce oogenesis from previtellogenic oocytes to produce viable larvae in a teleost, the flathead grey mullet (Mugil cephalus). Aquaculture 736418. https://doi.org/https://doi.org/10.1016/j.aquaculture.2021.736418

Schulz, R.W., de França, L.R., Lareyre, J.-J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411. https://doi.org/https://doi.org/10.1016/j.ygcen.2009.02.013

TRANSCRIPTOME ANALYSIS OF *Mugil cephalus* OVARIAN DEVELOPMENT INDUCED BY RECOMBINANT GONADOTROPINS

S. Ramos-Júdez[1], T. Manousaki[2], T. Danis[2], N. Angelova[2], A. Tsakogiannis[2], I. Giménez[3], N.J. Duncan[1*] and C. Tsigenopoulos[2*]

[1]IRTA-Sant Carles de la Ràpita, 43540 Tarragona, Spain
*neil.duncan@irta.cat
[2]Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (H.C.M.R.), 71003 Heraklion, Greece
*tsigeno@hcmr.gr
[3]Rara Avis Biotec, S.L., 46002 Valencia, Spain

Introduction

The flathead grey mullet (*Mugil cephalus*) exhibits a severe reproductive dysfunction in intensive captive conditions that arrests gametogenesis in the early stages. Homologous single-chain recombinant gonadotropins (rGths) produced in Chinese Hamster Ovary (CHO) cells provided a solution to the dysfunction. Treatment with recombinant follicle stimulating hormone (rFsh) followed by recombinant luteinizing hormone (rLh) has been used to induce the entire process of vitellogenesis in order to produce viable eggs and larvae (Ramos-Júdez *et al*., 2021), with up to 80 % egg fertilization and a mean of 96 % hatching from fertilized eggs (unpublished data). Considering these promising results by the application of rGths to induce complete oogenesis in the flathead grey mullet, the present study aims to characterize the molecular pathways and temporal gene expression patterns throughout the rGths-induced vitellogenesis.

Materials and Methods

Oogenesis was induced from previtellogenic stages through to oocyte maturation and ovulation as described by Ramos-Júdez *et al*., (2021). Repeated ovarian samples were collected by cannulation from the same five females at four sampling points in this induced ovarian development; (i) from initial arrested gonad before rGths application (previtellogenesis at primary growth and cortical alveoli stage), (ii) from early-mid vitellogenic follicles after rFsh administration, (iii) from late secondary-growth follicles after combined treatment with rFsh and rLh, and (iv) from full-grown follicles after rLh administration that completed the vitellogenic growth. The RNASeq libraries were constructed for all the aforementioned stages, sequenced on an Illumina HiSeq4000 and a *de novo* transcriptome assembly was constructed. Quality check of raw reads was performed using FastQC v0.11.8, and reads were pre-processed through a pipeline using Trimmomatic v0.39. Trimmed reads from all gonadal stages were assembled together to obtain a single transcriptome. Trinity software v2.8.5 was used to construct the *de novo* assembly and BUSCO v3.1.0 was utilized to assess the transcriptome completeness (the vertebrate orthologs database was used as reference). Trimmed reads were back-mapped to the assembly with Bowtie2, and the relative abundances of transcripts were calculated by means of RSEM. Transcripts with less than 1 FPKM were excluded. The assembled transcripts were functionally annotated using Trinotate pipeline v3.2.1 with e-value cut-off of 10^{-5}. The SwissProt, GO and KEGG databases were employed to annotate the sequences that formed the final assembly. Count data matrix from the filtered transcriptome was constructed and imported in R v3.6.1. Genes with < 30 reads in all samples were excluded from the differential expression gene analysis which was performed by DESeq2 v1.26.0 under the Bioconductor package. Pairwise differential expression analyses were performed with special attention to comparisons of gonadal stages in time lap sequence. Genes with an adjusted P-value < 0.05 were considered as statistically significant differentially expressed genes.

Results and Discussion

Illumina HiSeq4000 paired-end sequencing generated a total of 614,942,156 raw paired reads of which 506,875,944 paired reads were maintained after trimming. The assembled transcriptome produced by Trinity consisted of 513,643 transcripts with an average contig length of 919.18 nucleotides (nt) and N50 value of 1,561 nt. BUSCO revealed an 86.4% of transcriptome completeness. Moreover, an average of 89.68 % of the reads were back-mapped on the transcriptome. The final assembly was constituted of 287,089 transcripts with an expression value of FPKM≥1. The BLASTx against the Swiss-Prot databases resulted in 58,306 (20.3 %) transcript gene assignments. A total of 57,021 (19.9 %) sequences had a match against the GO database, of which 50,268 (88.2 %) represented biological processes, 53,992 (94.7 %) were associated with cellular components, and 48,190 (84.5 %) matched molecular function (MF). A total of 51,237 (17.8 %) sequences were associated to a KEGG pathway. The analysis of differentially expressed genes (DEGs) throughout oogenesis showed that 6147 genes were significantly up-regulated in the stage from previtellogenic follicles to vitellogenic

(Continued on next page)

follicles obtained after rFsh treatment. From vitellogenic follicles to late-vitellogenic follicles, obtained after rFsh and rLh combined treatment, 814 genes were up-regulated, while 994 genes were up-regulated in the transition from late-vitellogenic to full-grown follicles after rLh application. The corresponding numbers for downregulated genes were 2807, 299, and 593, respectively. Throughout oogenesis more genes were up-regulated than down-regulated. To gain insight into the biological roles of the most significantly up- or down-regulated genes, DEGs will be linked with GO terms and classified into categories. In addition, DEGs will be mapped to the reference pathways of KEGG.

The analysis will examine a wide range of genes focusing on those related to the Fsh/Fshr and Lh/Lhcgr pathways that may reflect temporally distinct functions during vitellogenesis, such as genes associated with electron transport chain, estrogen receptors, mitochondrial function, lipid uptake and oocyte maturation. This study will enable a better understanding of rGths-targeted genes to improve induction protocols and facilitate the control of flathead grey mullet reproduction as well as of other species with similar reproductive dysfunctions.

Acknowledgements: The study was funded by the European Union's Horizon 2020 research and innovation program under grant agreement No. 652831 (AQUAEXCEL2020). All computations were performed at the high-performance computing bioinformatics platform of HCMR (Crete, Greece). Ovary samples were provided by the Project RTI2018-094710-R-100 funded by the Spanish Ministerio de Ciencia, Innovación y Universidades. Participation of S.R. was funded by a PhD grant from AGAUR (Government of Catalonia) co-financed by the European Social Fund.

References

Ramos-Júdez, S., Chauvigné, F., González-López, W.Á., Rosenfeld, H., Cerdà, J., Giménez, I., Duncan, N., 2021. Providing recombinant gonadotropin-based therapies that induce oogenesis from previtellogenic oocytes to produce viable larvae in a teleost, the flathead grey mullet (*Mugil cephalus*). Aquaculture 736418. https://doi.org/https://doi.org/10.1016/j.aquaculture.2021.736418

CAN *ARTEMIA* PRODUCE ESSENTIAL FATTY ACIDS? ROLES OF ELONGASES IN THE BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Marc Ramos-Llorens[1*], Alberto Ribes-Navarro[1], Juan C. Navarro[1], Francisco Hontoria[1], Naoki Kabeya[2] and Óscar Monroig[1]

[1] Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain
marc.ramos@csic.es
[2] Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo, Japan

Introduction

Artemia nauplii are one of the most commonly used live preys in marine larviculture. However, a major drawback associated to using *Artemia* nauplii is their deficient nutritional value for marine fish larvae that is primarily linked to suboptimal levels of long-chain (C_{20-24}) polyunsaturated fatty acids (LC-PUFA) (Sorgeloos et al., 2001). LC-PUFA including eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) are essential compounds for normal development of vertebrates including fish (Monroig and Kabeya, 2018). Since *Artemia* contains low LC-PUFA, with only trace levels of DHA, nauplii must be enriched with LC-PUFA-rich products prior use as live prey for marine larvae. However, it remains unknown whether *Artemia* has some capacity for LC-PUFA biosynthesis itself (Ito and Simpson, 1996; Reis et al., 2013). Elongases (Elo) are key enzymes in the LC-PUFA biosynthetic pathway that catalyse the limiting step reaction in the fatty acid (FA) elongation pathway resulting in a 2-carbon extension of the FA substrate (Monroig and Kabeya, 2018). In the present study, we aimed to clarify the repertoire and function of Elo with putative roles in the LC-PUFA biosynthesis of *Artemia franciscana*, arguably the most commonly used *Artemia* species in aquaculture.

Materials and methods

To collect the full-length sequences of candidate elongases from *A. franciscana*, putative transcripts were retrieved through *tblastn* searches against Sequence Read Archive (SRA) database in the *A. franciscana* BioProject (PRJNA524488). Putative sequences of the encoded enzymes were analysed to identify specific domains and conserved motives (Hashimoto et al., 2008). Six Elo gene candidates were identified and selected for further functional characterisation in yeast. Briefly, the open reading frames (ORF) of each Elo were amplified by PCR using *A. franciscana* complementary DNA (cDNA) as template and with primers containing specific restriction sites designed for further cloning into pYES2 expression vector. Purified pYES2-ORF constructs were transformed in yeast that were subsequently supplemented with potential PUFA substrates. After two days at 30ºC, transgenic yeast was harvested and washed. Total lipids were extracted and processed to prepare fatty acid methyl esters for analysis by gas chromatography. Conversions of PUFA substrates to the corresponding products were calculated by the proportion of substrate FA converted to elongated FA product(s) as [areas of all products with longer chain than substrate/(areas of all products with longer chain than substrate + substrate area)] × 100.

Results

Six elongases, termed Elo1 to Elo6, were identified, cloned and characterised. Identified enzymes contained the motif [(Q/H)(V/I/L)(S/T)(F/L/V)LH(V/I/L)(Y/V/I)HH] and six transmembrane spanning regions. The ability of the *A. franciscana* Elo enzymes to elongate PUFA substrates was assayed in yeast. The capacity of the *A. franciscana* Elo to elongate the exogenously supplemented PUFA varied among enzymes (Table 1). Both Elo2 and Elo5 were able to convert all PUFA assayed (18:3n-3, 18:2n-6, 18:4n-3, 18:3n-6, 20:5n-3, 20:4n-6, 22:5n3 and 22:4n-6) to the corresponding 2-carbon elongated products (Table 1). Moreover, both Elo1 and Elo6 had the ability to elongate all C_{18} PUFA substrates, as well as 20:5n-3, although Elo1 but not Elo6 elongated 22:5n-3 to 24:5n-3 (Table 1). Finally, the *A. franciscana* Elo3 and Elo4 recognised only two out of four C_{18} PUFA as substrates, namely 18:4n-3 and 18:3n-6 for Elo3, and 18:2n-6 and 18:3n-6 for Elo4, with no activity detected towards any C_{20} and C_{22} PUFA (Table 1).

(Continued on next page)

Table. Functional characterisation of the six *A. franciscana* elongases (% conversion).

FA substrate	Product	Elo1	Elo2	Elo3	Elo4	Elo5	Elo6	Activity
18:3n-3	20:3n-3	0.75	0.57	nd	nd	0.45	0.45	C18 → C20
18:2n-6	20:2n-6	0.29	0.39	nd	0.59	0.51	0.84	C18 → C20
18:4n-3	20:4n-3	1.09	0.92	0.36	nd	0.58	2.47	C18 → C20
18:3n-6	20:3n-6	0.33	0.52	1.66	0.30	0.43	1.55	C18 → C20
20:5n-3	22:5n-3	0.12	0.12	nd	nd	0.03	0.24	C20 → C22
20:4n-6	22:4n-6	nd	0.11	nd	nd	0.06	nd	C20 → C22
22:5n-3	24:5n-3	0.12	0.66	nd	nd	0.07	nd	C22 → C24
22:4n-6	24:4n-6	nd	0.25	nd	nd	0.30	nd	C22 → C24

nd, not detected

Discussion and conclusions

PUFA elongases have been found and characterised in several invertebrates including crustaceans (Monroig and Kabeya, 2018). The *A. franciscana* Elo genes share the motif [(Q/H)(V/I/L)(S/T)(F/L/V)LH(V/I/L)(Y/V/I)HH], which is characteristic from PUFA elongases (Hashimoto et al., 2008). The *A. franciscana* Elo1 can be classified in the group of Elovl4, an Elo type that is widespread in animals including the branchiopod crustacean *Daphnia magna* (Monroig and Kabeya, 2018). Interestingly, the *A. franciscana* Elo2-6 belong to a group of unclassified elongases arbitrarily named "novel" elonganses, which have been found extensively in crustaceans such as the marine gammarid *Echinogammarus marinus* (Ribes-Navarro et al., 2021). Our functional data clearly show that the *A. franciscana* Elo1-6 can catalyse all the elongation reactions involved in the LC-PUFA biosynthetic pathways and, while further studies are required to clarify the presence of fatty acyl desaturases, we can conclude that *Artemia* has some capacity for biosynthesis of essential FA.

References

Hashimoto K, Yoshizawa AC, Okuda S, Kuma K, Goto S, Kanehisa M. The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. 2008. J. Lipid Res. 49, 183–191.

Ito MK, Simpson KL. The biosynthesis of **ω**3 fatty acids from 18:2ω6 in *Artemia* spp. 1996. Comp. Biochem. and Phys. -B Biochem. and Mol. Bio. 115, 69–76.

Monroig, Ó, Kabeya N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. 2018. Fish. Sci. 84, 911–928.

Reis DB, Acosta NG, Almansa E, Navarro JC, Tocher DR, Monroig **Ó**, Andrade JP, Sykes AV, Rodríguez C. Endogenous metabolism of unsaturated fatty acids in *Artemia* nauplii as determined through incubation with 14C-labelled fatty acid substrates. 2013. Commun Agric Appl Biol Sci. 78,392-5.

Ribes-Navarro A, Navarro JC, Hontoria F, Kabeya N, Standal IB, Evjemo JO, Monroig Ó. Biosynthesis of long-chain polyunsaturated fatty acids in marine gammarids: molecular cloning and functional characterisation of three fatty acyl elongases. 2021. Marine Drugs. 19(4), 226.

Sorgeloos P, Dhert P, Candreva P. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. 2001. Aquaculture, 147–59.

LIVE AQUAFEED PRODUCTION IN A NOVEL, SHORT LIGHT-PATH, ANNULAR COLUMN PHOTOBIOREACTOR WITH INTERNAL LED ILLUMINATON

Ranglová K.[1]*, Bureš M.[1], Yanes-Roca C.[2], Lakatos G.[1], Grivalský T.[1], Manoel J.[1,3] and Masojídek J.[1,3]

[1]Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Algal Biotechnology, Center Algatech, Třeboň, Czech Republic
[2]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic
[3]University of South Bohemia in České Budějovice, Faculty of Science, České Budějovice, Czech Republic
E-mail: ranglova@alga.cz

Instroduction

During growth, microalgae are able to synthetize high value compounds, such as pigments and polyunsaturated fatty acids (PUFA) giving them significant potential for nutritional purposes, e.g. in aquaculture. For aquafeed preparation the most important fatty acids are linoleic acid (LA; C18:2, n6), α-linoleic acid (ALA; C18:3, n3), arachidonic acid (AA; C20:4, n6), eicosapentaenoic acid (EPA; C20:5, n3) or docosahexaenoic acid (DHA; C22:6, n3) which are essential for fish larvae development (Nogueira et al. 2020). Pikeperch (*Sander lucioperca*) is highly demanded fresh and brackish water fish. Most of pikeperch production currently comes from wild fisheries, but the production in recirculating aquaculture systems (RAS) has been increasing, due to its high market value and fast growth rate in RAS. The survival rate of pikeperch larvae is usually low, below 20%, due to unsuitable nutrition. The introduction of some rotifers (e.g. *Brachionus plicatilis*) as suitable microalgae feed has been successfully performed, increasing the survival rate of pikeperch and overall physiological conditions (Yanes-Roca et al. 2020). In general, rotifers retain the nutritional composition of their diets. They are, hence, regarded as live-feed capsules for the transfer of crucial nutrients, mainly polyunsaturated fatty acids (20:5, n-3 and 22:6, n3), to fish larvae. Thus, microalgae with substantial fatty acid profile might be used as a feed for rotifers enhancing the rearing successfulness of pikeperch in RAS.

Material and methods

Five different microalgae strains, either Chlorophyceae or Eustigmatophyceae *(Chlorella vulgaris, Monoraphidium* sp., *Trachydiscus minutus, Vischeria helvetica and Monodopsis* sp.) were cultivated in a novel, short light path, annular column photobioreactior (AC-PBR) with internal LED illumination and full control of cultivation variables. The annular interspace (photostage) for microalgae culturing was only 4.6 cm thick and the highest PAR light intensity that could be achieved was 1600 μmol photons m^{-2} s^{-1}. During the cultivation of microalgae diets, the physiological status was monitored by several Chl fluorescence techniques. The effect of cultivation conditions on the fatty acid profile was investigated. The microalgae cultures produced in AC-PBR were used for rotifer (*Brachionus plicantilis*) feeding. These were introduced to pikeperch larviculture in RAS as live feed and the effect on overall fitness of larvae was studied.

Results

All microalgae cultures revealed high photosynthetic activity during the trial due to precious adjustment of required cultivation conditions and reached biomass density up to about 5 g DW L^{-1}. Under continuous illumination the volumetric productivity was found to be about 0.3 g DW L^{1} d^{1} during trials, corresponding to the areal productivity of about 15 g DW m^{-2} d^{-1}. Based on effective cooling, the temperature of cultures could be kept at the optimum value, potentially down to 10 °C within the trial. The proposed model of AC-PBR could even be placed outdoors to further increase cell irradiance for even higher biomass productivity in dense cultures.

References:

Nogueira N, Nascimento FJA, Cunha C, Cordeiro N (2020) *Nannochloropsis gaditana* grown outdoors in annular photobioreactors: Operation strategies. Algal Res 48:101913.

Yanes-Roca C, Holzer A, Mráz J, Veselý L, Malinovskyi O, Policar T (2020) Improvements on Live Feed Enrichments for Pikeperch (*Sander lucioperca*) Larval Culture. Animals 10, 401.

IN SEARCH OF STRESS BIOMARKERS: LABEL-FREE SHOTGUN PROTEOMICS ANALYSIS OF GILTHEAD SEABREAM HEPATIC AND SKIN MUCUS PROTEOME

Cláudia Raposo de Magalhães*, Ana Paula Farinha, Raquel Carrilho, Denise Schrama, Marco Cerqueira, Pedro M. Rodrigues

Centre of Marine Sciences (CCMAR), Universidade do Algarve, Faro, Portugal
csraposo@ualg.pt

Introduction

Managing fish stress is crucial to ensure a sustainable aquaculture production. In a research context, high plasma levels of cortisol, glucose and/or lactate are universally used to assess fish welfare. However, the heterogeneity of responses demonstrate that these should be interpreted with caution in cases of long-term stressors. The standardization of stress biomarkers would be an important contribute to the existing species-specific stress management protocols. Proteomics was employed in this study as a tool to discover more robust fish stress biomarkers, since proteins are ubiquitously affected by abiotic and biotic stimuli in a slower timescale when compared with endocrine responses. The analysis of proteome changes in different tissues, e.g. liver and mucus may offer not only tissue-specific protein fingerprints, but can be also highly advantageous in the context of fish welfare, as skin mucus allows for sampling in a non-invasive way. Moreover, this combined proteomic analysis provides a more detailed insight into the molecular mechanisms, as a complementary picture of the animal´s physiological state under stress.

Methods

Sparus aurata was exposed to different suboptimal rearing conditions in three separated trials: overcrowding (OC30 and OC45), repetitive net handling (NET2 and NET4), and hypoxia (HYP30 and HYP15), using fish reared under optimal conditions for the species, as control. By the end of the trials, fish were sampled and protein extracts from liver and mucus samples were prepared for further analysis by reverse phase nano liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Proteins identified with high confidence (protein FDR <0.5%; peptide FDR <0.1%) within each trial were analyzed by One-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Proteins were screened for enriched KEGG and Reactome pathways. Pairwise comparisons between control and stressed fish samples were also established within each trial (T-test, $q < 0.05$) and up- and down-regulated proteins were further classified according to Gene Ontology Enrichment (Fisher's Exact test, FDR <0.05). Cortisol, glucose and lactate were measured from blood samples, and glycogen stores were assessed in the liver.

Results and conclusions

Label-free shotgun proteomics reproducibly identified a mean of 1300 proteins in at least 4 out of 6 fish per treatment, either in the liver or in the skin mucus of gilthead seabream. A total of 297 (liver) and 250 (skin mucus) differentially regulated proteins were identified between stressed and control fish across the three trials. A tissue-specific stress regulation was observed, although 40 common proteins, mostly implicated in translation and protein folding processes, were found to be differentially regulated in both tissues. Liver-specific proteins were involved in metabolism regulation, while mucus-unique proteins were implicated in signal transduction and immune response. Plasma cortisol levels and liver glycogen storages were significantly altered exclusively in net-handled fish. The overall results suggest that the net-handling was the most impactful stressor, and the fish physiological stress response was tuned according to the challenge's severity. This integrated approach provides a starting point for the development of more reliable fish welfare assessment measures in comparison to cortisol levels solely, to further improve aquaculture sustainability.

CONTRIBUTIONS TO THE INTENSIVE CULTIVATION OF *Hediste diversicolor*

I. Rasines-Pérez*[1], I. Martín-Montero[1] and F. Aguado-Jiménez[1*],

[1]Spanish National Research Council. National Center Spanish Institute of Oceanography. Oceanographic Centre of Santander. "El Bocal" Marine Aquaculture Station. Monte-Corbanera, 39012, Santander, Spain
E-mail: inma.rasines@ieo.es

Introduction

The "ragworm" *Hediste diversicolor* is a suitable species for industrial aquaculture because of its adaptability to wide environmental conditions, feeding flexibility and high growth rates. Its use can be the traditional one, as fishing bait or the more recently as feed supplements for farmed fish and crustaceans (Batista *et al*. 2003).

The relationship between growth rate and density is well known and no more than 1000 individuals/m^2 is recommended for the cultivation of this species (Nesto *et al*. 2012). Nevertheless, production with this low density is scarce an intensification of the cultivation is needed to achieve industrial profitability. Depending on the final commercial use, production objectives can be larger individuals or higher biomass/m^2.

This species lives buried in the substrate and burrow depth increases with size (Esselink and Zwarts, 1989), so an increase in depth culture bed could result in increased space for growing worm. The relationship between density and substrate height has not been studied in this species and we hypothesise that there will be an interactions between these two factors.

Material and methods

Two substrate heights (60 and 120 mm) and two densities (1000 and 4000 ind. m^{-2}) were evaluated in a factorial design in the culture of *H. diversicolor*. Independent replicates (three for high density and six for low density) were considered for each of the substrate height x density combinations. Juveniles of wet body weight (BW) 48 ± 3 mg, (mean ± SEM), obtained from a captive population stock, were used in the experiment. Each replicate consisted of 10 or 40 worms stocked in cylindrical containers (0.01 m^2 and 0.2 m high) made with PVC frame closed at the base and sides by 335 μm mesh. Substrate was quarry sand (0.25 - 1.0 mm grain size). These cylindrical containers were immersed in 3 polycarbonate trays (width: 0.35 m; height: 0.30 m; length: 0.54 m) provided with aeration, top water inlet (2.5 renewals/hour) and bottom drainage connected to a Recirculating Aquaculture System (RAS).The RAS had biological and mechanical filtration, UV sterilization, and temperature control.

Temperature, salinity and photoperiod were fixed at 20 °C, 36 and 16/8 h light/dark, respectively. Dissolved oxygen (DO), pH, temperature and salinity were monitored daily and ammonia and phosphates once a week.

Polychaetes were fed with sole (*Solea senegalensis*) feed (0.35-0.50 mm in size) in a total amount equivalent to 4% of the daily wet biomass, distributed three times a week. Fortnightly, wet BW and survival was estimated and the diet adjusted to account for increases in mean polychaete weight throughout time.

Table I. Physico-chemical parameters during experimental period (57 days).

Temperature	DO	Salinity	pH	Ammonia	Phosphate
18.7±1.0 ºC	94±3 %	36.1±0.9 ppm	8.06±0.18	<0.025 mg/l	<0.25mg/l

Table II. Growth performance and productions parameters calculated in *H. diversicolor* reared at different densities. Data are expressed as mean ± SEM. Mean values with different superscript letters in the same column were significantly different ($p < 0.050$).

Density (ind/m^2)	Mean wet weight (mg)	Survival (%)	SGR (%/day)
1000	459±20[a]	92±2	4.01±0.07[a]
4000	384±11[b]	90±2	3.71±0.05[b]

(Continued on next page)

Mean wet BW, survival and specific growth rate (SGR= (ln BW_f- ln BW_i)*100/ T_f-T_i) at day 57 were calculated as growth performance, and number of individuals greater than 0.6 g and total biomass production (g/m^2) as a proxy of productivity. The factors height and density were analysed by two-factorial ANOVA. The Mann-Whitney test was used for non-parametric data. Values with $p < 0.050$ were considered statistically significant.

Results and Discussion

The physico-chemical parameters were in the correct range for the culture of this species (table I).

At day 57, the two-factor ANOVA showed no significant interactions between density and substrate height (p=0.392), nor significant substrate height effects (p=0.207) but a significant effect of density on BW (p=0.021). Similar results were obtained for SGR: interaction p=0.435, height p=0.179 and density p=0.010. No significant differences were found in survival (Mann-Whitney test, density p=0.615, height p=0.920) (Table II).

Nevertheless, the total final biomass was 415±16 and 1378±46 g/m^2 for low and high densities respectively, i.e. about 3 times higher when an initial stocking density 4 times higher was used. Nesto *et al*. 2012 found a higher SGR and similar final biomass when the stocking density was 1000 or 3000 ind /m^2. In our case, the similar survival rate between the two densities tested could have counterbalanced the lower growth. Additionally, in 8 weeks, the number of individuals that can be sold as bait (greater than 0.6 g) is 2.75 times higher with the high density (550±43 ind>0.6g)/m^2) than whit the low (200±30 ind>0.6g)/m^2).

The high survival rates obtained in this work could be due to the culture system used that allows a very good water quality.

We conclude that *Hediste diversicolor* can be reared at a high stocking density in order to obtain a major profitability, especially if low-cost feed, e.g. waste of fish aquaculture, will be used.

References

Batista, F.M., Fidalgo e Costa, P., Ramos, A., Passos, A.M., Pousão Ferreira, P., & Cancela da Fonseca, L. (2003). Production of the ragworm *Nereis diversicolor* (O.F. Müller, 1776), fed with a diet for gilthead seabream *Sparus aurata* L., 1758: survival, growth, feed utilization and oogenesis. Bol. Inst. Esp. Oceanogr. 19(1-4): 447-451.

Esselink, P. & Zwarts, L. 1989. Seasonal trend in burrow depth and tidal variation in feeding activity of *Nereis diversicolor*. Marine Ecology Progress Series, 56: 243-254.

Nesto, N., Simonini, R., Prevedelli, D., & Da Ros, L. 2012. Effects of diet and density on growth, survival and gametogenesis of *Hediste diversicolor* (O. F. Müller, 1776) (Nereididae, Polychaeta). Aquaculture, 362-363: 1-9

Acknowledgments

This study is co-funded (75 %) by the European Maritime & Fisheries Fund (EMFF) and the CN-IEO-CSIC.

A MULTIOMIC APPROACH REVEALS ESSENTIAL HOST-MICROBIOTA INTERACTIONS IN THE INTESTINAL ENVIRONMENT OF RAINBOW TROUT (*Oncorhynchus mykiss*)

Jacob Agerbo Rasmussen*[1,2], Kasper Rømer Villumsen[3], Madeleine Ernst,[4] Martin Hansen[5], Torunn Forberg[6], Shyam Gopalakrishnan[2], M. Thomas P. Gilbert[2,7], Anders Miki Bojesen[3], Karsten Kristiansen[1,8], Morten Tønsberg Limborg[1,2]

[1] Laboratory of Genomics and Molecular Medicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark
[2] Center for Evolutionary Hologenomics, GLOBE institute, Faculty of Health and Medical Sciences,
[3] Department of Veterinary and Animal Sciences, University of Copenhagen, Veterinary Clinical Microbiology, Denmark
[4] Section for Clinical Mass Spectrometry, Danish Center for Neonatal Screening, Department of Congenital Disorders, Statens Serum Institut, 2300 Copenhagen, Denmark
[5] Department of Environmental Science, Aarhus University, Aarhus, Denmark
[6] BioMar Group, Trondheim, Norway
[7] University Museum NTNU, Trondheim, Norway
[8] Institute of Metagenomics, BGI- Shenzhen, Shenzhen, China
Email: jacob.rasmussen@bio.ku.dk

Introduction
The microbial communities that inhabit the vertebrate gastrointestinal tract are tightly connected to many traits displayed by its host[1,2]. Applying a multi omic framework to investigate host-microbe interactions could be used in aquaculture related to improve growth, health, and sustainable production[3]. Here, we investigate the functional effects of pro- and synbiotic feed additives on microbiome associated functions in the commercially important rainbow trout (*Oncorhynchus mykiss*). We combine complementary insights from multiple omics datasets from gut content samples, including 16S bacterial profiling, genome resolving metagenomes, and untargeted metabolomics, to investigate bacterial metagenomic assembled genomes (MAGs) and their molecular interactions with host metabolism.

Methods
An experimental feeding trial was carried out over an eight-week period. Three experimental, proprietary feed formulations were selected: I) a control feed without any pre- or probiotic additives (CTRL), II) control feed plus the commercial probiotic with commercially available *Pediococcus acidilactici* MA18/5M (PRO), and III) control feed with a synbiotic additive, consisting of same probiotic and galactooligosaccharides (SYN). Bacterial profiling using the V3-V4 region of the bacterial 16S rRNA gene, were applied to investigate microbial profiles related to usage of supplemented feed in two gut sections of 120 individuals (40 individuals per feed group) of rainbow trout. A subset of these samples was whole-genome sequenced to investigate bacterial functionality of MAGs. Furthermore, A combination of two untargeted metabolomics approaches, including UHPLC-MS/MS and IC HR-MS/MS, were applied to 30 individuals (10 individuals per feed group) in order to decipher the metabolic landscape in the intestinal environment.

Results
Our findings reveal, that **(I)** feed additives changed the microbiome and that rainbow trout reared with feed additives had a significantly reduced relative abundance of the salmonid related species *Candidatus* Mycoplasma salmoninae in both the mid and distal gut content (Figure 1a), **(II)** genome resolved metagenomics revealed that alterations of microbial arginine biosynthesis and terpenoid backbone synthesis pathways were directly associated with presence of the native gut bacteria *Candidatus* Mycoplasma salmoninae (Figure 1a), **(III)** differences in the composition of intestinal microbiota among feed types were directly associated with significant changes of the metabolomic landscape, including lipids and lipid-like metabolites, amino acids, bile acids, and steroid-related metabolites (Figure 1b-d).

Conclusions
Our results demonstrate how use of multi-omics to investigate complex host-microbiome interactions enable us to better evaluate the functional potential of probiotics compared to studies that only measure overall growth performance or that only characterise the microbial composition in intestinal environments.

(Continued on next page)

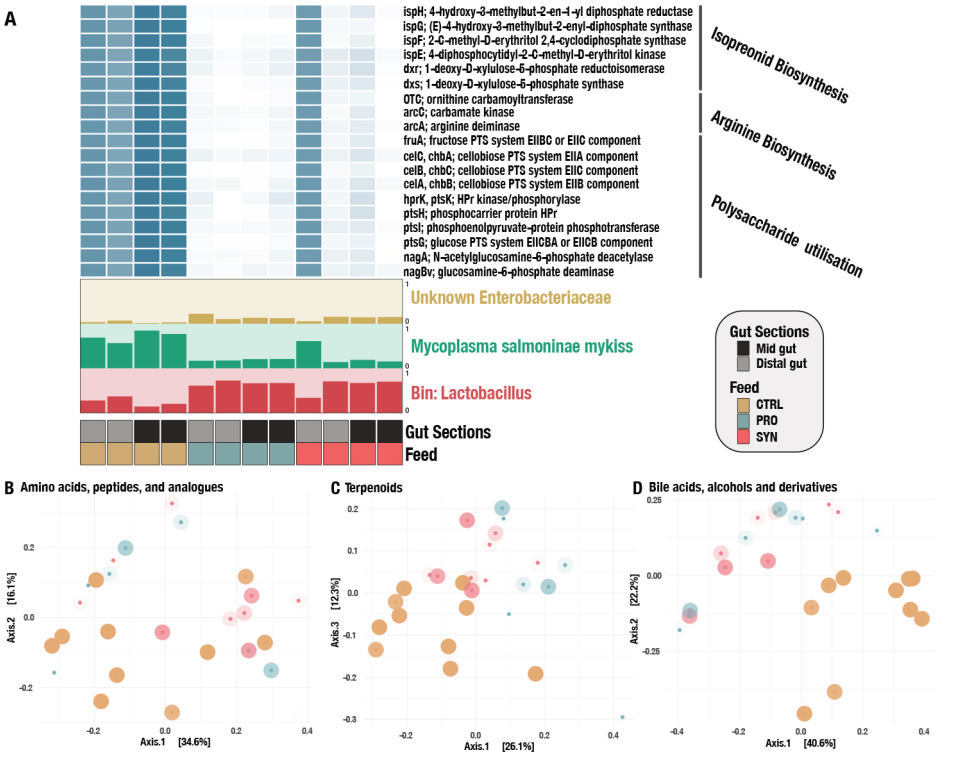


Figure 1 Feed related metagenomic and metabolomic variation in the intestine of rainbow trout. A) Heatmap visualises a series of genes of interest, which are related to isoprenoid biosynthesis, arginine biosynthesis, and polymer utilisation. Intensity of blue colour indicates log10 of coverage of genes across samples. Bar plots indicate relative abundance of the two MAGs and the bin within each sample. Intestinal sections (gut sections) were coloured as black for mid gut and grey for distal gut. B-D) Principal Coordinate Analysis (PCoA) of *in silico* classified metabolites across all three feeding types based on B) Amino acids and analogues, C) Terpenoids, and D) Bile acids, alcohols, and derivatives. Opacity of nodes is related to relative abundance of Mycoplasma. Grouping of rainbow trout reared on different feeding types were visualised as orange for CTRL, blue for PRO, and red for SYN.

References

1. Huang, Q. *et al*. Diversity of gut microbiomes in marine fishes is shaped by host-related factors. *Mol. Ecol*. (2020) doi:10.1111/mec.15699.
2. Perry, W. B., Lindsay, E., Payne, C. J., Brodie, C. & Kazlauskaite, R. The role of the gut microbiome in sustainable teleost aquaculture. *Proc. Biol. Sci*. **287**, 20200184 (2020).
3. Limborg, M. T. *et al*. Applied Hologenomics: Feasibility and Potential in Aquaculture. *Trends Biotechnol*. **36**, 252–264 (2018).

ELECTROPHYSIOLOGICAL RESPONSES TO AMINO ACIDS: THE "ELECTRO-OSPHRADIOGRAM" IN THE PACIFIC OYSTER (*Crassostrea gigas*)

Ana Rato[1,2*], Sandra Joaquim[2,3], Domitília Matias[2,3], Peter Hubbard[1]

[1] Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal
[2] Department of Sea and Marine Resources, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.), Av. 5 de Outubro s/n, 8700-305, Olhão, Portugal
[3] Interdisciplinary Centre of Marine Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

E-mail: ana.rato@ipma.pt

Introduction

In the sea, sometimes devoid of light and with high turbidity, organisms depend heavily on chemosensory systems to detect food, avoid predators and for reproduction (Hara, 1994). In vertebrates, the olfactory system is essential to sense the chemical environment, whereas in molluscs chemoreception is thought to be mediated by the osphradium. Although several studies have investigated chemical communication and the role of the osphradium in gastropods and cephalopods, there is little knowledge of chemical communication or chemosensory systems in bivalves. The detection of chemical cues is essential in several aspects of bivalve biology, such as food selection and detection of pheromones. To address this lack, we have adapted an electrophysiological technique used extensively in vertebrates - the electro-olfactogram - to record from the osphradium in the oyster (*Crassostrea gigas*). This was validated using amino acids as stimulants. The 'electro-osphradiogram' (EOG) may prove to be a powerful tool in the isolation and characterization of pheromones and other important chemical cues for bivalves.

Material and methods

Adult oysters were anaesthetized with magnesium chloride (50g L^{-1}) as suggested by Suquet et al. (2009). The right valve was then carefully removed by cutting the adductor muscle. The oysters were then replaced in normal seawater overnight. Amino acid solutions were prepared by dissolving directly in charcoal-filtered (natural) seawater (10^{-3} M to 10^{-7} M) to use as chemosensory stimulus. L-cysteine (10^{-3} M) was used as a positive control (or standard) whereas the blank or negative control was prepared in the same way, but without addition of any amino acid. The chemosensitivity of the osphradium was assessed through the EOG, a D.C. field potential. The electrodes were borosilicate glass micropipettes filled with 3 M NaCl bridged to solid-state electronics via an Ag/AgCl pellet (Hubbard et al., 2011). The recording electrode was placed close to (but not touching) the osphradium, near the ventral area of the adductor muscle and the reference electrode was placed close by on the mantle (not the osphradium) (Haszprunar, 1987). The optimal positions for electrodes and stimulus-delivery tube were determined using 10^{-3} M L-cysteine as stimulus. 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. All stimuli were administered randomly, but each amino acid was given in order of increasing concentration (10^{-7} M – 10^{-3} M). The EOG peak amplitude was measured in millivolts. All recorded responses were blank-subtracted and normalized to the standard (10^{-3} M L-cysteine). Concentration-response curves and thresholds of detection were calculated for each odorant.

Results

The osphradium proved to be highly sensitive to some amino acids (L-cysteine, L-leucine, L-proline, L-methionine, L-serine and L-tryptophan), evoking EOGs with a slow negative deflection at stimulus exposure onset, followed by a tonic response during which the EOG showed little or no sign of accommodation (in stark contrast to vertebrates). When stimulus delivery ended, the potential returned to baseline levels within seconds. However, other amino acids (L-glutamate, L-arginine and L-aspartic acid), and the neurotransmitter serotonin, did not evoke any response. Conspecific sperm evoked a strong EOG response similar in form and magnitude to those evoked by amino acids. The amplitude of EOG responses was strongly concentration-dependent. Thresholds of detection varied between $10^{-6.36}$ M and $10^{-5.30}$ M for L-methionine and L-cysteine, respectively. Different isomers of alanine (L, D and β) and leucine (L-; D-) evoked different EOG responses. β-Alanine evoked significantly lower response than L- and D-isomers, whereas L-leucine evoked significantly larger responses than its D-isomer.

(Continued on next page)

Discussion

To our knowledge, this was the first time an EOG recording was successfully carried out in any bivalve, and strongly supports the hypothesis that the osphradium is a chemosensory organ (Haszprunar, 1987) in this taxon, as it is in other molluscs. Subsequently, a whole series of questions about chemoreception in bivalves may finally be answered. As in the olfactory system of fishes (Caprio, 1978; Hubbard et al., 2011), the chemosensory organ of oysters proved to be sensitive to amino acids; however, the EOG in oysters is much slower, of somewhat lower amplitude and is more tonic. Apparently, oysters are more selective in which amino acids they detect than fishes; the responses varied substantially between amino acids, with some of them not eliciting any response. Furthermore, the rank order of potency is different from that of fishes; for example, L-proline was highly potent in the oyster, but is of minor potency in fishes (e.g., Hubbard et al., 2011). Like fishes, oysters seem to be more responsive to L-amino acids, probably due to its involvement in food identification and location (Hara, 1994).

Acknowledgements

This work was financially supported by the Portuguese Foundation for Science and Technology (FCT) through a PhD grant (SFRH/BD/147215/2019) to A.R. and project grant (UID/ Multi/04326/2021).

References

Caprio, J., 1978. Olfaction and taste in the channel catfish: An electrophysiological study of the responses to amino acids and derivatives. J. Comp. Physiol. - A 123, 357–371.

Hara, T.J., 1994. The diversity of chemical stimulation in fish olfaction and gustation. Rev. Fish Biol. Fish. 4, 1–35.

Haszprunar, G., 1987. The fine morphology of the osphradial sense organs of the mollusca. III. Placophora and bivalvia. Philos. Trans. R. Soc. London. B, Biol. Sci. 315, 37–61.

Hubbard, P.C., Barata, E.N., Ozório, R.O.A., Valente, L.M.P., Canário, A.V.M., 2011. Olfactory sensitivity to amino acids in the blackspot sea bream (*Pagellus bogaraveo*): A comparison between olfactory receptor recording techniques in seawater. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 197, 839–849.

Suquet, M., Kermoysan, G. De, Araya, R.G., Queau, I., Lebrun, L., Souchu, P. Le, Mingant, C., 2009. Anesthesia in Pacific oyster, *Crassostrea gigas*. Aquat. Living Resour. 22, 29–34.

IS BLUE GROWTH A DRIVER OR A CONSTRAINT TO THE SUSTAINABILITY OF MARINE AQUACULTURE DEVELOPMENTS? REVIEWS OF BOTTLENECKS AND LESSONS FROM PAST DEVELOPMENTS

Pascal Raux*, José Antonio Pérez Agúndez•

*Université de Brest (UBO), UMR 6308, AMURE, IUEM, rue Dumont d'Urville, 29280 Plouzané, France
•Ifremer, Unité d'Economie Maritime, UMR 6308, AMURE, IUEM, rue Dumont d'Urville, 29280 Plouzané, France
E-mail : pascal.raux@univ-brest.fr

After an initial rapid and strong growth, the development of marine aquaculture in Europe (EU) has shown signs of stagnation, even regression in some Member States. The explanatory factors of this stagnation have often taken the form of technical constraints, disease control and environmental management, constraints on production factors (access to capital, access to space, access to knowledge…). In addition to these constraints, there are significant conflicts of use with other activities, both for access to resources and for environmental impacts suffered or emitted. However, the explanatory factors have paid little attention to socio-economic forcing, whether it be the adequacy between production systems and markets, or even the consumption modes, or the adequacy of aquaculture developments with the territories where they take place. In addition, the growth of production has often been accompanied by increasing difficulties in terms of farms' profitability.

Notwithstanding these issues and following the financial crisis of 2008, Blue Growth has been perceived as a development opportunity through a series of key sectors that are expected to boost the economy and provide jobs and welfare (EC, 2017). Among these sectors, marine aquaculture raises high expectations through Blue Growth and its declination into the European Union's integrated maritime policy and its marine spatial planning tool (MSP). Seen as a tool for integration, and even integrated management, the MSP is also put forward as a factor for improving the social acceptability of aquaculture developments, especially through the approach of Allocated Zones to Aquaculture (AZAs), reinforced by communication actions aimed at mitigating the misperceptions that consumers and citizens might express about aquaculture and its products. The stagnation of catches from fisheries, the increasing demand per capita in seafood products supported by an ever increasing world demography and confirmed by the various models reinforce the role henceforth devolved to aquaculture to provide for this growth.

A review of issues and bottlenecks to the development of marine aquaculture in Europe and in the world nevertheless makes it possible to underline an important number of constraints which can break and question the desired or planned development, but also a certain number of contradictions between the stated objectives and the reality of developments. This long-standing dynamic linked to the first developments related to the Blue Revolution still seems to be at work. The same causes leading to the same effects, the adequacy of the objectives assigned to aquaculture developments with a sustainability approach is questionable. A review of development experiences within the H2020 MedAID project reinforces an initial statement related to the lack of attention paid to the adequacy of aquaculture objectives with the territories that support them, with markets and their evolution and with consumer expectations. If technological solutions and innovations remain at the heart of aquaculture developments, these constraints underline their necessary but not sufficient property and the needs for more holistic approach centred on governance.

EC (2017). Report on the Blue Growth Strategy, Towards more sustainable growth and jobs in the blue economy. Commission Staff working document on Blue Growth 2013-2016, SWD(2017) 128 final, 61p.

DIFFERENCES IN THE CIRCULAR RNA TRANSCRIPTOME BETWEEN WILD AND CAPTIVE-BRED NILE TILAPIA

Rbbani, G.M*[1], Nedoluzhko, A.V.[1], Sharko, F.S.[2], Konstantinidis, I.[1], Raeymaekers, J.A.M.[1], Galindo-Villegas, J.[1], and Fernandes, J.M.O[1]

Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia
Email: golam.rbbani@nord.no

Introduction

Domestication of organisms started as early as in the Neolithic period (c.14 000 years ago). Historically, fish domestication began almost 1000 years later than many other species, such as cattle, dogs, pigs, and horses. Domestication is a complex and long process in which organisms are subject to human control in many aspects, including feeding and breeding, thus changing their phenotype compare to their wild ancestors. Accumulating shreds of evidence suggest that domestication plays crucial roles in modulating behaviour, size, colouration, morphology, and physiology in teleosts (Balon et al. 2004). These modifications are often linked to changes in genetic structure through mutations and allele fixation. Investigations into more recently domesticated species reveal that phenotypic change is relatively fast, and expression of hundreds of gene can be altered in a single generation. Epigenetic modifications, potentially heritable modifications of the chemical structure of the genome without affecting the nucleotide sequence, are important in the shaping phenotypic differences during the domestication process. The role of methylation, hydroxymethylation in domestication of fish has been described in artificial selection, environmental adaptation, and genome evolution (Podgorniak et al. 2019; Konstantinidis et al. 2020). In addition, non-coding RNAs can also influence phenotypic diversity and fitness by regulating transcription mechanisms and give rise to the emergence of domestication traits. Circular RNAs (circRNAs) are a class of endogenous non-coding RNAs that have attracted interest in transcriptional regulation. They act as miRNA sponges and play a regulatory role in normal physiology as well as in pathological conditions. Despite the importance of circRNAs in several biological processes, their role in fish domestication remains largely unknown. In the present study, we compared wild and first-generation Nile tilapia (*Oreochromis niloticus*) reared in captivity to determine the potential role of circRNAs in domestication.

Materials and methods

The RNA-seq dataset for the wild and domesticated group (twelve samples) were obtained from ribosomal RNA depleted RNA-seq libraries published by Konstantinidis et al. (2021) under accession number GSE135811 in Gene Expression Omnibus. After filtering low-quality reads and adapters, sequences were mapped into the Nile tilapia reference genome (ASM185804v2). CircRNA prediction was performed for each RNA dataset with the circRNA in silico prediction tools CIRI2. Differential expression of circRNAs between the groups was assessed using circMeta (Chen L. et al., 2020), and the host genes of circRNAs were predicted using circParser (Artem et al., 2020).

Results

A total of 7,862 circRNAs were identified from a muscle RNA-seq dataset (n=6) using the CIRI2 algorithm. Among them, 26 circRNAs were present in both wild fish and their progeny undergoing domestication (Fig. 1). Further analysis revealed 39 differentially expressed (DE) circRNAs. Several DE circRNAs originate from host genes known to be involved in domestication, such as *MHC class I* and *glutamate receptor-interacting protein 2*. Furthermore, a number of DE circRNAs orthologues were found to be conserved between many fish species. Taken together, our findings reveal a potential role for circRNAs in fish domestication.

(Continued on next page)

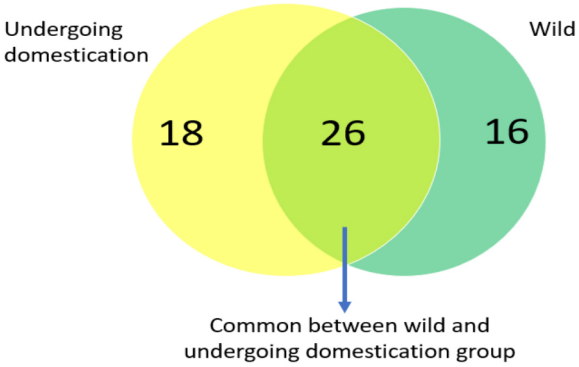


Figure 1: Venn diagram showing common and group-specific circular RNAs identified in wild Nile tilapia and their counterparts undergoing domestication (n=6).

Acknowledgements
This study has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme [grant agreement no 683210] and from the Research Council of Norway under the Toppforsk programme [grant agreement no 250548/F20].

References
Balon, E.K., 2004. About the oldest domesticates among fishes. Journal of fish Biology, 65, pp.1-27.
Chen, L., Wang, F., Bruggeman, E.C., Li, C. and Yao, B. (2020). circMeta: a unified computational framework for genomic feature annotation and differential expression analysis of circular RNAs. Bioinformatics, 36(2), pp.539-545.
Konstantinidis, I., Sætrom, P., Mjelle, R., Nedoluzhko, A.V., Robledo, D. and Fernandes, J.M. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. Epigenetics, 15(10), pp.1052-1067.
Nedoluzhko, A., Sharko, F., Rbbani, M.G., Teslyuk, A., Konstantinidis, I. and Fernandes, J.M. (2020). CircParser: a novel streamlined pipeline for circular RNA structure and host gene prediction in non-model organisms. PeerJ, 8, p.e8757.
Podgorniak, T., Brockmann, S., Konstantinidis, I. and Fernandes, J.M., 2019. Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication. Epigenetics, 14(8), pp.818-836.

ANTIOXIDATIVE ACTIVITIES, PHENOLIC COMPOUNDS AND MARINE FOOD ALLERGENS IN THE MACROALGAE *Saccharina latissima* PRODUCED IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS

C. Rebours*, J. Mildenberger and J.K. Stangeland

Møreforsking AS, Borgundveien 340, 6009 Ålesund, Norway
E-mail: Celine.Rebours@moreforsking.no

INTRODUCTION

The concept of "Integrated Multi-Trophic Aquaculture" (IMTA) has been given great interest in recent decades and is one of several strategies to increase biomass production from the sea by reducing the environmental impact of fish farming and by increased production of low-level trophic organisms such as macroalgae. However, due to lack of data and scientific evidence, appropriate food regulations are lacking for IMTA to guide the production of macroalgae and their application as food products. Macroalgae are however recognized to have valuable bioactivities including antioxidative activity. Due to variations in bioactivities between species, geographic location and season, characterization of the quality of specific IMTA products will be important for further marketing purposes. Besides valuable compounds, macroalgae can also contain elements with unwanted effects such as allergens. Thus, there is a growing concern from the food industry and food safety authorities regarding the potential occurrence of contaminating marine allergens in seaweed food products. As seaweed is produced in the ocean, allergens of marine species, such as crustaceans, molluscs or even fish might find their way into the production line and these hazards must be monitored to evaluate the risk for allergenic incidents and the need for appropriate labelling for future food products. Here, the occurrence of marine food allergens as well as total phenolic compounds and antioxidant activity have been assessed in *S. latissima* produced in three different locations in 2020 in the vicinity of salmonids farms in Norway.

MATERIALS AND METHODS

In 2020, 10 kg of seaweed was collected at three different Norwegian IMTA farms located at three different sites (Bjønnspjotneset, Dyrholmen Vest, Furholmen) owned respectively by Osland Havbruk AS, Sulefisk AS and Engesund Fiskeoppdrett. The seaweed was washed, freeze dried and ground. Extractions and analyses were conducted in triplicates. For analysis of phenolic compounds and antioxidants, seaweed samples were dissolved at 25 mg/ml in dH_2O or MeOH:dH_2O (4:1), incubated in an ultrasonic water bath (Branson 2200, 40kHz) for 30 min where indicated, and filtered. Phenolic compounds were measured by the Folin-Ciocalteu method[1,2]which can replace synthetic ones due to their potential implications for health problems in children, have gained significant popularity. Therefore, the antioxidant potential of extracts obtained from three brown macroalgae (Ascophyllum nodosum, Fucus vesiculosus and Bifurcaria bifurcata with gallic acid (Sigma Aldrich G7384) standard curve and undiluted samples. Antioxidative activity was assessed by ORAC assay, according to the BioTek Application Note 2006[3]cosmetics, supplements and pharmaceutical agents has become of particular interest. This is the result of the evidence demonstrating the relationship of reactive oxygen/nitrogen species (ROS. Samples were diluted to 25 μg/ml (1:1000) for MeOH extracts and to 5 mg/ml (1:5) for water extracts.

For allergen detection, ELISA kits for the antigens fish parvalbumin, mollusc tropomyosin or crustacean tropomyosin (Demeditec DEFISE1, DEMOLE1 and DECRUE1) were used. Dried and grinded seaweed (0.2 g, exact weight) was dissolved in 20 ml extraction buffer, filtered, and diluted 2-times to limit matrix effects.

RESULTS

The content of phenolic compounds was in a range of 1-2 mg GAE/g dry weight for all three locations and all three extraction methods. Antioxidative capacity was significantly increased when extracted in aqueous methanol as compared to water extracts in all samples. In the methanol extracts, the seaweed from Osland contained the significantly highest antioxidative activity with 1665.1 ± 39.4 μmol TE/g, followed by Sulefisk with 1243.2 ± 68.9 and Engesund with 630.9 ± 31.5 μmol TE/g. The difference in ORAC values between Osland and Sulefisk was not consistent for all extraction methods, while the sample from Engesund had the overall significantly lowest values. The treatment in an ultrasonic water bath did not have a significant effect on the content of phenolic compounds or antioxidant capacity but decreased the viscosity of water extracts.

(Continued on next page)

Further, the seaweed samples were tested for the established food allergens crustacean, mollusc and fish. Values for crustacean tropomyosin were for all samples above the lower detection limit of 20 ppm in the assay (corresponding to 0.2 mg/kg sample) and ranged from 0.442 ± 0.05 mg/kg for Osland to 1.005 ± 0.39 mg/kg at Sulefisk. The measured values were not significantly different between the three IMTA sites, although the lowest values were all detected in the samples from Osland. The mollusc tropomyosin concentrations in the seaweed samples were numerically above, but very near the lower detection limit of 10 ppm in the assay or 0.1 mg/kg sample. Fish allergens, provided as cod concentrations, were below the lower detection limit of 4 ppm in the assay (or 40 mg/kg sample) for the seaweed samples from Sulefisk and Engesund. The concentration measured in the samples from Osland was above, but near the detection limit. Osland was also the location with the highest fish production in 2020. According to literature, cod contains parvalbumin at 2 mg/g4,5 with higher concentrations in muscle6. Based on this, 40 mg cod per kg as the detection limit would translate to 0.08 mg parvalbumin per kg.

CONCLUSIONS

Products with relatively high market value such as food and feed ingredients are predicted to play an important role in creating value from Norwegian-grown seaweed and kelp[7]industry and public authorities have been committed to develop a Norwegian bio-economy based on cultivated seaweed, focusing on cultivation and processing of the biomass. This review reports on the status of seaweed aquaculture in Norway, supported by production data collected since the delivery of the first commercial cultivation permits at sea in 2014. Although novel product developments are currently limited, future industrial perspectives based on cultivated biomass are being discussed. Upscaling from experimental cultivation schemes to commercial production requires a thorough assessment of the risks and benefits associated with seaweed aquaculture, as well as the development of a regulative framework adapted to this industry. Issues associated with upscaling the macroalgal production that needs to be addressed includes (i. This work confirmed the presence of phenolic compounds and antioxidative activity in *S. latissima,* produced in IMTA systems, in a similar range for all assessed locations and comparable to previous studies, indicating that this species and production method can play a role for the development of natural food additives, functional food or health products from seaweed. The here reported bioactivities should be further investigating to fully understand the potential and limit of using *S. latissima* as functional ingredients in food or feed formulas.

Our analyses of *S. latissima* bulk samples with commercial sandwich ELISA kits for known food allergens have shown acceptable detection limits, linearity and recovery. Only crustacean allergens could be detected in all tested locations in this study, while mollusc tropomyosin was very near the detection limit, thus assumed as not detectable. Cod parvalbumin was detected slightly above detection limit only in one location. In the context of realistic incorporation in final food products and resulting daily ingestion, the presence of contaminating marine species in seaweed raw products does not seem to be critical, but should be further followed through seasons, locations and productions as more processed products are developed.

REFERENCES

1. Agregán *et al*. *Medicines* **5,** 33 (2018).
2. Aslan *et al*. *Mar. Pollut. Bull*. **141,** 313–317 (2019).
3. Held. *Appl. Note, Biotek* 9 (2006).
4. Kuehn *et al*. *Allergol. Sel*. **1,** 120–126 (2017).
5. Kuehn *et al*. *Int. Arch. Allergy Immunol*. **153,** 359–366 (2010).
6. Koppelman *et al*. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess*. **29,** 1347–1355 (2012).
7. Stévant *et al*. *Aquaculture International* **25,** 1373–1390 (2017).

CONSTRAINTS AND OPPORTUNITIES OF SUSTAINABLE INTEGRATED MULTI-TROPHIC AQUACULTURE IN NORWAY: SALMONID SEA CAGE FARMING AS A DRIVING FORCE

Céline Rebours[a*], Siv Anina Etter[b], Jan Sunde[a], Aleksander Handå[c], Kjell Inge Reitan[b]

a. Møreforsking AS, Postboks 5075, Larsgården, 6021 Ålesund, Norway
b. Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway
c. SINTEF Ocean, 7465 Trondheim, Norway
E-mail: celine.rebours@moreforsking.no

INTRODUCTION

Strategic development and research actions are needed to realize the Norwegian government's long-term aim for a predictable and sustainable growth within the aquaculture industry[1]. Fish farming in Norway is dominated by sea cage farming of salmonid species (Atlantic salmon and rainbow trout). In recent years, the industry has experienced an augmentation in production volumes per farm while the number of farm locations has decreased[2]. This augmentation of production leads to increased feed use, and subsequently to augmented release of nutrients and organic particles released into the marine environment[3,4]. These nutrients and organic particles released from aquaculture represent a possible resource for new biomass cultures, but also a risk to the environment and sustainability of the fish production. Thus, improved resource and energy utilization must be investigated in order to transform the wastes into a resource for producing other organisms at other trophic levels with potential for creating high value products[5]. In Norway, IMTA systems could also be a way to increase seafood production in areas already occupied by/allocated for salmonid aquaculture.

MATERIALS AND METHODS

The data were collected through a desktop study of peer-reviewed and 'grey' literature to identify the reported IMTA research and industrial activities and investigate the incentives already in place to develop this technology in order to outline the upscaling potential of each technological approach in Norway.

RESULTS

Various research initiatives, both nationally and internationally, have been addressing IMTA related questions and are contributing to a gradual development of the sector, including the transition from research to commercial scale IMTA systems. In Norway, the IMTA research activities currently focus on increasing the knowledge about new potential aquaculture species and appropriate organism for co-culturing based on their trophic levels and complementary functions in the ecosystem, as well as their economic value or market potential. However, most studies on IMTA have so far been based on experimental designs with limited biomass of extractive species not yet allowing for thorough evaluations of biological and economical pros and cons of implementing salmon based IMTA as a production strategy.

There is still a reluctance to this concept of ecological engineering in Norway reflected by the lack of research on IMTA and development of new technologies for integration of species at lower trophic levels with today's intensively fed monocultures of fish. At present there is uncertainty about the potential use of bivalves in IMTA, while macroalgae and deposit feeders seems more promising and are the scope for research environments and industry.

Seaweed has demonstrated having a high capacity for absorption and metabolism of inorganic nutrients excreted by fish aquaculture, integrating seaweed cultures in IMTA and seems promising for further upscaling of IMTA in Norway while producing valuable products of marine origin[6]. However, based on the current production technology, the seaweed value-chain will require extensive innovation and economies of scale to become energy competitive[7,8] and to be competitive as a biomass source for the production of fish feed ingredients[9]. Further research should investigate the predictive environmental impacts of a fully developed seaweed value-chain and account for the emissions and multi-functionality of the system (e.g. lost biomass, impact on biodiversity, ecosystem services).

The particulate excess from salmon aquaculture can also be regarded as a food source for filter feeders in an IMTA system. Whereas feed waste constitutes only 3-5% of the feed distributed, most of the particles originate from feces production. Fish feces has a low nutritional value, but this food source still can support the growth of bivalves such as blue mussels in nutrient limited areas. Thus, to the best of our knowledge it has not been investigated whether running a whole cultivation cycle of mussels or scallops in IMTA with salmon in Norway yields higher bivalve biomass at harvest compared to monoculture production. Nevertheless, collected sludge from production of larger smolts in land-based systems before deployment at sea can be further fed as substrate and utilized by deposit feeders (e.g. polychaetes, sea cucumber) and currently presents one of the new opportunities for developing IMTA in Norway[10].

(Continued on next page)

CONCLUSIONS

Upscaling of pilot experiments with new sophisticated technological solutions and systems at industrial scale is a prerequisite for the quantification of the potential for bioremediation services and increased biomass production of the low-trophic species in IMTA in Norway. Although the aquaculture regulations are intended to secure a sustainable industry, they have not yet been fully adapted to suit the emerging integrated systems. Norwegian aquaculture activity is thoroughly regulated, and spatial issues related to minimum distance between aquaculture farms, co-location of species at different trophic levels, and zoning strategies has been used as main management tools to control disease transfer. Therefore, restrictions on co-location of multiple species on the same site and the limited environmental, economic and societal pressures and incentives from the industry and the public are important challenges to the develop commercial IMTA in Norway.

REFERENCES

Parliament message nr. 16 (2014-2015) Forutsigbar og miljømessig bærekraftig vekst i norsk lakse- og ørretoppdrett p.15.

Gullestad P et al. (2011). Effective and sustainable use of areas for aquaculture – land of desire (in Norwegian). Report from an expert panel appointed by Ministry of Fisheries and Coastal Affairs, Oslo, Norway. 198 pp.

Holmer M (2010) Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. Aquacult Environ Interact, 1:57-70.

Wang X et al. (2012). Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquacult Environ Interact, 2: 267-283

Chopin T et al. (2006). Integrated multi-trophic aquaculture: Seaweeds and beyond the need of an inter-disciplinary approach to develop sustainable aquaculture. J Phycol, 42:11-33).

Handå A et al. (2013). Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture: Implications for macroalgae cultivation in Norwegian coastal waters. Aquaculture 414-415: 191-201.

Philis G et al. (2018) Comparing the primary energy and phosphorus consumption of soybean and seaweed-based aquafeed proteins – A material and substance flow analysis. Journal of Cleaner Production 200:1142-1153.

Koesling M et al. (2021) Environmental impacts of protein-production from farmed seaweed: Comparison of possible scenarios in Norway. Journal of Cleaner Production 307:127301.

Emblemsvåg J et al. (2020) Strategic considerations for establishing a large-scale seaweed industry based on fish feed application: A Norwegian case study. JAPH, 32, 4159-4169.

Wang H et al. (2019). Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Müller, 1776) cultivated on waste from land-based salmon smolt aquaculture. Aquaculture 502:232-241

PRODUCER-LEVEL EFFECTIVENESS OF AQUACULTURE ECO-CERTIFICATION

M. Rector[1]*, R. Filgueira[1], J. Grant[2]

[1] Marine Affairs Program, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada
[2] Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada

Email: megan.rector@dal.ca

Introduction

Eco-certification programs provide eco-certified status to aquaculture farms that meet a set of sustainability criteria based on the assessment of a third-party auditor. These programs are certifying an increasing volume of farmed seafood and claim to address both environmental and social issues in the sustainable development of aquaculture, but their success in creating positive sustainability outcomes remains uncertain.

Tröster & Hiete (2018) provide a definition of success for eco-certification that includes four dimensions of sustainability: problem solving effectiveness is the ability of eco-certification to address the issues it is intended to address, behavioural effectiveness is change in the activity of the eco-certified producer, process effectiveness is the uptake of eco-certification by producers, and constitutive effectiveness is the acceptance of certification amongst stakeholders. While each of these types of effectiveness are necessary for the overall success of eco-certification, problem solving and behavioural effectiveness are required to improve sustainability without limiting eco-certification to creation of a 'market for sustainable seafood' only (Ponte, 2012) (Figure 1). Given the growth of aquaculture and the increasing volume of eco-certified farmed seafood, this review provides an overview of research on the effectiveness of eco-certification, in particular, producer-level effectiveness, which is necessary if eco-certification is to create positive ecosystem-level outcomes.

Approach

A scoping review was used to review and assess the status of research literature on the success of aquaculture eco-certification. Tröster & Hiete's (2018) four dimensions of effectiveness were used to categorize types of research on the effectiveness of eco-certification and summarize findings. Research literature related to producer-level effectiveness was further reviewed and themes related to challenges in the application of eco-certification to aquaculture sustainability issues were identified. Where relevant, the way in which these challenges are addressed by eco-certification in other resource sectors were also reviewed to discuss potential approaches for improving producer-level effectiveness of aquaculture eco-certification.

Results and Discussion

Evaluating the effectiveness of eco-certification is challenging due to difficulty identifying appropriate non-certified farms (counterfactuals) for comparison with certified farms (Blackman & Rivera, 2011). However, there has been increasing interest in this topic in recent years, including research on the potential producer-level effectiveness of aquaculture eco-certification based on analysis of eco-certification criteria and processes, which has provided insight into specific shortfalls. These shortfalls include advancing an incomplete definition of sustainability, risking future improvements by labelling farms as sustainable, and not accounting for the potential far-field effects of aquaculture or local context in the application of eco-certification criteria.

Recommendations for addressing these shortfalls include (1) applying an ecosystem services framework to the selection and development of eco-certification criteria, (2) differentiating the level of compliance or stringency of criteria between and/or within eco-certification schemes, (3) using benchmark criteria and requirements for improvement over time, (4) including more criteria related to the far-field effects of aquaculture on the ecosystem, and (5) recognizing the role of local context in achieving sustainability outcomes.

While several recommendations for the improvement of eco-certification criteria are identified, it is important to consider the implications of these recommendations for *process effectiveness*, since as compliance criteria become more stringent and complex, producer participation can be expected to decline (Kalfagianni & Pattberg, 2013). For example, while moving beyond farm scale is necessary if eco-certification is to include an ecosystem perspective of sustainability, producer capacity to address increasingly complex eco-certification criteria may affect the willingness of farmers to embrace eco-certification.

(Continued on next page)

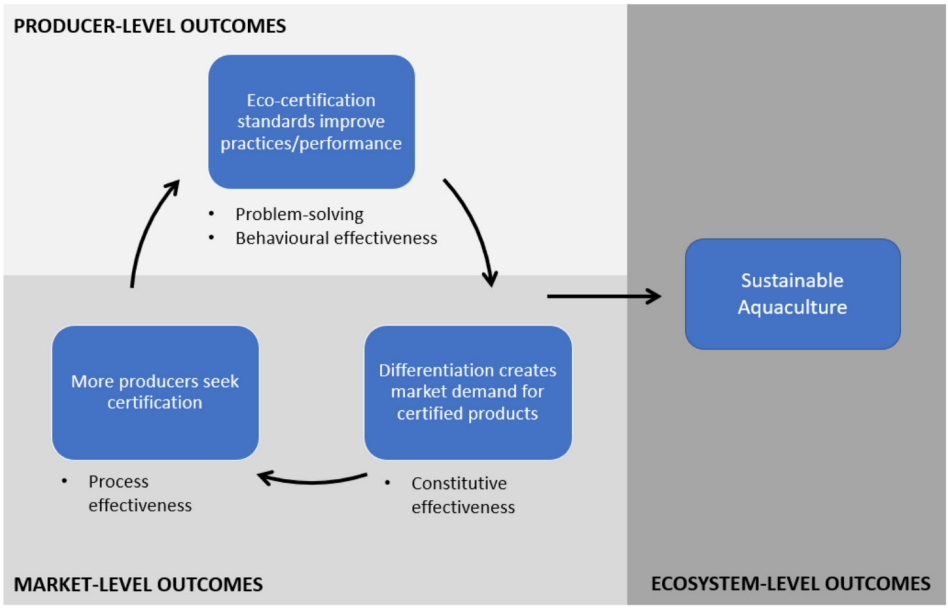


Figure 1. Types of eco-certification effectiveness and associated levels of potential sustainability outcomes.

References

Blackman, A., & Rivera, J. (2011). Producer-Level Benefits of Sustainability Certification. *Conservation Biology*, *25*(6), 1176–1185.

Kalfagianni, A., & Pattberg, P. (2013). Fishing in muddy waters: Exploring the conditions for effective governance of fisheries and aquaculture. *Marine Policy*, *38*, 124–132. https://doi.org/10.1016/j.marpol.2012.05.028

Ponte, S. (2012). The Marine Stewardship Council (MSC) and the Making of a Market for "Sustainable Fish." *Journal of Agrarian Change*, *12*(2–3), 300–315. https://doi.org/10.1111/j.1471-0366.2011.00345.x

Tröster, R., & Hiete, M. (2018). Success of voluntary sustainability certification schemes – A comprehensive review. *Journal of Cleaner Production*, *196*, 1034–1043. https://doi.org/10.1016/j.jclepro.2018.05.240

LOCAL AND SYSTEMIC IMMUNE RESPONSE OF GILTHEAD SEABREAM *Sparus aurata* JUVENILES FED MICROALGAE-DERIVED B-GLUCANS

B. Reis[1,2,3,4]*, A. Gonçalves[1], P. Santos[2], M. Sardinha[1], L.E.C. Conceição[1], R. Serradeiro[5], J. Pérez-Sánchez[6], J. Calduch-Giner[6], U. Schmid-Staiger[7], K. Frick[7], J. Dias[1], B. Costas[2,3]

[1]SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[2]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros de Leixões. Av. General Norton de Matos s/n 4450-208 Matosinhos, Portugal
[3]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[4]Sorgal S.A., Estrada Nacional 109, Lugar da Pardala 3880-728, São João de Ovar, Portugal
[5]Riasearch, Rua do Farol, 131, Torrão do Lameiro 3880-394 Ovar, Portugal
[6]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, IATS-CSIC, 12595, Castellón, Spain
[7]Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Dept Environmental Biotechnology and Bioprocess Engineering, Nobelstraße 12, 70569 Stuttgart, Germany

*E-mail: breis@ciimar.up.pt

Introduction
Animal health related issues are nowadays the major constraint for aquaculture expansion and sustainability (Adams 2019). Besides vaccination, prophylactic measures such as the incorporation of immunostimulants and prebiotics in feeds (Meena et al. 2013; Song et al. 2014), have also been used to prevent disease outbreaks. To enhance fish disease resistance and general health, diets are often supplemented with β-glucans, which are compounds with known beneficial effects in fish innate immune response (Guzmán-Villanueva et al., 2014). β-glucans show repeating patterns on their structure that are recognized in the gut by cell pattern recognition receptors (PRR), leading to the activation of the host´s innate immune cells enhancing its immune response (Dalmo et al., 2008). The present work aimed to evaluate the effects of both short- and mid-term feeding diets supplementated with microalgae (*Phaeodactylum tricornutum*) extracted β-glucans on gene expression, oxidative stress biomarkers and plasma immune parameters in gilthead seabream (*Sparus aurata*) juveniles.

Material & Methods
A practical commercial-like diet was used as control (CTRL), whereas 3 others based on CTRL were further supplemented with a constant concentration of β-glucans, derived from *Saccharomyces cerevisiae* (diet MG1000) and different extracts of *P. tricornutum* (diets Phaeo21 and Phaeo37). Diets were randomly assigned to quadruplicate groups of 95 gilthead seabream (initial body weight: 4.1 ± 0.1g) that were fed to satiation three times a day for 8 weeks in a pulse feeding regimen. Therefore, fish were fed the different experimental diets intercalated with the CTRL dietary treatment every 2 weeks. After 2 and 8 weeks of feeding, 3 fish/tank were sampled for blood and tissues collection.

Results & conclusion
All groups showed equal growth performance and no significant changes in plasma innate immune status. Nonetheless, seabream fed β-glucans supplemented diets showed an improved anti-oxidant status compared to those fed CTRL at both sampling points. Furthermore, diet Phaeo37 seems to induce an immune tolerance effect in the gilthead seabream gut, causing a general down-regulation of immune related genes, without compromising systemic immune response. In conclusion, results suggest that the dietary administration of a *P. tricornutum* 37% enriched-β-glucans extract might be relevant in a context of gut inflammation due to its immune tolerant and anti-oxidative effects.

Acknowledgements
This work has received funding from the Bio Based Industries Joint Undertaking (BBI JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 745754 (project MAGNIFICENT). This output reflects the views only of the author(s), and the European Union and BBI JU cannot be held responsible for any use which may be made of the information contained therein. BR and BC were supported by FCT - Foundation for Science and Technology (PD/BDE/129262/2017 and IF/00197/2015, respectively).

(Continued on next page)

References

Adams A (2019) Progress, challenges and opportunities in fish vaccine development. Fish Shellfish Immunol 90:210-214.

Dalmo, R.A., Bogwald, J., 2008. Beta-glucans as conductors of immune symphonies. Fish & Shellfish Immunology, 25, 384–396.

Guzmán-Villanueva,L. T., Tovar-Ramírez, D., Gisbert, E., Cordero, H., Guardiola, F. A., Cuesta, A., Meseguer, J., Ascencio-Valle, F., Esteban, M. A., 2014. Dietary administration of β-1,3/1,6-glucan and probiotic strain Shewanella putrefaciens, single or combined, on gilthead seabream growth, immune responses and gene expression. Fish & Shellfish Immunology, 39, 34-41.

Meena DK, Das P, Kumar S, Mandal SC, Prusty AK, Singh SK, Akhtar MS, Behera BK, Kumar K, Pal AK, Mukherjee SC (2013) Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol Biochem 39 (3):431-457.

Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E (2014) Prebiotics as immunostimulants in aquaculture: A review. Fish Shellfish Immunol 40 (1):40-48.

FATTY ACID PROFILE COMPARISON OF THREE MARINE BY-CATCH FISH SPECIES AVAILABLE IN INDIAN WEST-COAST WATERS WITH THREE INDIAN FOOD FISH

Steffi Reji*, Phibi Philip Naduvathu, Aldon Baby, R. Anandan and S. Bharathi.

*Scottish Association for Marine Science, Oban PA37 1QA, Agryl, United Kingdom
*Email: steffireji97@gmail.com

Introduction

The total capture fisheries production of India in 2019 was about 13.7 million tonnes (NFBD, 2020) of which 3.56 million tonnes was contributed by marine sector (CMFRI, 2019). In 2011, trawl fisheries contributed to around 50% of the total marine capture production and the low valued bycatch contributes to around 25% to the total trawl fisheries production in India (Dineshbabu *et al*, 2013). *Canthidermis maculata*; an epi-pelagic (1-110m), reef associated fish (Lieske and Meyer, 1994), *Gephyroberyx darwinii*; a meso-pelagic (200-500 m) deep-sea fish (Maul, 1990) and *Satyrichthys adeni* a demersal (58-300 m), deep sea fish are found to constitute a good quantity of by-catch discards from deep sea trawlers operating at off south west coast of India. Relatively lower flesh content, lack of consumer demand and market, smaller size at catch of fish are the major reasons behind discarding. New studies can reveal new utility of by-catch but discarding bycatch is complete wastage of natural resources and can cause environmental concerns too. Nutrient profiling of these by-catch fish is the primary step to find some utility that can lead to by-catch or waste utilisation. Most of the deep-sea fish stocks remain underexploited and harvesting of these fish resources can increase the capture fish production. Shifting the effort towards exploiting deep sea fish stocks shall reduce the exploitation pressure on other pelagic fish stocks allowing them to recover. In this regard the work tried to analyse and compare the chemical composition and fatty acids profiles of three marine fish species *viz Canthidermis maculata, Gephyroberyx darwinii* and *Satyrichthys adeni* with three available Indian food fish *viz Channa striata, Systomus serana* and *Gerres subfasciatus*.

Materials and methods

The fish muscles were homogenized well in a blender to prepare the fish samples for nutritional analyses after filleting and skinning. The moisture content, total nitrogen, crude fat and ash content of the samples were carried out according to the standard procedures of Association of Official Analytical Chemists (AOAC, 2005). Fatty acid methyl esters were derived from the samples through lipid extraction suggested by Folch *et al* (1957), followed by saponification and esterification (Metcalfe *et al*, 1966). The sample were then injected and run in Gas Chromatography (Perkinelmer clarus 580) to derive the fatty acid profile. Analyses were triplicated and the results were expressed as mean ± standard deviation.

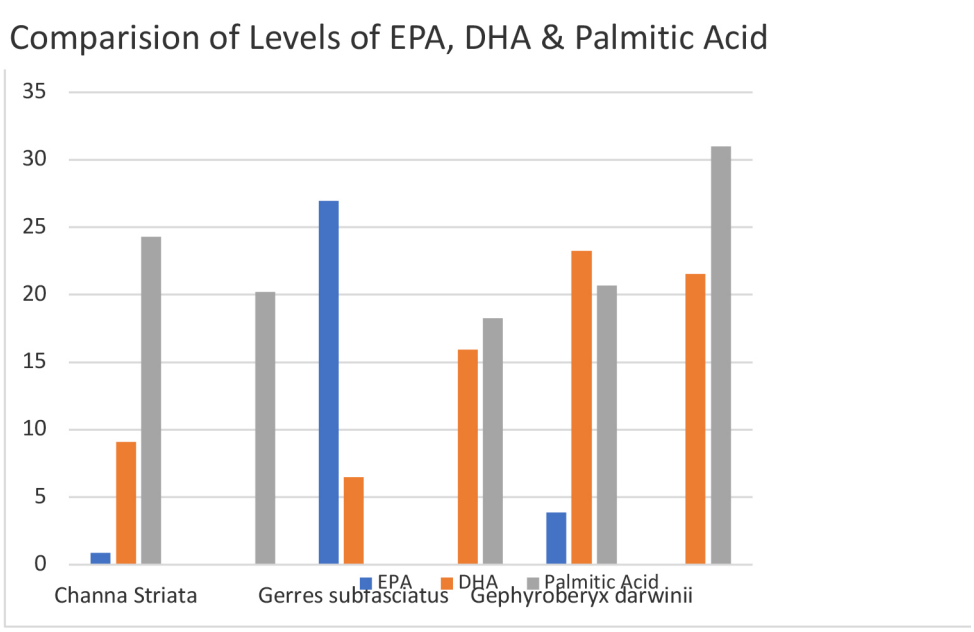


Fig 1: Comparison of levels of EPA, DHA and Palmitic Acid in *Channa striata, Systomus serana, Gerres subfasciatus, Canthidermis maculata, Gephyroberyx darwinii* and *Satyrichthys adeni*

(Continued on next page)

Results and Discussions:

The crude protein content expressed in percentage of *Canthidermis maculata, Gephyroberyx darwinii* and *Satyrichthys adeni* were found to be 21.99%, 19.97% and 20.99% respectively which were comparable with the crude protein content of the food fish *viz Channa striata* (17.97%), *Systomus serana* (21.93%) and *Gerres subfasciatus* (22.01%). The significant protein level implies that it can be a good raw material for fish meal production. Levels of docosahexaenoic acid (DHA) in the total fatty acid level of *Canthidermis maculata*, *Gephyroberyx darwinii* and *Satyrichthys adeni* were 15.94%, 23.23% and 21.52% respectively which were significantly higher than the levels in the food fish. The level of palmitic acid (in brackets) in *Canthidermis maculata* (18.26%), *Gephyroberyx darwinii* (20.68%) and *Satyrichthys adeni* (30.98%) were comparable with that of the food fish *viz Channa striata* (24.28%) and *Systomus serana* (20.20%). Among the six fish, significant levels of eicosapentaenoic acid (EPA) were only found in *Gephyroberyx darwinii* (3.87%), *Channa striata* (0.89%) and *Gerres subfasciatus* (26.98%). Caproic acid was found in in significant level in *Canthidermis maculata* (59.70%) and *Satyrichthys adeni* (14.64%). The significant levels of DHA, EPA and palmitic acids (see figure 1) in the marine fish can be extracted to be used as food or feed additive, nutrition supplements, pharmaceutical products, emollient or surfactant in cosmetics etc.

References:

Association of Official Analytical Chemists (AOAC). 2005. Official Methods of Analysis of AOAC,18th edn.,(Horwits, W. and Latimer, G.W.Jr.,Eds), AOAC, Gaithersburg, Maryland, USA.

CMFRI, Kochi (2019) *CMFRI Annual Report 2019*. Technical Report. CMFRI, Kochi.

Dineshbabu, A.P., Radhakrishnan, E.V., Thomas, S., Maheswarudu, G., Manojkumar, P.P., Kizhakudan, S.J., Pillai, S.L., Chakraborty, R.D., Josileen, J., Sarada, P.T. and Sawant, P.B., 2013. Appraisal of trawl fisheries of India with special reference on the changing trends in bycatch utilization. *Journal of the Marine Biological Association of India*, *55*(2), pp.69-78.

Folch, J., Lees, M. and Stanley, G., 1957. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *Journal of Biological Chemistry*, **226**(1), pp.497-509.

Lieske, E. and R. Myers, 1994. Collins Pocket Guide. Coral reef fishes. Indo-Pacific & Caribbean including the Red Sea. Haper Collins Publishers, 400 p

Maul, G.E**.**, 1990. Trachichthyidae. p. 620-622. In J.C. Quéro, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 2.

Metcalfe, L. D., Schmitz, A. A., & Pelka, J. R. 1966. Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis. *Analytical Chemistry*, **38**(3), 514–515.

National Fisheries Development Board (NFDB). 2020. *Annual Report 2020*. NFBD, Hyderabad.

ANTIOXIDANT SYNERGY OF MEDITERRANEAN HERBS AND ALGAL POLYSACCHARIDES: POTENTIAL IN THE DEVELOPMENT OF FUNCTIONAL BEVERAGES

M. Repajić[a*], A. Dobrinčić[a] and V. Dragović-Uzelac[a]

[a]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia
Email: maja.repajic@pbf.unizg.hr

Introduction

Herbal extracts and algae contain a wide range of bioactive molecules with positive effects on human health. However, their application in the production of functional beverages is still insufficient despite the fact that various plant species and their potential use as ingredients in different categories of functional beverages have been explored.

Salvia officinalis and *Thymus serpyllum* are aromatic herbs from the Lamiaceae family native to the Mediterranean area. Due to their high antioxidant capacity, which is directly correlated with a high content of various polyphenolic and volatile compounds (Mrkonjić et al., 2021), they present a valuable source for the production of functional beverages. On the other hand, green alga *Ulva lactuca* is an important source of sulfated polysaccharide ulvan, while brown algae *Fucus virsoides* and *Cystoseira barbata* are important sources of sulfated polysaccharide fucoidan. All of these polysaccharides show a wide range of biological activities such as antioxidant, anti-inflammatory and antitumor, however they are strongly dependent on chemical composition of polysaccharides.

In order to test the possibility in the development of functional beverage on the basis of selected Mediterranean herbs extracts enriched with algal polysaccharides, the aim of this research was to evaluate antioxidant capacity of *S. officinalis* and *T. serpyllum* extracts, polysaccharides of *U. lactuca*, *F. virsoides* and *C. barbata* and their mixtures.

Materials and methods

S. officinalis and *T. serpyllum* were purchased from Suban Ltd. (Strmec, Croatia), while *U. lactuca*, *F. virsoides* and *C. barbata* were harvested from coastal region of Zadar (Croatia) in February 2020. *S. officinalis* and *T. serpyllum* (2 g) were each extracted with ddH_2O (100 mL) using ultrasonic bath at 50 °C/30 min. Obtained extracts were analysed for total polyphenols using Folin-Ciocalteau method (Repajić et al., 2018) and mixed in 1:1 ratio for further experiment. Conventional polysaccharide extraction was performed at 80 °C/3 h using 0.1M H_2SO_4 (Dobrinčić et al., 2021). Extracted polysaccharides were analyzed as follows: total sugars by colorimetric phenol-sulfuric acid method (Dubois et al., 1956), L-fucose concentration by colorimetric assay with L-cysteine (Dische and Shettles, 1948), sulfate content was quantified after acid hydrolysis (1M HCl at 105 °C for 5h) by turbidimetric $BaCl_2$-gelatin method (Dodgson and Price, 1962) and uronic acid content was measured with modified sulfamate/m-hydroxydiphenyl colorimetric method (Filisetti-Cozzi and Carpita, 1991). In order to evaluate antioxidant synergy of Mediterranean herbs and algal polysaccharides, three different amounts of polysaccharides (0.25, 0.5 and 1 g) from each alga were mixed with 10 mL of herbal extract. The ability of prepared extracts (herbal extract, algal polysaccharides and their mixtures) to scavenge the DPPH radical was assessed spectrophotometrically.

Results

Content of total sugars in all three algal polysaccharides ranged from 57.28 to 61.21%, while sulfate group content ranged from 33.55 to 41.86%. *U. lactuca* polysaccharides had significantly lower content of fucose and uronic acid, while *C. barbata* polysaccharides contained the highest fucose content. The highest content of uronic acid was found in *F. virsoides* polysaccharides. Herbal extracts contained 27.00 (*S. officinalis*) and 15.03 mg GAE g^{-1} (*T. serpyllum*) of total polyphenols and their DPPH radical scavenging activity was 242.03 and 215.9 mg L^{-1}, respectively. Furthermore, mixture (1:1 ratio) of these extracts showed DPPH radical scavenging activity of 230.53 mg L^{-1}, while DPPH radical scavenging activity of *U. lactuca*, *F. virsoides* and *C. barbata* polysaccharides was 43.51, 67.64 and 71.14 mg L^{-1}, respectively. Addition of polysaccharides into the herbal extract increased DPPH radical scavenging activity (280.03 to 299.9 mg L^{-1}) regardless of the algal species and the amount added. However, increase of polysaccharides addition from 0.25 to 1 g increased scavenging activity in *F. virsoides* and *C. barbata* herbal mixtures, but not in herbal mixture of *U. lactuca*.

(Continued on next page)

Discussion and conclusion

Various biological activities of algal polysaccharides are often associated with their chemical composition, e.g. high levels of L-fucose, high degree of sulfation and low levels of contaminants such as uronic acid and protein (January et al., 2019). However, significant discrepancies in structural properties makes it impossible to precisely relate the bioactivity of polysaccharides with just one structural property but rather a combination of several properties. Green algae *U. lactuca* is a source of ulvan whose major monosaccharide unit is L-rhamnose, while L-fucose is predominant monosaccharide in brown algae polysaccharides. Lower fucose and sulfate group content, despite low level of uronic acid, could explain lower DPPH radical scavenging activity of *U. lactuca* polysaccharides. On the other hand, *S. officinalis* and *T. serpyllum* extracts contain various polyphenols which are known as strong antioxidants. The chemical structure of polyphenols varies upon number of aromatic rings and hydroxyl substituents which enable polyphenols to scavenge free radicals and reacting oxygen species through direct reaction with free radicals and from the chelation of free metals (Leopoldini et al., 2011).

Obtained results revealed a synergistic action of polyphenols from Mediterranean herbs and algal polysaccharides by which antioxidant activity of herbal extracts increased with the addition of algal polysaccharides, even in low amounts. These observations present an excellent base for the further development of functional beverages enriched with natural antioxidants.

References

Dische, Z., and Shettles, L.B. 1948. A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination. Journal of Biological Chemistry 175, 595–603.

Dobrinčić, A., Dobroslavić, E., Pedisić, S., Balbino, S., Elez Garofulić, I., Čož-Rakovac, R., and Dragović-Uzelac, V. 2021. The effectiveness of the Fucus virsoides and Cystoseira barbata fucoidan isolation as a function of applied pre-treatment and extraction conditions. Algal Research 56, 102286.

Dodgson, K.S., and Price, R.G. 1962. A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochemical Journal 84, 106–110.

Dubois, M., Gilles, K., Hamilton, J., Rebus, P., and Smith, F. 1956. Colorimetric method for the determination of sugars and related substances. Analytical Chemistry 28, 350–356.

Filisetti-Cozzi, T.M.C.C., and Carpita, N.C. 1991. Measurement of uronic acids without interference from neutral sugars. Analytical Chemistry 197, 157–162.

January, G.G., Naidoo, R.K., Kirby-McCullough, B., and Bauer, R. 2019. Assessing methodologies for fucoidan extraction from South African brown algae. Algal Research 40, 101517.

Leopoldini, M., Russo, N., and Toscano, M. 2011. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry 125, 288-306.

Mrkonjić, Ž., Rakić, D., Kaplan, M., Teslić, N., Zeković, Z., and Pavlić, B. 2021. Pressurized-liquid extraction as an efficient method for valorization of Thymus serpyllum herbal dust towards sustainable production of antioxidants. Molecules 26, 2548.

Repajić M., Hajdinjak I., Lončarić P., Ivanković M., Dragović-Uzelac V. and Levaj B. 2018. Stability of olive leaf drink upon storage at different temperatures. Glasnik zaštite bilja, 41, 86-94.

DIETARY INCLUSION OF 3% BLOOD HYDROLYSATES IMPROVES THE DISEASE RESISTANCE TO TENACIBACULOSIS IN EUROPEAN SEABASS

Daniela Resende*[1,2,3], Benjamin Costas[1,2], Tiago Sá[1], Umberto Golfetto[1,2], Marina Machado[1,2], Miguel Pereira[3], Carlos Pereira[4], Bianca Marques[5], Cristina Rocha[5], Manuela Pintado[3], Luísa M.P. Valente[1,2]

[1]CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal
[2]ICBAS, UP, Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[3]CBQF, Laboratório Associado, ESB-UCP, Rua Diogo de Botelho, 1327, 4169-005 Porto, Portugal
[4]Politécnico de Coimbra/ESAC, Bencanta, 3045-601 Coimbra, Portugal
[5]CEB, UM, Campus de Gualtar, 4710-057 Braga, Portugal
*Presenting author. Email: danielaresende@outlook.com

Introduction

Bioactive peptides are small amino acid chains with valuable properties (beyond their nutritional value), including antioxidant, mineral-binding, immunomodulatory or antimicrobial activities. They can be obtained from protein-rich by-products and included in aquafeeds, contributing to circular economy and waste reduction. In this work, we hypothesised that including blood hydrolysates (BH) in sustainable plant-based aquafeeds could promote fish robustness, increase the economic value of animal blood (which is often discarded) and minimize waste, contributing to a circular economy. Additionally, BH may address the *T. maritimum* infections that occur in aquaculture farms, with high impact on fish health, well-being and aquaculture production.

Methods

Three fractions of swine BH obtained by autohydrolysis (AH) or enzymatically were selected. AH was oven dried, whilst the enzymatically obtained BH were further submitted to a micro- (MF) and nanofiltration (NF) and the respective retentates freeze-dried. Dried hydrolysates were then included in five isolipidic and isoproteic diets for European seabass: a fishmeal (FM) based diet (positive control, PC), a commercially-based diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g), fed three times daily until apparent satiation in a recirculating saltwater system (RAS). Growth, nutrient utilisation and whole-body, liver and muscle composition were evaluated after 12 weeks. Plasma was also collected from 9 fish per treatment for evaluation of immune parameters. At the end of the trial, ten fish per tank were infected with *Tenacibaculum maritimum* (3.5×10^5 cfu/mL), in a two-hour water bath, and mortality was assessed for 8 days. A digestibility trial was carried out with the remaining fish from the growth trial in a Guelph system, for evaluation of macronutrient and mineral digestibility. All diets were supplemented with 200 mg of Y_2O_3 per kg, as an inert marker.

Results

Dry matter apparent digestibility coefficient (ADC) was significantly lower in hydrolysates-containing diets compared to the PC; NF did not differ from the NC. Protein ADC was generally high (>91%) and both MF and NF had similar values to the PC and NC diets. MF and AH had a significantly lower lipid ADCs when compared to PC and NC, while the NF only differed from the PC. Mineral ADC values of the PC, NC and NF did not differ statistically. AH diet led to a significantly lower iron ADC value compared to the controls. Calcium ADC was significantly lower for MF and AH diets, when compared to all other diets. MF also displayed lower copper and potassium ADC.

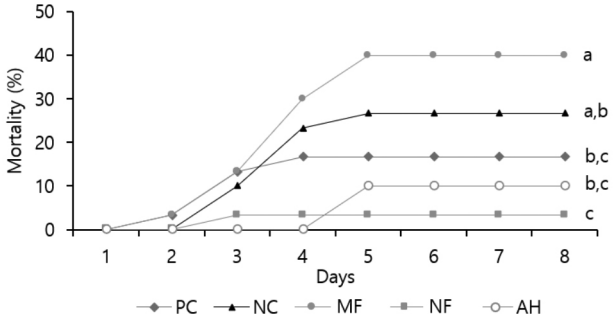


Figure 1 - Mortality rate of seabass fed the experimental diets for 12 weeks and challenged with *T. maritimum*. Different letters indicate significant differences among treatments.

(Continued on next page)

Fish fed PC had the highest final weight, followed by NC and NF that had similar final weights. Specific growth rate (SGR) and feed conversion ratio (FCR) of fish fed NF showed no significant differences from those fed PC and NC. Diet MF induced the lowest final body weight and highest FCR.

Despite no significant differences among treatments regarding final body composition, lipid and energy retention and gain were lowest in fish fed MF, which also had the lowest condition factor. No differences were found in muscle and liver lipid content.

Innate immunity markers were evaluated in the plasma. Peroxidase was increased by the MF diet, but lysozyme was significantly lower in fish fed this diet, compared to all other treatments. Regarding the infectious challenge, NF had the lowest mortality rate that differed significantly from the NC and MF groups (figure 1).

Conclusions

Results clearly show that the NF diet was able to significantly reduce European seabass mortality after exposure to a very common pathogen in fish farms (*T. maritimum*). It should be noted that the higher pathogen resistance was achieved without affecting fish growth compared to a commercially-based diet (NC). Contrarily, MF diet reduced nutrient digestibility and fish growth; this diet also led to decreased plasmatic lysozyme, which supports the lower resistance towards disease that was evidenced in the bacterial challenge, as this diet led to the highest mortality. In conclusion, the enzymatically obtained blood hydrolysate further submitted to a nanofiltration (NF) has a high potential to be included in aquafeeds for European seabass, corresponding to a new and effective way to reduce fish susceptibility to *T. maritimum*.

Acknowledgments

Work supported by Project MOBFOOD, POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT, SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019).

EFFECT OF THE DIETARY INCLUSION OF BLOOD HYDROLYSATES ON EUROPEAN SEABASS RESPONSE TO STRESS

Daniela Resende*[1,2,3], Ricardo Pereira[1,2,3], Cristina Velasco[1], David Domínguez[1], Miguel Pereira[3], Carlos Pereira[4], Bianca Marques[5], Cristina Rocha[5], Manuela Pintado[3], Luísa M.P. Valente[1,2]

[1]CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal
[2]ICBAS, UP, Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[3]CBQF, Laboratório Associado, ESB-UCP, Rua Diogo de Botelho, 1327, 4169-005 Porto, Portugal
[4]Politécnico de Coimbra/ESAC, Bencanta, 3045-601 Coimbra, Portugal
[5]CEB, UM, Campus de Gualtar, 4710-057 Braga, Portugal
*Presenting author. Email: danielaresende@outlook.com

Introduction

In aquaculture farms, fish are subjected to several stress situations, including high farming densities, periodic handling, routine procedures or even changes in temperature, oxygen and other environmental parameters. All these stressors may induce oxidative stress by causing an imbalance between the generation of reactive oxygen species (ROS) and the scavenging activity of antioxidants [1]. The accumulation of ROS may damage biomolecules such as proteins, lipids or nucleic acid, impairing growth, immune response and flesh quality [2]. Bioactive peptides, small amino acid chains with properties including antioxidant, mineral-binding, immunomodulatory or antimicrobial activities, can be obtained from protein-rich by-products, making them attractive ingredients for inclusion in aquafeeds within the context of a circular economy. Thus, supplementation of diets with functional ingredients able to modulate fish oxidative stress has been considered in this work, using bioactive peptides included in swine blood hydrolysates (BH). We have investigated the potential of the dietary inclusion of BH to improve European seabass (*Dicentrarchus labrax*) oxidative status after air exposure.

Methods

Three fractions of swine BH obtained by autohydrolysis (AH) or enzymatically were selected. The enzymatically obtained BH were further submitted to a micro- (MF) or nanofiltration (NF). Dried hydrolysates were then included in five isolipidic and isoproteic diets for European seabass: a fishmeal (FM) based diet (positive control, PC), a commercial-based diet where 50% of FM was replaced by plant proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g), and fed to apparent satiation in a recirculating saltwater system (RAS). After 12 weeks, 9 fish per treatment were either immediately sampled or air-exposed for 1 minute and let to recover for 6 hours prior to sampling. Plasma and liver were collected to evaluate oxidative stress indicators.

Results

Plasmatic cortisol and lactate were elevated for all diets after the air exposure although differences among diets were not found. Glucose levels were unaffected by either diet or stress. Regarding oxidative stress markers in the liver, lipid peroxidation (LPO), measured by TBARS, tended to increase after air exposure. Considering the non-stressed fish, MF diet led to the lowest LPO levels, being significantly lower than the AH, but without differing significantly from the remaining diets. In stressed fish, the dietary impact on LPO levels was limited. Protein oxidation, evaluated by carbonyl content, decreased in stressed fish, irrespectively of the dietary treatment. Liver catalase was significantly lower in the NC, NF and AH compared to the PC, and increased after stress in all treatments. Glutathione peroxidase was unaffected by diets or stress condition. Fish fed MF had a significantly higher superoxide dismutase activity than those fed PC and AH diets and was reduced in all stressed fish compared to the non-stressed ones.

Conclusions

The stress response triggered by air exposure involved an increase in cortisol levels, which was followed by an increment in plasma lactate; however, the tested BHs could not enhance plasma response to stress. Regarding liver oxidation markers, the stress challenge increased LPO, as ROS can accumulate in the liver under oxidative stress. Carbonyls decreased post-stress, likely due to a feedback interaction with the radicals produced during LPO, which acted to reduce protein oxidation. The NF, composed of smaller peptides than the other BH, may modulate European seabass antioxidant defences by lowering catalase levels without increasing LPO, suggesting this diet could be providing exogenous antioxidants to counteract ROS-induced oxidative stress.

(Continued on next page)

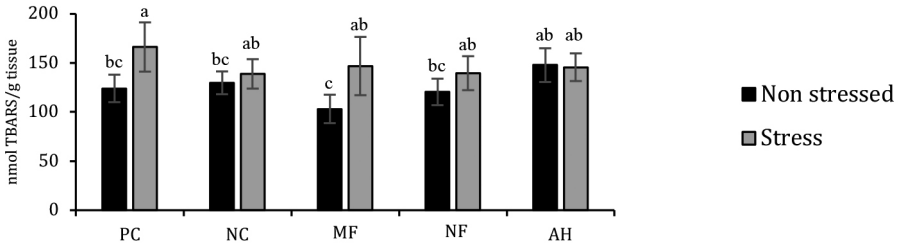


Figure 1 - Lipid peroxidation (LPO) in the liver of fish sampled before (non-stressed) or after (stress) 1 minute air exposure, and after feeding the experimental diets for 12 weeks. Values presented as mean ± standard deviation (n=9). Different lowercase letters indicate significant differences for Diet x Stress after a two-way ANOVA ($p<0.05$).

References

1. Hanke, I., et al., *Chronic stress under commercial aquaculture conditions: Scale cortisol to identify and quantify potential stressors in milkfish (Chanos chanos) mariculture*. Aquaculture, 2020. **526**: p. 735352.
2. Abdel-Tawwab, M., et al., *Dietary garlic and chitosan enhanced the antioxidant capacity, immunity, and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass*. Fish Shellfish Immunol, 2021. **113**: p. 35-41.

Acknowledgments

Work supported by Project MOBFOOD, POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT, SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019). RP thanks FCT for his PhD grant (SFRH/BD/144631/2019).

BIOFORTIFIED FISH PRODUCTS FOR CONSUMERS WITH NATURAL IODINE, SELENIUM AND FATTY ACIDS: IMPACTS ON FISH PHYSIOLOGY

Laura Ribeiro[1]*, Marisa Barata[1], Ravi Araujo-Luna[1], Vera Barbosa[1], Narcisa Bandarra[1],Florbela Soares[1], Cátia Marques[1], Jorge Dias[2], António Marques[1], Pedro Pousão- Ferreira[1]

[1]IPMA- Instituto Português do Mar e da Atmosfera, I.P. / Estação Piloto de Piscicultura de OlhãoEPPO, Av. Parque Natural da Ria Formosa, s/n 8700 - Olhão, Portugal
[2]SPAROS - SPAROS, Lda., 8700-221 Olhão, Portugallribeiro@ipma.pt

Introduction
Tailor-made fortified seafood products can contribute to overcome one third of global population nutritional deficiencies, particularly on iodine, selenium and iron (1). Therefore, eco-innovative biofortified diets in iodine (macroalgae), selenium (yeast), EPA and DHA (microalgae), were enhanced with levels of these essential nutrients within the legal limits for consumers (2) and tested with gilthead seabream (*Sparus aurata*). These nutrients are known to interfere in vertebrates important metabolic and physiological functions, thus affecting both humans and fish. Iodine is an essential mineral for thyroid function (metabolism), selenium is involved in different and important enzymatic reactions of different pathways (deiodinases, oxidative stress, digestive enzymes, etc) and EPA and DHA are essential for membranes formation, lipid metabolism, synthesis of important bioactive molecules, among others. Therefore, biofortification of farmed fish products must assure that levels used in diets do not interfere with fish physiological functions, to avoid compromising fish health and welfare. The aim of this study was to assess the effect of the biofortified diets on different aspects of seabream physiology namely on digestive physiology, blood analysisand liver condition.

Material and Methods
Trial was carried out at IPMA's aquaculture research station in Olhão, for 3 months, using gilthead seabream (2). Fish were fed four experimental diets manufactured by SPAROS, one based on commercial formulation (CTRL), and three enriched diets (B1, B2 and B3) supplemented with different blends of iodine-rich macroalgae (0.40% in B1 and B2; 0.80% in B3) and selenized yeast (0.015% in B1 and B2; 0.035% in B3). At the end of the trial six fish were anesthetized, blood collected from caudal vein, and afterwards sacrificed to collect intestine and liver samples for analysis. Histological sections of intestine and liver stained with H-E were made to analyse tissue structure andintegrity. Aminopeptidase and alkaline phosphatase activities were determined using specific substrates. Liver fatty acid profile was determined by GC-FID. EPOC multiparameter blood reader (Siemens Healthcare) was used for blood analysis (haematocrit, haemoglobin, pH, pCO_2, pO_2, K^+, glucose and lactate). Plasma was used for total lipids, triglycerides, and phospholipids determination using clinical diagnostic kits from SPINREACT (Spain), whereas enzyme immunoassay kits from IBL(Germany), were used for quantitative determination of triiodothyronine and free triiodthyronine (fT3).

Results
The histological structure of gilthead seabream intestine and the number of mucous cells scattered on the villi epithelium ($P>0.05$) were similar among the different dietarytreatments. The activity of digestive enzymes was differently affected by the diet, since fish fed B2 diet exhibited higher aminopeptidase activity ($P<0.05$), whereas alkaline phosphatase was higher for fish fed CTRL and B3 diets. Liver histological structure of fish was similar regardless the diet. Liver fatty acid profile exhibited few differences among treatments, where higher levels of n-3 fatty acids reflected the biofortification process.

Blood analysis of gilthead seabream indicated that parameters varied within similar intervals of values. Still, pH, oxygen and glucose were higher on fish fed B1 diet ($P<0.05$). Similar values were observer for the analysed plasmatic parameters (lipid metabolism and thyroid hormones) among treatments, although FT3 level tended to increase in the biofortified diets.

Discussion
Physiological biomarkers values observed for gilthead seabream fed biofortified diets were largely similar when compared to control diet. Biofortified diets induced different physiological responses by gilthead seabream for the different parameters studied. The different composition of biofortified diets, manufactured with different ingredients and doses, might have triggered biological pathways differently, resulting in different physiological responses by fish. Integrating some of the physiological biomarkers (alkaline phosphatase activity and FT3 levels) with zootechnical parameters (2), suggeststhat B3 diet enhanced fish metabolism but with low energy efficiency, due to the higher FCR and lower weight attained at the end of the trial.

Overall the biofortification of fish farmed products for consumers is possible withoutcompromising fish health and welfare.

(Continued on next page)

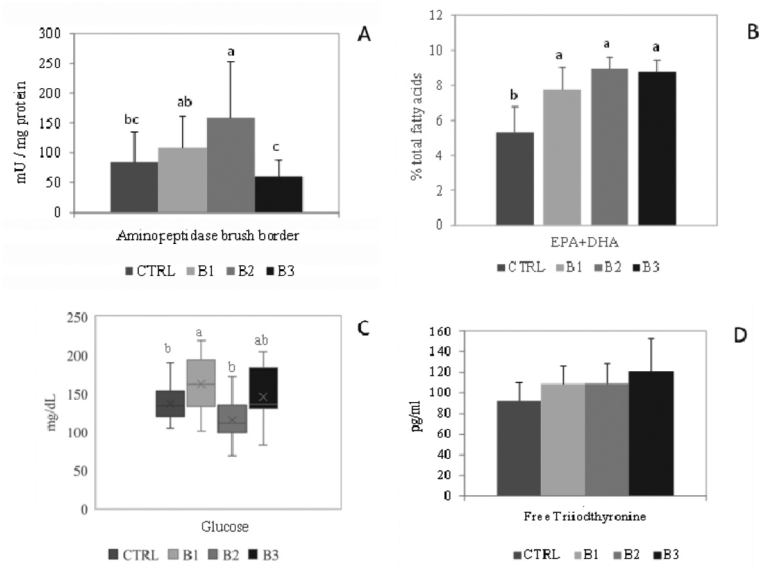


Figure 1 – Different physiological biomarkers measured on different tissues of *Sparus aurata* fed experimental diets; A - aminopeptidase activity on intestine; B- EPA+DAH on liver; C – & D – plasmatic levels, respectively of glucose and free triiodothyronine.

Acknowledgments

To Horizon 2020 and MAR2020 programmes, respectively SEAFOODTOMORROW (G.A. no. 773400) and DIVERSIAQUA II project (MAR-02.01.01-FEAMP-0175) for funding this study.

References

Pinkaew and Karrila, 2015. Key properties of iodine-, iron- and zinc- fortified fish cracker: effects of ambient shelf storage on iodine retention and quality indicators. doi:10.1111/ijfs.12871

Barbosa V. et al., 2020. Enriched feeds with iodine and selenium from natural and sustainable sources to modulate farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) fillets elemental nutritional value. doi. org/10.1016/j.fct.2020.111330

PROJECT AQUA&AMBI 2 –SUPPORT THE WETLAND MANAGEMENT IN THE IBERIAN SOUTHWEST COASTAL AREA: INTERACTIONS BETWEEN AQUACULTURE AND THE ENVIRONMENT IN THE EUROREGION ALENTEJO-ALGARVE-ANDALUSIA

L. Ribeiro[1]*, P. Pousão-Ferreira[1], D. Matias[1], S. Joaquim[1], J. Garcês[1], G.M Arroyo[2], S. Haro[2], A. de la Cruz[2], F. Zurita[3], M.L. Lara[3], M. Fernandez[3], P. Noronha[4], P. Gaspar[4], J.-L. Oviedo[5], E. Malta,[6] M.M. Agraso[6], O. Moreno[7], M. Herrera[7], and Maria E. Cunha[1]

[1]IPMA- Instituto Português do Mar e da Atmosfera, I.P. / Estação Piloto de Piscicultura de Olhão EPPO, Av. Parque Natural da Ria Formosa, s/n 8700 - Olhão, Portugal
[2] Dept. Biology, Universidad de Cádiz (UCA) / University Institute of Marine Research INMAR-UCA, Spain
[3] Agencia de Gestión Agraria y Pesquera de Andalucía (AGAPA), Spain
[4] Portuguese Environment Agency (APA), Portugal
[5] Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Spain
[6] Fundación Centro Tecnológico de Acuicultura de Andalucía (CTAQUA), Spain
[7] Instituto de Formación Agraria y Pesquera (IFAPA, Agua del Pino), Spain

Email:lribeiro@ipma.pt

Introduction

The Iberian southwest coast possesses important and strategic zones for biodiversity conservation that are under the umbrella of different environment conservation instruments such as Natura 2000, RAMSAR, Special Protection Areas (SPA and National Parks. Traditionally these were sources of local socio-economic profits, such as extensive/semi-intensive aquaculture in earthen ponds using old salterns (1). Currently, their low profitability resulted in the neglect of the ponds and loss of biodiversity since, due to sedimentation, they become unproductive diked areas. Although intensive production systems are known to be responsible for biodiversity loss, recent studies acknowledge the importance of low-intensive land-use systems as important elements for large-scale conservation programs (2). It is, therefore, important to understand the environmental effects of land use for the conservation of biodiversity, and its relation to ecosystem services.

Project approach

The idea behind AQUA&AMBI project (phase 1 and phase 2) was to develop tools and knowledge to support decision on the management of wetlands, and to increase the profitability of these wetland by implementing ecological approaches to aquaculture systems. A multidisciplinary approach was adopted combining expertise from natural and social scientists, from various cross-border institutions of Portugal and Spain.

During phase 1, AQUA&AMBI 1 contributed for the creation of a Geographic Information System that enabled the recognition of areas with different legal protection levels, as well as their compatibility with territory uses, activities and occupation. Main ecosystem services provided by aquaculture were assessed, whit special focus on those related to ornithological biodiversity. Also public consultation, to ascertain scores of the natural capital associated with threatened biodiversity and to generators of ecosystem services (conservation of avifauna and natural oyster banks), was performed to establish basis for future conservation programs.

The actual phase 2 of the project, AQUAAMBI 2 (Fig. 1) will bring out information to support coastal spatial planning, assess the role of aquaculture and salt production in the carbon balances, identify new species for production and/or with biotechnological importance, and ecological approaches to aquaculture production like organic and integrated multi-trophic farming. The technologies and knowledge obtained within the project will be transferred to stakeholders in specific actions.

Expected results

The information obtained with AQUA&AMBI will allow the development of innovative methodologies to enhance the productive identity of these spaces and their dynamism as an engine of socio-economic development and a factor of competitiveness for other regions. In addition, the collective linked to the sea sector has been enriched with tools to access new opportunities that generate employment and economic growth and increase opportunities for transition to sustainable aquaculture activities.

Overall, the concept of AQUA&AMBI is to contribute to preserve and even enhance biodiversity thru optimization of profitability of abandoned areas under the actual legal framework and the spreading knowledge and use of ecological approaches to aquaculture production.

(Continued on next page)

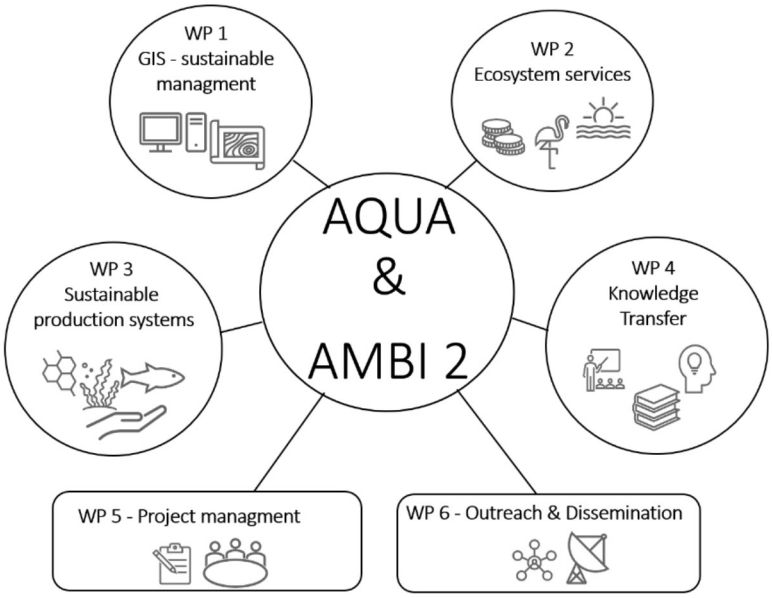


Figure 1 – Structure of the project AQUA&AMBI 2

Acknowledgments
The authors acknowledge to INTERREG V A Espanha Portugal (POCTEP) program for funding through project 0750_AQUA_AMBI_2_5_P and project 0240_AQUA_AMBI_5_P

References

1. Sousa C.A.M., Cunha M. E., Ribeiro L., 2020. Tracking 130 years of coastal wetland reclamation in Ria Formosa, Portugal: opportunities for conservation and aquaculture compatibilization. Land Use Policy, 94:104544. doi. org/10.1016/j.landusepol.2020.104544
2. Tscharntke,T. Klein, A.M., Kruess A., Steffan-Dewenter I., Carsten T., 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. Ecology Letters, 8: 857–874 doi: 10.1111/j.1461-0248.2005.00782.x

WILL CONSUMERS BUY FISH ENRICHED WITH OMEGA 3 FATTY ACIDS?

A.R.A. Ribeiro*, G. Teixeira, J. Tomé and E. Matos

*B2E Associação para a Bioeconomia Azul – Laboratório Colaborativo. Av. da Liberdade s/n 4450-718 Leça da Palmeira
E-mail: geral@b2e.pt

Introduction

Global aquaculture is growing at a fast rate and is a fundamental sector as source of healthy food and nutrients for humans (FAO, 2018). All fish are rich in macronutrients, vitamins, and minerals and are our main source of long-chain omega-3s. This characteristic associates fish consumption with numerous health benefits, distinguishing itself from other agri-food products. The aquaculture industry, particularly of carnivorous fish, still depends on fishmeal (FP) and fish oil (OP) obtained from wild fish, standing a huge and 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. Therefore, it is essential to investigate strategies to overcome this problem, since one of the main goals of aquaculture is to guarantee the consumer the supply of omega 3, recommended by the World Health Organization to promote health. OmegaPeixe project aims at mitigating this problem through 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. For this strategy to have a real impact, this innovative product must be viable on the market, which means that the increase in costs per kg of fish produced should be inferior to the price increase that the consumers are willing to pay for a high-quality product, rich in omega 3s. In order to revisit the market's acceptance of differentiated aquaculture products, a comprehensive consumer survey was carried out nationally and in the relevant European countries, considering the general consumer segment, but also the BIO 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 to allow the collection of information about the target audience - socio-economic characterization data, consumption data for fish and aquaculture products, availability to pay for value-added products, level of confidence 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, entities relevant to consumers (e.g., DECO, Portuguese Nutrition Association, Portuguese Cardiology Association, FIPA), aquaculture sector entities (e.g., FAO, Portuguese Aquaculture Association, DOCAPESCA, European Aquaculture and Technology Platform), and NGOs (e.g., WWF, Quercus, ZERO) were approached to participate in the dissemination of the questionnaire, so that it was as comprehensive as possible. The answers were collected for 6 months, and then the collected information was statistically treated in order to develop the product study report.

This questionnaire was developed according to Hill[1]. Pre-tests of 20-30 quizzes were undertaken and a final sample size of 385 valid questionnaires were considered as the minimum number, in order to undertake a multivariate analysis and decreasing the error. Questions were simple, short and without conjunctions or disjunctions, for a better understanding of the respondent. Types of quantitative and qualitative responses were obtained, and the response scale was chosen in even number to better understand the trends. Moreover, questions were elaborated based on the indications and objectives of the OmegaPeixe project and previous surveys carried out in reference documents such as scientific articles[2,3] and publications by UE[4] and FAO[5].

The collected data will be treated using SPSS Statistics, by applying a Multivariate Analysis so that the hypotheses and relationships between the variables can be evaluated.

Results

The collection of survey responses is still ongoing. Results will elucidate the perception of consumers about aquaculture products, their positioning regarding their wild counterparts, and identify trends in fish consumption. Furthermore, the survey will establish how much consumers are willing to pay for a high-quality product, rich in omega-3s.

Acknowledgements

Work supported by Project OmegaPeixe, funded by Portugal 2020, financed by the European Regional Development Fund (FEDER) through the Operational Competitiveness Program (COMPETE) - POCI-01-0247-FEDER – 069748.

References

1. Hill, A. Hill, M.,2009. Investigação por Questionário, 2ª Edição.Edições Sílabo, Lisboa.
2. Gholifar, E., Abbasi, E., Pezeshki Rad, GH., Salehi, H., & Rezaei, A., 2017. Development and validation of sustainable aquaculture indicators: Case of Alborz Dam Basin, Mazandaran, Iran. International Journal of Agricultural Management and Development, 7(2), 191-199. URL: http://ijamad.iaurasht.ac.ir/article_527216_3f90b13efdaa111fbf74c5969da10b4b.pdf
3. Verbeke, W., Sioen, I., Pieniak, Z., Van Camp, J., & De Henauw, S., 2005. Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. Public Health Nutrition, 8(4), 422-429. doi:10.1079/PHN2004697
4. European Commission., 2019. Assessment of voluntary claims on fishery and aquaculture products. Directorate-General for Maritime Affairs and Fisheries. ISBN 978-92-76-04615-8. doi: 10.2771/732718
5. FAO, 2018. The State of World Fisheries and Aquaculture 2018. Available at: http://www.fao.org/3/i9540en/I9540EN.pdf

INFLUENCE OF TEMPERATURE ON EGG PRODUCTION AND EMBRYO VIABILITY OF THE FISH PARASITE *Calceostoma glandulosum* (MONOGENEA)

M. C. Ribeiro[1,2], C. L. Marques[1], *T. Baptista[2], P. Pousão-Ferreira[1], F. Soares[1]

[1] Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO), Av. Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
[2] MARE –Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Rua do Conhecimento nº 4, 2520-641 Peniche, Portugal
E.mail: teresa.baptista@ipleiria.pt

Introduction

Fish consumption has increased over the past decades due to high nutritional value, but the large scale of the world population and the fast growth rate may lead to collapse the stocks in the sea, and there is a need to increase production in aquaculture. Despite the evolution of sustainable strategies and the implementation of new technologies in aquaculture, it has started to become more intensive, resulting in increased disturbances throughout the fish production process, making them more susceptible to disease outbreaks (Ariel & Olesen, 2002). Monogenean ectoparasites, such as *Calceostoma glandulosum*, can be found on the gills of hosts as well as on its skin and fins. One of the main environmental factors influencing parasite life cycle (egg production and free-living larvae (*oncomiracidium)* hatching) is the temperature (Turgut, 2012). Thus, the main objective of this study was to understand how this abiotic parameter could influence egg production and viability, embryo development and, finally, what are the optimum values for hatching.

Methods

Monogenea eggs were collected from cotton strips which were placed in the *Argyrosomus regius* broodstock tanks at EPPO (Aquaculture Research Station of Olhão). During the trial, five average sea water temperatures were recorded 13ºC, 14ºC, 16ºC, 17ºC, 22ºC and 24ºC. Egg viability was also performed for each temperature. Therefore, the eggs collected from the tanks were counted and classified according to Militz *et al* (2014). All eggs showing *oncomiracidium* with developed eye opercula were removed from the collecting substrate, with a needle. Then transferred to a Petri dish and after to six-well incubation plates, forming a stock of 100 larvae, by placing 10 eggs in 3ml of UV-filtered seawater. Five different controlled incubation temperatures of 17ºC, 19ºC, 21ºC, 23ºC and 25ºC were then used, with a photoperiod of 12:12 (day/night), to understand which would provide the fastest hatching rate in the first 48 hours. The water in the wells was carefully renewed by about 1/3, every day. The cultures were observed daily at the same time, recording the day of hatching of the larvae.

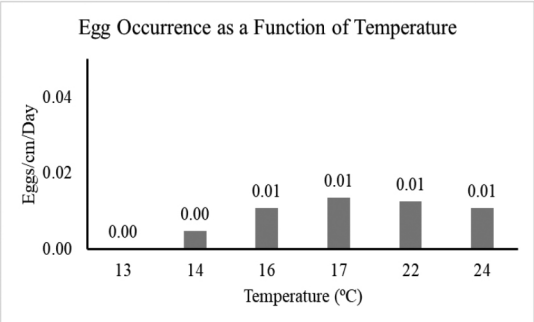


Figure 1 - Occurrence of *Calceostoma glandulosum* eggs in *Argyrosomus regius* broodstock at different temperature

23ºC**Table I** - Eclosion rate of Calceostoma glandulosum at different temperature

	Eclosion Rate (%)	
Temperature (ºC)	**24 H**	**48 H**
17	16	27
19	25	40
21	41	56
23	42	100
25	35	50

(Continued on next page)

Results

Along the trial it was observed that the number of eggs produced had oscillations, since at colder temperatures, such as 13ºC, egg production was almost non-existent and gradually increased at 14ºC, 16ºC and 17ºC, with the latter temperature presenting maximum values, decreasing gradually when the water reached 22ºC and the minimum was to 24ºC. Egg viability was also influenced by this factor as observed by the number of viable and hatched eggs with the rise of temperature values. With respect to egg incubation, where the conditions were complete controlled, a similar pattern for the hatching rate was observed, and the optimum value for total hatching, after 48 hours, was 23°C.

Discussion

In this study, *C. glandulosum* eggs hatching rate was found to be affected by fluctuations in water temperature, and a significant decrease in egg production was observed in the transition from autumn to winter, where minimum values were reached (13ºC), and then a significant increase at the beginning of spring (14ºC – 16ºC). Parasites, besides showing optimum values for egg production, exhibit a range in which they have the capacity to survive and produce eggs. In the case of *C. glandulosum* the range that this species is able to tolerate is situated between 14ºC and 25ºC. The viability of *C. glandulosum* eggs is also affected by temperature. Therefore, the number of hatched eggs decreased equally, as well as the embryonated eggs. This can be confirmed by Zhang *et al* (2021), as he states that when eggs are exposed to very low temperatures, outside the tolerable limits of the parasite, hatching fails completely, which may bring problems for the embryo that may eventually die, making the eggs unviable. On the other hand, when the temperature drops to levels, still within the range that the species can withstand, the eggs can be preserved and when this factor reaches optimal values the development of the embryo returns, since it has energy reserves and important lipids that facilitate larval longevity (Brazenor *et al*, 2020). To conclude, it was observed that *C. glandulosum* has a tolerable limit for temperature, particularly between 14ºC and 25ºC, demonstrating that, outside this range, the production and hatching of eggs is extremely low or does not occur. It was also possible to observe that the increase in temperature may be beneficial to a greater proliferation of this parasite, which may cause disease outbreaks.

Acknowledgments

The present work was financed by the projects SAUDE&AQUA (MAR-02.05.01-FEAMP-0009), Be4AQUAHEALTH (MAR-02.05.01-FEAMP-0013), Fundação para a Ciência e Tecnologia (FCT), through the strategic project UIDB/04292/2020 granted to MARE-Marine and Environmental Sciences Centre and DIVERSIAQUA II (MAR2020-P02M01-0656P).

References

Ariel, E., Olesen, N. J. (2002). Finfish in aquaculture and their diseases-a retrospective view on the European community. Bulletin-European Association of Fish Pathologists, 22(2), 72-85.

Brazenor, A. K., Francis, D. S., Conlan, J. A., Carton, A. G., Hutson, K. S. (2020). Temperature alters reproduction and maternal provisioning in a fish ectoparasite. International Journal for Parasitology, 50(10-11), 839-849.

Turgut, E., 2012. Influence of Temperature and Parasite Intensity on Egg Production and Hatching of the Monogenean. Israeli Journal of Aquaculture – Bamidgeh, 6, 1-5.

Militz, T. A., Southgate, P. C., Carton, A. G., Hutson, K. S. (2014). Efficacy of garlic (*Allium sativum*) extract applied as a therapeutic immersion treatment for *Neobenedenia sp*. management in aquaculture. Journal of Fish Diseases, 37(5), 451-461.

Zhang, X., Shang, B., Cheng, Y., Wang, G., Li, W. (2021). Effects of Different Strategies of Low Temperature on Egg Hatching of *Dactylogyrus vastator* (Monogenea: Dactylogyridae). Research Square, 2-9.

INVESTIGATING THE BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN MARINE GAMMARIDS

Alberto Ribes-Navarro [1], *, Juan C. Navarro [1], Francisco Hontoria [1], Naoki Kabeya [2], Inger B. Standal [3], Jan O. Evjemo [3] and Óscar Monroig [1]

[1] Instituto de Acuicultura de Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain
alberto.ribes@csic.es
[2] Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo, Japan
[3] Department of Fisheries and New Biomarine Industry, SINTEF Ocean, Trondheim 7010, Norway

Introduction

Gammarids are aquatic invertebrates that have been identified as promising candidates for their use in aquaculture. Gammarids have good growth performance, tolerate high densities and, importantly, can be fed on a wide range of sidestreams from bioindustries [1,2]sustainable nutrient sources for utilisation as dietary ingredients. Exploring the potential of under-utilised resources from other industries is imperative to replace finite natural resources, such as fish meal. Marine gammarids may be an excellent source of essential fatty acids; however, their aquaculture using formulated diets remains untested in terms of survival, growth and nutritional value of the cultured product. Here, juveniles of 2 marine gammarid species, Gammarus locusta and Echinogammarus marinus, were maintained in controlled feeding experiments with 2 marine diets (Ulva spp. and Fucus spp.. While marine gammarids contain relatively high levels of long-chain ($C_{20–24}$) polyunsaturated fatty acids (LC-PUFAs), physiologically essential compounds required for normal growth and development of vertebrates [3], it is unknown whether the LC-PUFA levels of gammarids grown on low LC-PUFA containing substrates can be maintained. Conversions of short-chain fatty acids (FAs) into LC-PUFAs in animals is determined by the complement and function of genes encoding enzymes that catalyse these multistep pathways [4]. Our main goal is to elucidate the LC-PUFA biosynthetic pathways in gammarids in order to identify species with high capacity to convert short-chain FAs available in sidestreams into LC-PUFAs that ultimately could allow obtaining a high nutritional value biomass. Specifically, this study aimed to characterise molecularly and functionally three genes encoding elongation of very long-chain fatty acid (Elovl) proteins, termed as *elovl4*, *elovl6* and *elovl1/7*-like, from *Echinogammarus marinus*.

Materials and methods

To obtain the full-length sequences of the *E. marinus elovl4*, *elovl6* and *elovl1/7*-like open reading frames (ORFs), BLAST searches were carried out on the Transcriptome Shotgun Assembly (TSA) of the *Gammarus* BioProject (PRJNA497972). The ORFs were identified and then isolated by PCR using *E. marinus* complementary DNA (cDNA) as template, with primers containing specific restriction sites for further cloning into the pYES2 vector. The *E. marinus* Elovl4, Elovl6 and Elovl1/7-like were functionally characterised by heterologous expression in yeast. Transgenic yeast containing the different *E. marinus elovl* ORFs were grown in the presence of a series of exogenously supplemented polyunsaturated fatty acid (PUFA) substrates to test their elongation capacity. After 2 day incubations, yeast were harvested and washed prior extraction of total lipids. An aliquot of total lipids was used to prepare fatty acid methyl esters for analysis by gas chromatography. Conversions of PUFA substrates to the corresponding products were calculated by the proportion of substrate FA converted to elongated FA product(s) as [areas of all products with longer chain than substrate/(areas of all products with longer chain than substrate + substrate area)] × 100.

Results

The molecular analyses showed that all the *E. marinus* Elovl sequences had the distinctive characteristics of fatty acyl elongases, including a histidine box (HXXHH). Interestingly, Elovl4 and Elovl1/7-like, but not Elovl6, contained diagnostic amino acids preceding the histidine box that are characteristic of PUFA elongases. Consistently, the functional characterisation results showed that the *E. marinus* Elovl4 and Elovl1/7-like are indeed PUFA elongases, since they elongated PUFA substrates ranging from C_{18} to C_{22}. However, the *E. marinus* Elovl6 only showed activities towards C_{18} PUFA substrates (Table 1).

(Continued on next page)

Table 1. Functional characterisation of the different *Echinogammarus marinus* elongases.

FA substrate	FA product	Elovl4	Elovl6	Elovl1/7-like	Activity
18:3n-3	20:3n-3	2.67	0.22	0.29	C18→C20
18:2n-6	20:2n-6	1.32	0.11	0.19	C18→C20
18:4n-3	20:4n-3	1.82	0.33	1.46	C18→C20
	22:4n-3	0.02	nd	0.02	C20→C22
18:3n-6	20:3n-6	1.21	0.35	0.87	C18→C20
20:5n-3	22:5n-3	2.52	nd	13.17	C20→C22
	24:5n-3	0.05	nd	0.17	C22→C24
20:4n-6	22:4n-6	1.61	nd	5.75	C20→C22
	24:4n-6	0.02	nd	0.09	C22→C24
22:5n-3	24:5n-3	0.92	nd	2.16	C22→C24
	26:5n-3	0.09	nd	nd	C24→C26
22:4n-6	24:4n-6	0.46	nd	0.72	C22→C24
22:6n-3	24:6n-3	0.38	nd	0.54	C22→C24

nd, not detected

Discussion and conclusions

The research described in this work demonstrates that gammarids possess at least three distinct elovl genes, namely elovl4, elovl6 and elovl1/7-like, with putative roles in the biosynthesis of LC-PUFAs. Molecular and functional characterisation of the set of elovl sequences from the marine gammarid E. marinus revealed that gammarids' Elovl4 and Elovl1/7-like are PUFA elongases with affinity towards PUFA substrates ranging from C18 to C22, and account, by themselves, for all the elongation reactions required for LC-PUFA biosynthesis from C18 biosynthetic precursors [5]. On the contrary, the gammarid Elovl6 sequence contained characteristics typically found in non-PUFA elongases [6] that, along with its elongation capacity being restricted to C18 PUFA substrates, indicated that this enzyme does not play major roles in LC-PUFA biosynthesis in gammarids. Elovl4 was the sole Elovl found in gammarids with the ability to produce PUFAs of up to 26 carbons. Overall, the present study provides insight into the endogenous machinery enabling gammarids to bioconvert dietary fatty acids into high value LC-PUFAs.

References

1. Alberts-Hubatsch, H.; Slater, M.J.; Beermann, J. Effect of diet on growth, survival and fatty acid profile of marine amphipods: Implications for utilisation as a feed ingredient for sustainable aquaculture. *Aquac. Environ. Interact.* **2019**, *11*, 481–49.
2. Jiménez-Prada, P.; Hachero-Cruzado, I.; Guerra-García, J.M. Aquaculture waste as food for amphipods: the case of *Gammarus insensibilis* in marsh ponds from southern Spain. *Aquac. Int.* **2021**, *29*, 139-153.
3. Guillou, H.; Zadravec, D.; Martin, P.G.P.; Jacobsson, A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog. Lipid Res.* **2010**, *49*, 186–199.
4. Gladyshev, M.I.; Sushchik, N.N.; Makhutova, O.N. Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins Other Lipid Mediat.* **2013**, *107*, 117–126.
5. Monroig, Ó.; Kabeya, N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. *Fish. Sci.* **2018**, *84*, 911–928.
6. Hashimoto, K.; Yoshizawa, A.C.; Okuda, S.; Kuma, K.; Goto, S.; Kanehisa, M. The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. *J. Lipid Res.* **2008**, *49*, 183–191.

718
HOW DIET AND TEMPERATURE AFFECT FATTY ACID BIOSYNTHESIS IN GAMMARIDS FED SIDESTREAM SOURCES

Alberto Ribes-Navarro [1]*, Hilke Alberts-Hubatsch [2], Óscar Monroig [1], Francisco Hontoria [1], and Juan C. Navarro [1]

[1] Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain
alberto.ribes@csic.es
[2] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany

Introduction

The fast and remarkable growth of global aquaculture in the recent years has created new economic and ecological challenges [1]. With regard to fish farming, such challenges have been mostly linked to guarantee the supply of raw materials used for feed formulation in order to reduce the current usage of finite resources such as fishmeal and fish oil. Gammarids, particularly their biomasses, have prompted interest as alternative sources for feed formulation due to their nutritional profile. Recent studies have reported the ability of gammarids to grow on a wide range of sidestreams while accumulating relatively high levels of long-chain (C_{20-24}) polyunsaturated fatty acids (LC-PUFA) [2,3], physiologically essential compounds for normal growth and development of vertebrates [4]. Diet and temperature have been suggested to modulate the abundance of LC-PUFA in aquatic invertebrates [5]. Here, we aimed to elucidate the effects of three diets (the seaweed *Fucus* sp., carrot leaves, coconut flesh) and four different temperatures (5, 10, 15, and 20°C) on LC-PUFA profiles of *Gammarus locusta*, a marine gammarid with capacity for trophic upgrading when grown in low-LC-PUFA diets [3].

Materials and methods

Offspring (42 days post hatch) from laboratory cultured *G. locusta* were starved for 2 d at 10°C to allow gammarids to empty their gut. Gammarids (20) were placed in 1 L buckets and cultured at the corresponding diet vs. temperature combination during 21 d. The combinations of three different diets, namely the seaweed *Fucus* sp., carrot leaves, coconut flesh, and four different temperatures (5, 10, 15, and 20°C), were tested. At the end of the experiment, gammarids (3-4 replicates per condition) were collected, rinsed twice in ddH_2O, and freeze-dried before analysis. Total lipids and fatty acids (FA) were analysed from gammarid samples, as well as experimental diets. Principal Component Analysis (PCA) was used to analyse and visualise the relations between the experimental conditions and FA profiles of gammarids. Moreover, Analysis of Variance (two-way ANOVA) was used to test the effect of temperature and diet on the *G. locusta* FA profiles and potential interactions between both factors.

Results

FA analysis of diets showed that coconut is rich in saturated fatty acids (SFA) in comparison to *Fucus* sp. and carrot leaves. Regarding LC-PUFA, *Fucus* sp. showed moderate levels of arachidonic acid (ARA, 11.96%) and eicosapentaenoic acid (EPA, 4.81%), and lower levels of docosahexaenoic acid (DHA). Both coconut and carrot leaves diets lack LC-PUFA. FA analysis of *G. locusta* showed that temperature did not significantly influenced the composition of gammarids irrespectively of the diet. However, the *G. locusta* FA profiles varied with diets. Statistical analysis revealed that diet is the leading factor accounting for the *G. locusta* FA profiles ($p < 0.05$). Interestingly, gammarids fed on *Fucus* sp. showed the highest content of total lipids among all dietary treatments, and a fairly similar FA profile to that of wild type specimens [3]. Furthermore, *G. locusta* fed on carrot leaves and coconut showed levels of DHA similar to those fed on *Fucus* sp. diet.

Discussion and conclusions

The study demonstrates that diet is the main modulator of FA composition in *G. locusta* when compared to temperature. Our analyses also revealed the presence of DHA, albeit in small proportions, in gammarids fed on either coconut or carrot leaves, regardless of the presence of this LC-PUFA in these sidestreams. These results suggest that *G. locusta* might have some endogenous capacity enabling the production of certain LC-PUFA from precursors present in diets devoid of these essential compounds. Therefore, gammarids can contribute to trophic upgrading of aquatic food webs by the production of physiologically essential FA such as EPA and DHA from primary sources. Thus, it is fundamental to fine tune the culturing conditions for LC-PUFA production in *G. locusta* regarding the optimisation of such biosynthetic mechanisms. We conclude that cultured gammarids fed on either *Fucus* sp. or carrot leaves are promising candidates culturing marine gammarids from which high nutritional value biomasses can be processed for their use as ingredients for aquafeeds.

(Continued on next page)

References

1. FAO The State of World Fisheries and Aquaculture 2020. *Sustainability in action*.; 2020.
2. Jiménez-Prada, P.; Hachero-Cruzado, I.; Guerra-García, J.M. Aquaculture waste as food for amphipods: the case of *Gammarus insensibilis* in marsh ponds from southern Spain. *Aquac. Int.* 2020, doi:10.1007/s10499-020-00615-z.
3. Alberts-Hubatsch, H.; Slater, M.J.; Beermann, J. Effect of diet on growth, survival and fatty acid profile of marine amphipods: Implications for utilisation as a feed ingredient for sustainable aquaculture. *Aquac. Environ. Interact.* 2019, *11*, 481–491, doi:10.3354/aei00329.
4. Guillou, H.; Zadravec, D.; Martin, P.G.P.; Jacobsson, A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog. Lipid Res.* 2010, *49*, 186–199, doi:10.1016/j.plipres.2009.12.002.
5. Monroig, Ó.; Kabeya, N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. *Fish. Sci.* 2018, *84*, 911–928, doi:10.1007/s12562-018-1254-x.

HOW UNIQUE OR COMMON ARE MICROBIAL COMMUNITIES IN BIOFILTERS? A TALE OF STATE-OF-THE-ART LONG-TERM MONITORING OF INDUSTRIAL RECIRCULATING AQUACULTURE SYSTEMS

D. Ribičić[a*], S. W. Dahle[a], K. Attramadal[b], T. Busche[c] and R. Netzer[a]

[a]SINTEF Ocean AS, Environment and new resources, 7010 Trondheim, Norway
[b]Norwegian University of Science and Technology (NTNU), Department of Biotechnology and Food Science, 7491 Trondheim, Norway
[c]Bielefeld University, CeBiTec, D-33615 Bielefeld, Germany
E- mail: deni.ribicic@sintef.no

Introduction

At present, microbiological analyses used for detection of microorganisms in biofilter are mainly limited to the detection of total bacteria, nitrifiers or certain potentially problematic representatives. Although nitrifying microorganisms are a very important component for the performance of a biofilter in RAS, these are not the only microorganism group in such a complex ecosystem. In this study, we have employed 16S rRNA gene amplicon sequencing on samples derived from five different full industrial scale RAS over a period of fifteen months to elucidate microbiota dynamics during standard production period and to investigate how different or common are microbial communities in different RAS systems. This study obtains data at an unprecedented immensity and longitudinal depth reported so far.

Materials and methods

Biofilters of five different RAS farms farming Atlantic salmon (*Salmo salar*) have been monitored over a period of fifteen months. Monitored farms, and corresponding biofilters, exhibit different designs, but also characteristics. Samples from each biofilter were collected bi-monthly and in triplicates. DNA was extracted, amplified (V3-V4 16S rDNA region), and sequenced using Illumina MiSeq platform. Obtained sequences were treated using Quantitative Insights Into Microbial Ecology 2 (QIIME2) pipeline. Downstream statistical analysis was performed in R using dedicated packages for microbiome analysis and multivariate statistics.

Results and discussion

Biofilters showed differences in regard to alpha diversity (Simpson and Shannon index). As seen from figure 1A. the smallest variations in diversity were observed for biofilters in farms A and C. Also, biofilters A and C showed significantly higher Simpson and Shannon diversity when compared to microbial diversity observed in biofilters B, D and E. This is also an indication that the most stable bacterial communities can be found in biofilters A and C. Principal Coordinate Analysis (PCoA) of microbial communities revealed unique microbial fingerprint for each of biofilters (Figure 1B). The clustering distance coincides also with geographical locations of the respective RAS farms, meaning that biofilters A and C are to be found on completely opposite sides of Norway, while biofilters B, D and E are geographically much closer. It has been hypothesized that each RAS biofilter (and RAS in general) has the potential to establish a unique microbial composition or "fingerprint" due to different operational procedures, components or design[1]. Exact explanation for the differences in microbial community, as for within and between biofilters, are rather inconclusive here as many different variables could have accounted for that. All farms investigated for this study are very different in biofilter design, capacity, maturity, day-to-day operations, disinfection methods and frequency, and even the fish rearing stages. However, there is an indication that geographical origin of water used to fill the systems and uniqueness of RAS itself could have played a substantial role as a driving force for shaping microbial community composition and subsequent maturation, as observed in Figure 1B and Figure 1C.

Most dominating phyla were Proteobacteria with relative abundance ranging from 38.1% up to 55.3% (Figure 1C). Proteobacteria were followed by Bacteroidetes, with 13.8% to 28.2% in relative abundance. Other phyla that showed abundances larger than 2 % were Plantomycetes, Cloroflexi, Verrucomicrobia, Actinobacteria, Acidobacteria, Nitrospirae, Chlamydiae, Chlorofexi and Gemmatimonadetes. Similar observations were obtained by Huang et al.[2] and Ruan et al.[3] where most dominant phyla in monitored biofilters were Proteobacteria, followed by Bacteroidetes, Nitrospirae, Planctomycetes, Actinobacteria, Chlorofelxi and Verrucomicrobia. Although beta-diversity analysis showed rather unique microbial composition between biofilters, there is an indication that certain microbial groups are omnipresent in monitored biofilters. To investigate this further, we have screened for common microbiota of all five biofilters. Interestingly few genera found to represent core microbiota are already well-known nitrifiers, i.e., Nitrospira and Nitrosomonas. For the other taxa found within the core microbiome, literature overview shows that these microbes are commonly found in wastewater and activated sludge, but also other RAS biofilters, where they demonstrated ability for the hydrolysis and utilization of complex carbon sources.

(Continued on next page)

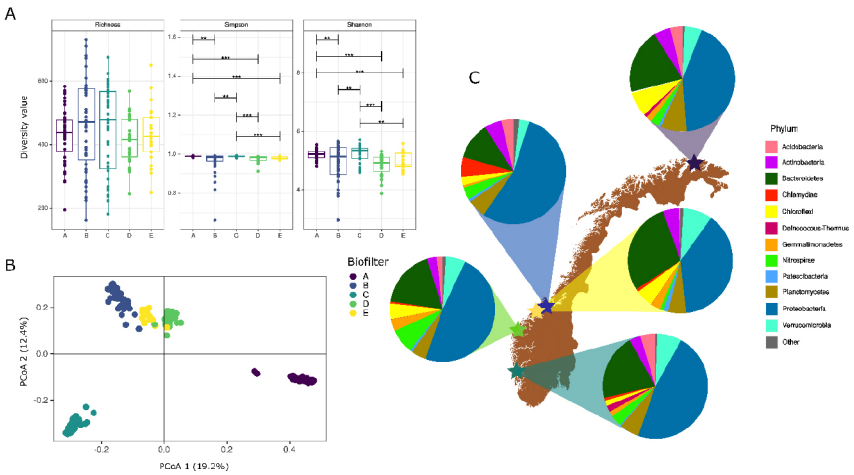


Figure 1. Alpha diversity represented as total observed number of ASVs ("Richness"), Simpson and Shannon diversity metric (A). PCoA plot based on unweighted UniFrac distance metric (beta-diversity) A map of Norway with indicated positions of five RAS and community composition at Phylum level for each of the biofilter represented as pie-charts (C).

Conclusions

In this study we have conducted deep and comprehensive mapping of microbial communities and their dynamics in biofilters of five full scale commercial RAS cultivating Atlantic salmon. Large differences in microbial communities and heir dynamics could be observed between monitored biofilters. Although, microbiologically different, core common microbiota could be established, with known nitrifiers representing it amongst other taxa.

References

1. Blancheton, J. P., Attramadal, K. J. K., Michaud, L., d'Orbcastel, E. R. and Vadstein, O. (2013). "Insight into bacterial population in aquaculture systems and its implication." Aquacultural Engineering 53: 30-39.
2. Huang, Z., Wan, R., Song, X., Liu, Y., Hallerman, E., Dong, D., Zhai, J., Zhang, H. and Sun, L. (2016). "Metagenomic analysis shows diverse, distinct bacterial communities in biofilters among different marine recirculating aquaculture systems." Aquaculture international 24(5): 1393-1408.
3. Ruan, Y.-J., Guo, X.-S., Ye, Z.-Y., Liu, Y. and Zhu, S.-M. (2015). "Bacterial community analysis of different sections of a biofilter in a full-scale marine recirculating aquaculture system." North American Journal of Aquaculture 77(3): 318-326.

A NON-LETHAL APPROACH UPON *Sparicotyle chrysophrii* BURDEN PREDICTION IN GILTHEAD SEA BREAM (*Sparus aurata)*

E. Riera-Ferrer[1], I. Estensoro[1], R. del Pozo[1], M.C. Piazzon[1], P. Moreno-Estruch[2], A. Sitjà-Bobadilla[1], O. Palenzuela[1]*

[1] Fish Pathology Group, Institute of Aquaculture Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC). Castellón, Spain
E-mail: oswaldo.palenzuela@csic.es
[2] Culmarex SAU, Murcia, Spain

Introduction

Sparicotyle chrysophrii is a polyopisthocotylean monogenean (Microcotylidae) parasite of gilthead sea bream (*Sparus aurata*) (GSB). It attaches to the gills and it can cause Sparicotylosis, which often involves severe anaemia and white-gill syndrome. The disease is ubiquitous across sea cages in the Mediterranean and it causes direct and indirect economic losses to the GSB farming sector. *S. chrysophrii* infection intensity determinations are necessary for appropriate application and coordination of farms' health management plans, aiming at keeping parasite presence below threatening thresholds. In the farms, these counts are tedious and time-consuming. Parasite loads are usually extrapolated from partial counts, e.g., of a number of gill arches from one or both sides. However, different protocols and indexes are used by the industry.

In previous studies, we have demonstrated the hematophagous nature of *S. chrysophrii* and its direct role in GSB anaemia. The aim of the current study is to explore for a fast, non-lethal approach to predict the parasitic burden of affected GSB using haematological parameters. Therefore, a large data set on infection intensity and parasite distribution, as well as biometrical and haematological data from laboratory experimental infections and from commercial cages, have been gathered and analysed.

Materials and methods

Fish sampling, Hematological data and Parasite Counts

Fish kept under experimental (N = 150) and farming conditions (N = 388) were euthanized and bled from the caudal vein. Haematocrit values were recorded after standard microhematocrit capillary tube centrifugation, and haemoglobin values obtained using the HemoCue ® Hb 801 System. Gill arches were dissected and either inspected thoroughly under stereomicroscopes, or processed by a high-throughput method based on an incubation of the gill arches in a diluted formalin solution, followed by concentration and final counting of parasites in a Sedgewick-Rafter chamber. The number and type of life stages (juveniles and adults) were registered and the performance of both counting methods compared to assess the total parasite burden.

Sparicotyle chrysophrii distribution

A Kruskal-Wallis H test was performed in order to analyse the distribution of adults, juveniles and the total parasite population throughout the gill arches.

Haematology analysis

Haematocrit and haemoglobin values were correlated using Pearson correlation coefficient and the R^2 value was calculated.

Prediction models

Partial counts sensitivity was assessed for farm data (N = 2 fresh gill arches per fish counted with scope) and laboratory data (N = 6 remaining arches processed for Sedgewick-Rafter counts) from commercial GSB cages. Several generalised linear models (GLMs) including haematocrit and haemoglobin variables were explored in order to predict the number of *S. chrysophrii* adults in affected GSB. All the statistical analyses, were conducted using R Statistical Software (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria).

Results

Sparicotyle chrysophrii distribution

Parasite presence in the different arches presented a non-normal distribution in the different arches of the left and right gills. No significant differences were found in neither adult, juvenile, nor total parasite counts among the different gill arches. Moreover, no significant differences were found in total parasite counts between the left and right gill arches.

$$N_{adults} = \exp\left(5.884 + (Hb \times -0.294) + (Hct \times -0.056)\right)$$

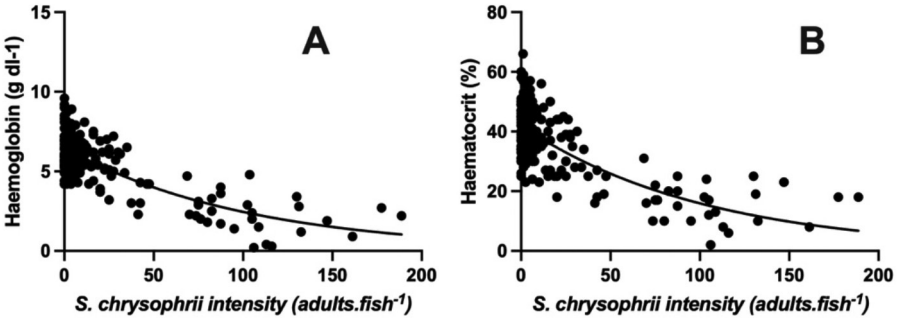


Figure 1. Exponential regression plot between infection intensity and haematological parameters (A: Haemoglobin, B: Haematocrit)

Haematology analyses

Correlations between haematocrit and haemoglobin values were highly significant in experimental, farm and in combined experimental and farm data.

Prediction models.

Different iterations were explored with subsets of data extrapolated from total or partial counts. Significant differences were found in the predicted burden values calculated from partial count data from two and six gill arches, being the latter the most reliable. Correlations between adult parasite load and haematocrit and between adult parasite load and haemoglobin values were highly significant for data from experimental and farming conditions (Fig. 1). For our entire dataset, a negative binomial regression model was obtained:

Discussion and Conclusions

No bias associated to specific gill arches of GSB could be found in the distribution of juveniles or adults of *S. chrysophrii*, and therefore, extrapolation of the total parasite load can be performed from parasite counts in a limited number of arches. However, the sensitivity of the partial counts in two gill arches was significantly inferior, showing up to 66.67% of false negatives in the earliest stages of infection. A high-throughput method was developed for *S. chrysophrii* counting from GSB gill arches, which delivers highly sensitive and reliable intensity values. Haemoglobin and haematocrit values proved to be reliable parameters, which are strongly correlated with the parasite load in GSB infected with *S. chrysophrii*. Furthermore, haematological data will allow the assessment of parasite burden in a fast and non-lethal manner by means of the prediction models developed.

Acknowledgements

This study was funded by the Spanish Ministry of Science and Innovation project no. RTI2018-098664-B-100 (SPARICONTROL).

PHARMACOKINETICS AND *IN VITRO* EFFICACY OF FLORFENICOL IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

G. Rigos*, D. Kogiannou, C. Nikoloudaki, P. Katharios, A. Triga and G. Pyrenis

Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, 46.7 Athinon-Souniou ave, 19013 Anavyssos, Attiki / Former American Base of Gournes, Heraklion 71003, Crete, Greece
Email: grigos@hcmr.gr

Introduction

Florfenicol (FLO) is a synthetic amphenicol antibiotic that exerts broad spectrum antibacterial activity against Gram-negative bacilli, gram-positive cocci and other atypical bacteria. Moreover, FLO is a highly lipophilic drug providing thus high concentrations to treat intracellular pathogens. The pertinent literature on the use of FLU in aquaculture indicates that the drug has promising properties in fish. In this study, the absorption and depletion of FLO in European seabass following a multiple oral dosing, were investigated. Minimum inhibitory concentration tests (MIC) were also carried out against some important bacterial pathogens of European seabass.

Materials and methods

European seabass kept at 24ºC, received a medicated diet with FLO (10 mg/kg fish) for 7 consecutive days. Blood samples were taken from 10 individuals in each time point from 2 to 24 hours and days 2, 3, 4, 5, 6 and 7. Following the completion of the treatment, 10 fish were killed and muscle plus skin samples were obtained for 5 consecutive days. An HPLC method with fluorescence detection was used for FLO measurements in plasma and muscle samples of individual fish at each time point. The MIC values of FLO were also determined for bacterial fish pathogens isolated from diseased European seabass in Greece including *Aeromonas veronii bv sobria*, *Vibrio anguillarum strain*, *V. harveyi, Photobacterium damselae* subsp. *damselae*, *P. damselae* subsp. *piscicida* and *Edwardisiella anguillarum*.

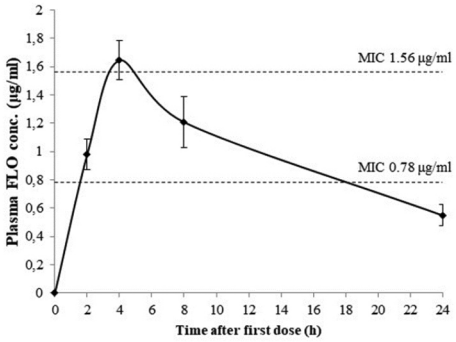


Fig. 1. Mean plasma concentrations of FLO (10mg/kg fish) during first treatment day.

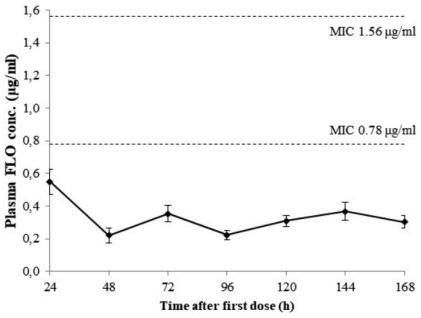


Fig. 2. Minimum plasma concentrations of FLO after multiple oral administrations at 10 mg/kg per day for seven consecutive days.

(Continued on next page)

Results

Maximum plasma concentrations of FLO in European seabass revealed values around 1.5 μg/ml at 4h post feeding during the first day (Fig.1), while during the seven-day therapy, mean plasma concentrations of FLO showed no statistical differences between 24h time intervals (Fig. 2).

Based on the muscle plus skin FLO levels (including the amine), withdrawal times for FLO were calculated to be less than 3 days post treatment. The MIC values of FLO were measured to be 0.78 μg/ml for *A. veronii* bv *sobria*, *P. damselae* subsp. *piscicida* and *E. anguillarum* and 1.56 μg/ml for *V. anguillarum, V. harveyi* and *P. damselae* subsp. *damselae*. Based on the MIC values alone, none of the tested strains can be considered as resistant to FLO in the current trial.

Conclusions

FLO is readily absorbed and rapidly eliminated from European seabass edible tissues. A PK/PD evaluation of FLO properties as a time-dependent bacteriostatic antibacterial, fits to a predictive model of Tc>MIC where Tc is the percentage of the inter-dosing interval during which the serum/plasma concentration exceeds the *in vitro* MIC against the target bacterium. Considering the above model, FLO could be a potentially efficient 'off label' antibacterial against some bacterial pathogens of European seabass tested herein. A double FLO dosing, administered twice a day may aid to obtain higher circulatory daily drug levels in European seabass, but this remains to be experimentally verified.

Funding

Project "MOdern UNifying Trends in marine biology - MOUNT" (MIS 5002470) which is implemented under the "Action for the Strategic Development on the Research and Technological Sector", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

REACHING HIGH SUSTAINABILITY STANDARDS IN FISH AQUACULTURE

João Rito[*,1], Nuno Leite [1], Daniela Santos [2], Carlos Estevão Simonka [2], Sónia Cotrim Marques [3], Sérgio Miguel Leandro [3] and John Griffith Jones [1]

[1]SEAentia
Parque Tecnológico de Cantanhede
Núcleo 04, Lote 2
3060-197 Cantanhede - Portugal
E-mail: joao.rito@seaentia.pt

[2]MARE — Marine and Environmental Sciences Centre, Polytechnic Institute of Leiria, Peniche, Portugal

[3]MARE — Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Peniche, Portugal

Background:
Just like in any other activity there are bad examples that spoil the aquaculture industry and good examples that must be followed. It's important to distinguish both and drift the industry towards the good practices to achieve sustainability.

Technology is able to improve aquaculture's practices and sustainability by aiming its focus in fish health and welfare. In turn, it will influence all the outcomes of the industry reaching increasingly better results and higher quality products.

Real case example:
How can a startup play a sustainability role in the industry?

SEAentia is a Portuguese aquaculture start-up, which aims to produce top quality fish in the most sustainable manner by combining novel aquaculture engineering with scientific research. SEAentia will pioneer in meagre (*Argyrosomus regius*) production from hatchery to commercial size in a sustainable and environmental-friendly RAS production system where animal health and welfare is a major concern.

We aim to become an international reference in aquaculture by exploring new species with great marketing potential and consumer acceptability. This will only be possible by combining new farming methods with novel technology and scientific knowledge, thereby contributing to address the global challenge of sustainably feeding the growing human population with high-quality, traceable and biosecure products.

SEAentia is paving its way to thrive, grow and lead by example.

GUT HEALTH IMPORVEMENT IN EUROPEAN SEABASS *Dicentrarchus labrax* FED ENZYMATICALLY PROCESSED SOY PROTEIN

R. Robles[1], L. Bermúdez[1], S. Husballe-Rasmussen[2].

1Testing Blue S.L. C/Holanda, 26.Puerto Real.11510. Cádiz. Spain.
2 Hamlet Protein A/S. Saturnvej 51, 8700 Horsens, Denmark.

Introduction

Aquaculture production is projected to reach 109 million tons in 2030. The need for high quality protein is increasing particularly to feed high-value species such as shrimp, marine fish and salmonids. The search for alternative protein sources of high quality is on and supported by decades of academic and private research in aquaculture nutrition around the globe.

Bioprocessing, fermentation or enzymatic treatment are some of the processes developed to increase the utilisation efficiency of soybean in fish. Enzymatic hydrolysis decreases the content of anti-nutritional factors (ANFs) and promotes nutrient utilization. An enzyme-treated soy protein (ESP; HP AquaSure) produced from dehulled and fat-extracted soybean meal has an average crude protein content of 52%, and a reduced ANF content of 2.0 mg/g trypsin inhibitor activity (TIA) and 10^3 ppm β-conglycinin.

This study reports on the evaluation of ESP in a feeding trial for the European seabass *Dicentrarchus labrax*.

Materials and methods

The trial has been carried out at the facilities of Testing Blue S.L. (Cádiz, Spain). ESP has been tested at 10 % inclusion level in two formulations: ESP M0 and ESP P0; in the first case ESP replaced soybean meal (SBM) in the formula and in the case of ESP P0, soy protein concentrate (SPC) was replaced. Both diets were evaluated against a Control diet including SBM and SPC. The formulations were made according to the current practice of using a mixture of vegetable protein sources in fish feeds (Table 1). Fishmeal has been included at 25% in all the diets.

European seabass fingerlings of 7.30±0.08 g average individual body weight were stocked in a RAS (recirculating aquaculture system) equipped with 12 units of 150 litre-tank and the appropriate solid separation system and water treatment and biofiltration. Each diet has been tested in 4 replicate tanks. The trial lasted 12 weeks. Histology (intestinal damage index and ratio IP/EP) and oxidative stress (LPO, CAT and GSH:GSSG) were evaluated at the end of the trial. Faeces production was also measured during the last week of the trial.

Results

No issues related to palatability was found during the trial. Feed intake was very similar for the three diets. Production results (Figure 1) namely specific growth rate (SGR) and feed conversion rate (FCR) were very similar among the three diets and no significant difference was found among diets (ANOVA $p>0.05$).

Replacing SBM by ESP resulted in 6% increase in the hepatosomatic index whereas replacing SPC resulted in 8% lower hepatosomatic index. For each ESP diet, there was no significant difference with the Control. No significant difference among diets was found in the eva luation of three oxidative stress biomarkers in the intestinal tissue. In the case of the histopathological evaluation of the intestinal epithelium, ESP sustains a better condition in the intestines of the seabass fed ESP products compared with the Control diet with an average 25% lower damage score. The ratio IP/EP for the ESP M0 and P0 were 7 % and 2 % higher than the IP/EP ratio for the Control diet. These results indicate a healthier intestinal epithelium and greater surface area for nutrient absorption in sea bass fed the ESP diets (Figure 2).

Another remarkable finding of the trial was the significantly reduced volume ($p< 0.00032$) of faeces produced by the ESP P0 diet compared with the Control diet. ESP M0 and P0 had a 2.6% and 9% lower faeces production than the Control (Figure 3). This result points to the potential advantage of using feeds including ESP to reduce the amount of solid waste in RAS.

(Continued on next page)

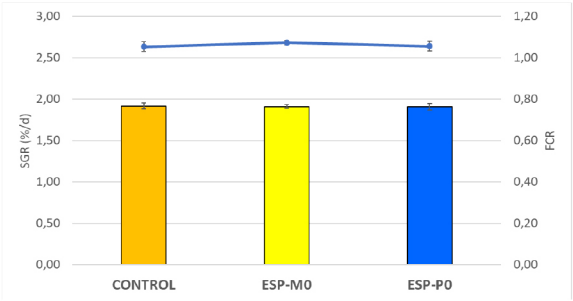


Figure 1. Specific growth rate (SGR, bars) and feed conversion ratio (FCR, line) of European seabass fed the different diets. Bars represent the average and standard deviation of 4 replicate tanks

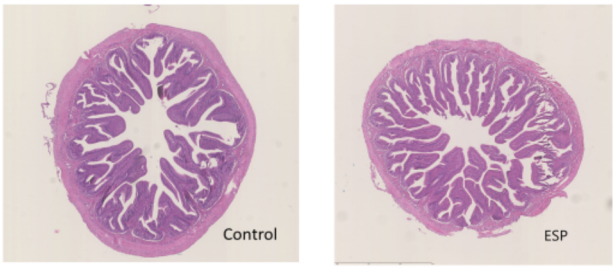


Figure 2. Section of posterior intestine with IP/EP ratio of 3.89 (left image from a specimen belonging to the Control group) and 4.93 (right, specimen from ESP M0 group).

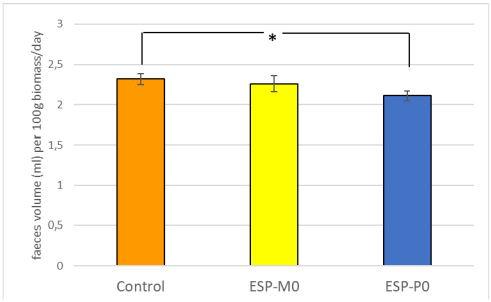


Figure 3. Faeces volume of European seabass fed the different diets. Data indicate average volume of faeces collected per tank (n=4) during three consecutive days, expressed per 100 g of fish biomass per day. Significant difference indicated with an asterisk.

REFINED GLYCEROL IN FEEDS FOR WHITELEG SHRIMP (*Penaeus vannamei*)

R. J. M. Rocha*[1]; A. Laranjeira[1], M. Sardinha[2,3], A. Santos[2], M. Viegas[2], J. Dias[2]

[1] RIASEARCH Unipessoal Lda, Murtosa (Portugal)
[2] SPAROS Lda., Olhão (Portugal)
[3] Current affiliation: BIOMAR AS, Trondheim (Norway)
Email: ruirocha@riasearch.pt

Introduction

The turn towards renewable energy sources has increased the European production of biodiesel from vegetable oils, leaving glycerol (also known as glycerine) as a valuable by-product. In the EU catalogue of feed materials, "Glycerol" is described as "Product of biodiesel production (methyl or ethyl esters of fatty acids), obtained by trans-esterification of oils and fats of unspecified vegetable and animal origin" with subsequent refining via distillation or ion exchange chromatography. Although at a higher cost, refined glycerol (glycerol >98% DM; methanol <0.2%), shows much lower levels of impurities than crude glycerol.

In the living organisms, glycerol is a precursor for synthesis of triacylglycerides and of phospholipids in the liver and adipose tissue. Additionally, when the body uses stored fat as a source of energy, glycerol can be converted to glucose by the liver and provides energy for cellular metabolism. At a metabolic level, glycerol must be converted to the intermediate glyceraldehyde 3-phosphate before it can enter the pathway of glycolysis or gluconeogenesis (depending on physiological conditions). Glycerol has been evaluated as a dietary energy source for several farm animals, including various fish species. However, little is known about the potential use of glycerol in shrimp feeds. A study was undertaken to assess the effect of dietary biodiesel-derived refined glycerol (from rapeseed oil) on the overall growth performance, nutrient retention and digestibility in whiteleg shrimp (*Penaeus vannamei*).

Methods

The trial comprised 3 dietary treatments: a control diet (CTRL) containing among others 15% by-products fishmeal, 30.5% solvent-extracted soybean meal and 25% whole wheat; and two additional diets with refined glycerol at 5 and 10% (GLY5 and GLY10, respectively). Glycerol was incorporated at the expenses of wheat and a slight upward adjustment of soybean meal to guarantee isonitrogenous conditions. All other ingredients were kept constant. Diets were extruded and were isonitrogenous (CP: 38% DM), isolipidic (CF: 7.5% lipid) and isoenergetic (GE: 19.4 MJ/kg DM).

Homogenous groups of 45 shrimp (IBW: 2.55 g) were stocked in 500 L tanks. Each experimental treatment was tested in quintuplicate tanks over 50 days. Rearing tanks were supplied with recirculated seawater (salinity: 19.4; temperature: 28.1 ± 0.5°C; dissolved oxygen >7.2 $mg \cdot L^{-1}$). Additionally, at the end of the trial the apparent digestibility of protein and energy of the various diets was measured by the indirect method with feeds containing 0.02% yttrium oxide and feces collected by siphoning. Data were treated by a one-way ANOVA and when appropriate, means were compared by the Student's-Newman-Keuls test. Statistical significance was tested at 0.05 probability level.

Results and discussion

After 50 days of experimental feeding, the final body weigh ranged between 12.97 and 13.65 g, which represents a 5-fold increase of their initial weight. No significant differences were found in terms of survival, final body weight and SGR among treatments ($P>0.05$). However, shrimp fed with the GLY5 and GLY10 diets showed a significantly higher FCR, feed intake and a significantly lower PER than those fed the CTRL diet ($P<0.05$).

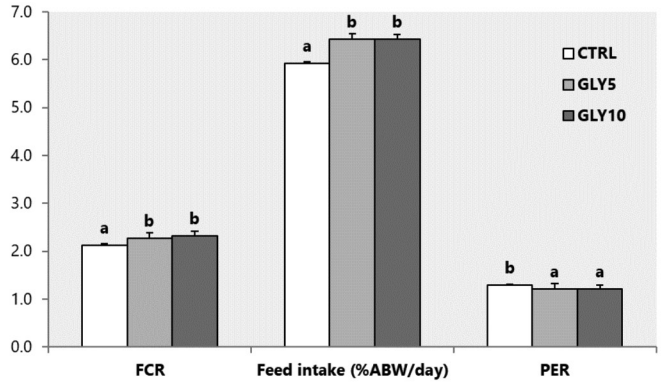
Conclusions

Data from this study indicates that refined glycerol used at 5 and 10%, and incorporated at the expenses of wheat, does not compromise weight gain criteria in whiteleg shrimp. However, glycerol inclusion tends to have a detrimental effect on FCR. This lower feed utilization could not be associated to a lower digestibility of protein or energy. This suggests that glycerol, in comparison to wheat, may present a lower efficiency at a metabolic level. Further studies, comprising a detailed assessment of the metabolic fate of glycerol in shrimp are warranted.

Acknowledgements

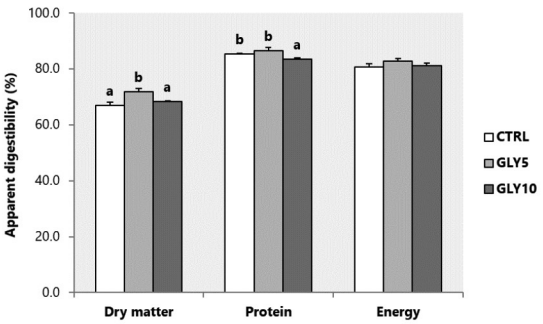
This work is part of project 47175_FICA, supported by Portugal and the European Union through FEDER/ERDF, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

(Continued on next page)



The whole-body composition of shrimp in terms of moisture, ash, protein, lipid and energy was not significantly affected by dietary treatments (P>0.05). Similarly, the whole-body retention of protein, lipid and energy was not significantly by dietary treatments (P>0.05).

The apparent dry matter digestibility of GLY5 was significantly higher than that of CTRL and GLY10 (P<0.05). Additionally, diets CTRL and GLY5 showed also a significantly higher digestibility than diet GLY10 (P<0.05). Energy digestibility was not affected by dietary treatment (P>0.05).



SPONTANEOUS SPAWNING OF THE BLACK SEA-CUCUMBER *Holothuria forskali* (DELLE CHIAJE, 1823) IN CAPTIVITY. PRELIMINARY RESULTS OF FERTILIZATION, EGGS INCUBATION AND LARVAL REARING

C. Rodríguez (*), J.M. Martínez, C. Lobo and F. Aguado-Jiménez.

Spanish National Research Council. National Center Spanish Institute of Oceanography. Oceanographic Centre of Santander. "El Bocal" Marine Aquaculture Station. Monte-Corbanera, 39012, Santander, Spain
E-mail: cristina.rodriguez@ieo.es

Introduction

Holothuria forskali (Delle Chiaje, 1823) is a native species in the Cantabrian Coast (Spain) that can also be found in the North East Atlantic Area and Mediterranean Sea. It is a detritivore species that feeds mainly at night. This gonochoric species has an annual reproduction cycle with a synchronous spawning for male and female in spring in the Atlantic Area (Tuwo and Conand, 1992). *H. forskali* is an interesting species from an aquaculture point of view, not only as a gastronomic product, but also for its bioremediation potential in integrated multitrophic (IMTA) configurations. Therefore, breeding control in captivity is basic for its integration in IMTA systems. With this aim we planned to create a stock of breeders obtaining them from their natural environment.

Material and methods

In April 2021, coinciding with the end of the reproductive season of the species in the Atlantic, 33 *H. forskali* adults were collected at La Maruca´s Beach (Cantabrian Sea, Spain: 43°28'47"N 3°50'11"W) during low tide (seawater temperature at 12.9 °C). Specimens were transferred in a 20 L cooler to the hatchery of the "El Bocal" Marine Aquaculture Station of the Oceanographic Spanish Institute of Santander (Cantabria, Spain). At all time, the sea-cucumbers were carefully handled to avoid expulsion of Cuvierian tubules which would induce stress.

Specimens were weighted (wet weight) and placed in a rectangular tank (3 m long x 1 m wide and an average depth of 0.25 m: aprox.600 L) with flowing seawater at 13 °C.

Unexpectedlly, two hours after arrival at our facilities, a spontaneous spawning event occurred, and 5 specimens (3 males and 2 females) began to release their gametes (Fig. 1). Naturally fertilized eggs were rapidly removed from the tank with 20 μm sieve and washed with seawater. Also, eggs and sperm were collected directly from the gonopore of those specimens that remained releasing their gametes naturally, and artificial fecundation was performed in a flask. Egg diameter was measured in one hundred fertilized eggs.

Swiftly and with the available means, egg incubation and larval rearing was planned. The seawater used for embryonic rearing was filtered at 1 μm and sterilized with a UV light system. Eggs were incubated at 14 °C (±2 °C) in 1 L flask with soft aeration and natural photoperiod.

Once embryonic development was completed, lyophilized *Nannochloropsis gaditana* at a final concentration of 3.10^3 cell $.mL^{-1}$ was used to feed the larvae.

Results

The mean weight of the specimens collected was 120.24 ± 33.50 g. No mortality was recorded after acclimation to captivity. The average diameter of the fertilized eggs was 156.37 ± 7.35 μm. Embrionic development started with elevation of the fertilization envelop and expulsion of the polar bodies (Figure 2). The morula stage took place 3 days after fertilization (DAF), early auricularia 4 DAF and mid auriculatia 5 DAF

After mid auricularia larval stage, larval rearing collapsed. Until that moment, embryonic development was slower than as Laguerre et al. (2020) described it. This could be due to our lower water temperature.

(Continued on next page)

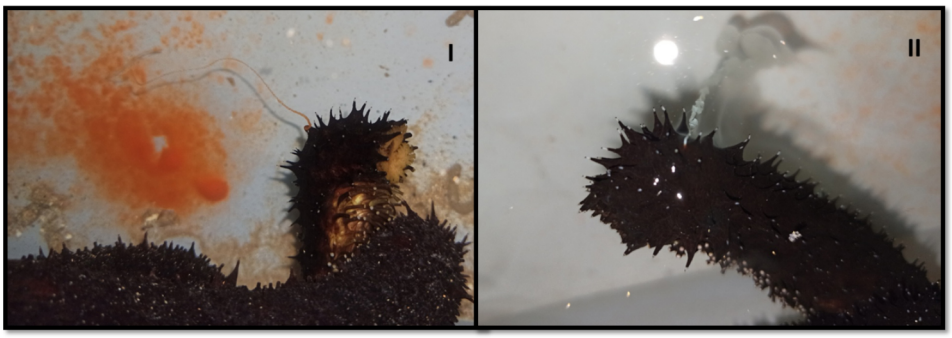


Fig 1. Female (I) and male (II) of *Holothuria forskali i*performming natural spawning.

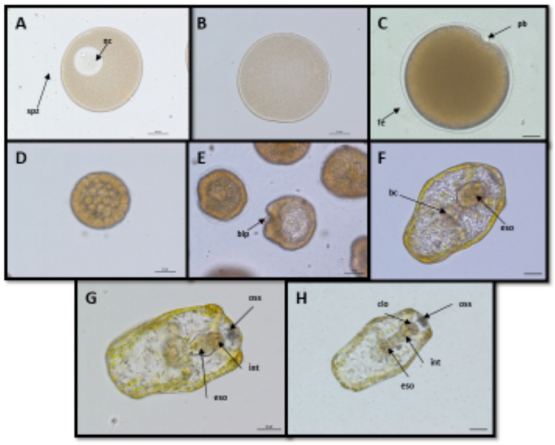


Figure 2: Embryonic development and early stages of larval development in *H. forskali*. A: Spawned oocyte with clearly visible nucleus (nc) and spermatozoons (spz). B: early fertilized egg. C: fertilized egg with the fertilization envelop developed (fe) and differenciated polar bodies (pb). D: Morula. E Gastrula with blastopore (blp). F: early auricularia, 4 days after fertilization (daf); buccal cavity (bc), esophagus (eso). G Mid auricularia, 5 daf; intestine (int) ossicle (oss). H: Mid auricularia 6 daf; cloaca (clo). Scale bars= 50 µm

Bibliografia

Laguerre, H., Raymond, G., Plan, P., Améziane, N, Bailly, X and Le Chevalier, P. (2020). First description of embryonic and larval development, juvenile growth of the black sea-cucumber *Holothuria forskali* (Echinodermata: Holothuroidea), a new species for aquaculture in the north-eastern Atlantic. Aquaculture, 521, 734961.

Ramofafia, C., Battaglene, S. C., Bell, J. D., & Byrne, M. (2000). Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. Mar. Biol., 136(6), 1045–1056.

Tuwo, A., and Conand, C. (1992). Reproductive biology of the holothurian *Holothuria forskali* (Echinodermata). J. Mar. Biol. Assoc. 72, 745–758.

FREEZE DRIED MICROALGAE: THE NOVEL OFF-THE-SHELF STANDARD IN BIVALVE AND CRUSTACEAN HATCHERIES AND NURSERIES?

L. Roef[1], M. Muys[1] , M. Wille[2], V. Vermeylen[2], C.T. Giang[3], J.D. Lima[4], M. Michiels[1]

[1]Proviron Holding NV, George Gilliotstraat 60, 2620 Hemiksem, Belgium
[2]Universiy of Ghent
[3] National Broodstock Center for Mariculture Spesies in Northern Vietnam, RIA1
[4]IMAQUA
Mailing address: luc.roef@proviron.com

Introduction

Although microalgae are indispensable in aquaculture when feeding mollusks and crustaceans during the larval stages, microalgae production within hatcheries and nurseries is still a major bottleneck. Many hatcheries struggle to combine microalgae culture with the core business of farmed animal production. The general experience with microalgae culture systems is one of variable product quality, 'crashes', culture upsets, contaminations, and most important, a general inability to culture the microalgae species that are most desirable as aquaculture feed. Even a brief 'crash' of the algal cultures can result in the loss of a large investment in the animals. These problems can be overcome with a reliable year-round external supply of high-quality freeze-dried (FD) microalgae.

Proviron has developed, engineered and patented a proprietary closed photobioreactor system for reliable indoor cultivation of freeze-dried microalgae, named ProviAPT (Proviron Advanced Photobioreactor Technology), that is particularly suited for the mass production of high quality algae biomass for aquaculture purposes. Rigorous control and standardization of all operational parameters within narrow boundaries, results in the production of pathogen-free freeze-dried microalgae featuring a stable and high quality. In contrast to fresh and frozen algae products, the application of freeze-dried microalgae allows for easy global distribution from a centralized production site and logistics hub and results in a higher flexibility due to the prolonged shelf life up to 3 years.

In the current presentation, a variety of feeding trials are reported that validate the performance of freeze-dried microalgae as an off-the-shelf solution, compared with the live algae alternative. Studied organisms include shrimp (*Litopenaeus vannamei*) larvae, oyster (*Crassostrea gigas*) and manila clam (*Venerupis philippinarum*) spat, ... An array of blends of different species of freeze-dried microalgae, as well as live microalgae were investigated. Furthermore, an automated algae dosing unit (ADU) was developed and tested to guarantee a continuous supply of microalgae to the larvae tanks. Using this ADU, different feeding regimes were investigated.

Table 1. Survival at the postlarval stage, growth performance of different tested organisms on a diet c omposed of FD algae.

Case	Organism	Diet	Survival rate (%)
1	*P. vannamei* larvae	FD *T. pseudonana* + FD *T. chuii*	61,5
		FD *C. muelleri* + FD *T. chuii*	50
		live *C. muelleri* + live *T. chuii*	44.9
		live *T. pseudonana* + live *T. chuii*	76,5
2	*C. gigas* larvae	70% live + 30% freeze dried algae	64,2
		50% live + 50% freeze dried algae	52,8
		70% live + 30% freeze dried algae	48,3
		100% live algae	32,3
		100% freeze dried algae	30,2

(Continued on next page)

Materials and methods

Larval cultivation of *L. vannamei* took place in a RAS set-up in a temperature- and light-controlled room. *N*auplii were stocked in 100-L cylindroconical PVC tanks at a density of 100-150 larvae. L^{-1}. Microalgae were dosed at ~10^5 cells/mL, while artemia were fed starting from zoea 3. Four microalgae feeds were formulated using Provirons ThalaPrime P, ChaetoPrime and TetraPrime FD microalgae and applied in triplicates: (1) FD *Thalassiosira pseudonana* and FD *Tetraselmis chuii*; (2) FD *Chaetoceros muelleri* and FD *T. chuii*; (3) live *C. muelleri* and live *T. chuii;* and (4) live *T. pseudonana* and live *T. chuii*.

Pediveliger larvae of Crassostrea gigas were fed 5 diets consisting of varying amounts of live and freeze dried algae. From day 1 to 6 diets contained the species *Nannochloropsis* sp., *Tisochrysis lutea* and *Chaetoceros muelleri* at 33,3% each. From day 7 onwards *Tetraselmis chuii* was added to the diet (25% each). All diets thus contained the same algae species at the same proportions, but with different percentages of the live algae being replaced by their freeze dried counterparts (NannoPrime, IsoPrime, ChaetoPrime and TetraPrime C). Algae were added at 2.500 cells per larvae at pediveliger stage up to 80.000 cells at setting.

Results and discussion

Table 1 gives an overview of the results of two case studies in which FD algae were used to replace or complement live algae diets.

For shrimp larvae, the use of diets consisting only of freeze dried microalgae resulted in a promising relative survival rate between 50 and 62%, with values that were slightly higher (ChaetoPrime) or slightly lower (ThalaPrime) compared to the live microalgae control treatment. For *Crassostrea gigas*, a diet composed of only freeze dried algae fed from pediveliger stage on resulted in settling rates of 30,2% comparable to a diet consisting of only live alga (32,3%), be it with a delay of one day (18 versus 17 days). A clear synergistic effect was noted however when using diets consisting of a mixture of live and dried algae. A diet of 70% live + 30 % freeze dried algae resulted in setting at day 14 with a survival rate of 64,2 %.

These results demonstrate for the first time that the complete replacement of live algae with FD algae is possible for a variety of organisms in aquaculture. The synergistic effect between live and freeze dried algae that is noted may further increase performance. The use of FD algae could become the novel standard in hatcheries, reducing the costs related to live algae cultivation, while improving reliability and feed quality.

Acknowledgements

Results on shrimp larvae and oyster are collected as part of the BlueMarine^3.Com project funded by the Flemish government through Flanders Innovation and Entrepreneurship (VLAIO) and is facilitated by the Blue Cluster program.

MARKET INTERGRATION WITHIN THE VALUE CHAIN

K.H. Roll

University of South Eastern Norway
Raveien 215
3184 Borre, Norway
krr@usn.no

In this article, we will investigate the market integration between high value aquaculture species (salmon and tuna) and lover value spices that are used as input to the aquaculture industry (sardine and anchovy). Sardine and anchovy are low value species, where a large share of it landing traditionally has been utilized as bait for higher value fish (such as tuna) or reductions used as in ingredient in the feed for aquaculture (such as salmon), in addition to human consumption. To investigate how the price of the small pelagic spices are integrated with the price of higher values spices, where it is utilized as bait and feed, we are conducting a traditional Johansen market integration analyses. For the analyses we aggregate trip level landings and revenue data to monthly price indexes, testing for Law of One Price (LOP) and price leadership

Understanding how small pelagic species are related to this market would be a next step in understanding how the fish markets are related, since the small pelagic are an important input factor in the farmed salmon market. Understanding how the different small pelagic are related are also interesting it itself. While a number of studies has investigated the market integration between different spices of whitefish fish (Asche et al 2004), between wild and farmed fish (Bjørndal and Guillen 2016), there has been little focus and the market interaction between the small pelagic. A few exceptions to however exist: Mulazzani et al (2012) investigate spatial interaction for the European anchovies and sardines and Pincinato and Asche (2018) presents a market integration and price transmission analysis for the Brazilian sardine market, comprising the fresh and canned sardines' segments.

A PROXY TO CARRYING CAPACITY FOR MEDITERRANEAN AQUACULTURE

F. Romero*[1], P. Sanchez-Jerez[1], G. Martínez[2], V. Fernandez-Gonzalez[1], M.M. Agraso[2] and K. Toledo-Guedes[1].

[1] Department of Marine Science and Applied Biology, University of Alicante, Alicante, SPAIN
[2] Andalusian Aquaculture Technology Center (CTAQUA). Muelle Comercial S/N, 11500, El Puerto de Santa María, Cádiz, Spain
E-mail: francisca.romero@ua.es

Introduction
Over the last two decades, the European fish farming industry has been experiencing a progressive stagnation in production levels due to the rare of suitable areas for aquaculture because of the confluence of maritime space uses. Therefore, the planning and management of aquaculture areas aims to play a key role in their successful sustainable development. In this sense, its management should take into account not only the selection of the allocated zones for aquaculture (AZAs) if not the application of carrying capacity studies (Sanchez-Jerez *et al*., 2016; Macias *et al*., 2019; Weitzman *et al*., 2021). Therefore, in this study is intended to develop a mathematical model for the calculation of carrying capacity, integrating productive, ecological and socio-economic factors, which can be used by public administrations to regulate aquaculture activity in the open sea on the Spanis Mediterranean cost.

Material and Methods
This study used a modified Delphi approach structured in three rounds. It started with the formation of a network of contacts through semi-structured interviews and ended with two rounds of questionnaires discussed in sectoral roundtables and workshops with experts to obtain a consensus on the value of the maximum allowable production (baseline production) and the different factors involved in increasing or reducing it.

Results and Discussion
During the first round, the experts identified a total of 39 indicators classified according to their type of carrying capacity (technical-productive, environmental, social and economic) and their degree of importance. After the first round, 18 of the 39 indicators were excluded from the study and in addition, 3 new ones were also identified, bringing the total of 11 factors as possible estimators of carrying capacity. After a consensus analysis by the Delphi expert panel, Round 2 ended with a model proposal based on a baseline production and 8 factors carrying capacity estimators, together with their respective ranges and weighting values. Due to its high restrictive power and observing the possible inconsistencies of the model, Round 3 ended with a final readjustment of the model (Table 1) in order to provide a new tool for the Spanish fish farming, available for producers and administrators for the innovative management of the Spanish aquaculture since its formulation is based not only on ecological, but also on social and economic aspects selected and defined by marine aquaculture stakeholders.

This project is carried out with the collaboration of Fundación Biodiversidad, of the Ministerio para la Transición Ecológica y el Reto Demográfico, through Pleamar Program, cofinanced by FEMP. We thank the collaboration of the three companies that gave us access to their facilities.

References
Macias, J.C., Avila Zaragozá, P., Karakassis, I., Sanchez-Jerez, P., Massa, F., Fezzardi, D., Yücel Gier, G., Franičević, V., Borg, J.A., Chapela Pérez, R.M., Tomassetti, P., Angel, D.L., Marino, G., Nhhala, H., Hamza, H., Carmignac, C. & Fourdain, L. (2019). Allocated zones for aquaculture: a guide for the establishment of coastal zones dedicated to aquaculture in the Mediterranean and the Black Sea. General Fisheries Commission for the Mediterranean. Studies and Reviews. No 97. Rome, FAO. 90 pp

Sanchez-Jerez, P., Karakassis, I., Massa, F., Fezzardi, D., Aguilar-Manjarrez, J., Soto, D., Chapela, R., Avila, P., Macias, J. C., Tomassetti, P., Marino, G., Borg, J. A., Franičević, V., Yucel-Gier, G., Fleming, I. A., Biao, X., Nhhala, H., Hamza, H., Forcada, A., & Dempster, T. (2016). Aquaculture's struggle for space: The need for coastal spatial planning and the potential benefits of Allocated Zones for Aquaculture (AZAs) to avoid conflict and promote sustainability. Aquaculture Environment Interactions, 8, 41–54.

Weitzman, J., Filgueira, R., & Grant, J. (2021). Development of best practices for more holistic assessments of carrying capacity of aquaculture. Journal of Environmental Management, 287, 112278.

(Continued on next page)

Table[1]. Final carrying capacity model. * This factor has no associated dimension or it is governed by qualitative or semi-quantitative measures and lacks of measurement. ** The values of this factor are valid for sea bream/bass farming, and the ranges and values must be adjusted for different species.

CARRYING CAPACITY = BP * V_{T1} * V_{T2} * V_{T3} * V_{A1} * V_{A2} * V_{A3} * V_{S1} * V_{E1}

BASAL PRODUCTION ALLOWED (BP) = 50 t / ha			
Factor of the model	**Calculation**	**Range**	**Weighting value (V)**
TECHNICAL AND PRODUCTION-RELATED			
Tons feed per hectare (T1)	Tonnes of feed per hectare of concession per annum	35-45 t/ha	1.38
		45-55 t/ha	1.18
		55-65 t/ha	1.00
		65-75 t/ha	0.87
		<75	0.50
Space arrangement (T2)	Average free area between two floating nursery trains / average area of floating nursery trains	>3.5*	1.33
		2.5-3.5*	1.14
		1.5-2.5*	1.00
		0.5-1.5*	0.86
		<0.5*	0.66
Distance between facilities (T3)	Distance (nm) between one installation and the next nearest installation	>20 nm	1.47
		10-20 nm	1.27
		2-10 nm	1.05
		1-2 nm	0.82
		<1 nm	0.62
ENVIRONMENTAL			
Distance to habitats considered of high-priority for conservation (A1)	Distance (nm) of the installation to marine phanerogams, maerl beds and gorgonians	>3 nm	1.48
		2-3 nm	1.32
		1.2-2 nm	1.10
		0.5-1.2 nm	0.97
		<0.5 nm	0.72
Depth (A2)	Average depth (m) of the installation	<50 n	1.57
		40-50 m	1.32
		30-40 m	1.08
		25-30 m	0.83
		>25 m	0.42
Current (A3)	Annual average current intensity (cm/s) in the installation at a depth of 15m	>10 cm/s	1.35
		10-6 cm/s	1.18
		6-4 cm/s	1.02
		4-2 cm/s	0.80
		<2 cm/s	0.37
SOCIAL			
Quality of employmet provided (S1)	Annual Work Units / number of inhabitants of the coastal province (mil.)	> 800	1.10
		600-800	1.05
		400-600	1.00
		200-400	0.95
		0-200	0.90
ECONOMIC			
Unit Cost of Production (E1)	Amount of money (€) it costs to produce one kilo of fish in each facility.	3.25-3.5	1.09
		3.5-3.75	1.04
		3.75-4	1.00
		4-4.25	0.98
		4.25-4.5	0.93

EPIDEMIOLOGICAL MODELLING OF WATERBORNE SPREAD OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN FARMED SALMON POPULATIONS

J.Romero[1*], I. Gardner[1], S. Saksida[1], D. Price[2], K. Garver[3], K.Thakur[1]

[1]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada.[2]Aquaculture Management Division, Fisheries and Oceans Canada, Canada. [3]Pacific Biological Station, Fisheries and Oceans Canada (DFO), Nanaimo, British Columbia V9T 6N7, Canada.
*E-mail: joao.fps.romero@gmail.com

Introduction

Three high-mortality epidemics of infectious hematopoietic necrosis (IHN) occurred in farmed salmon populations in British Columbia (BC), Canada from 1992 to 1996 (St-Hilaire et al., 2002), 2001 to 2003 (Saksida, 2006), and in 2012 (Garver & Wade, 2017). Wild Pacific salmon were hypothesized to be the source of IHN virus to farmed populations (Saksida, 2006; Garver & Wade, 2017). Since the 2012 epidemic, IHN has been well controlled through vaccination on salmon farms. In 2010, a Viral Disease Management (VDMP) was developed by BC salmon companies to rapidly and effectively minimize infection transmission and prevent spread to more distant Management Zones (MZ); however, to date the effectiveness of VDMP practices in mitigating IHN epidemics has not been evaluated (Garver & Wade, 2017)"autho r":[{"family":"Garver","given":"K"},{"family":"Wade","given":"J"}]},"label":"page"},{"id":628,"uris":["http://zotero.org/users/2112239/items/XYAPA36R"],"uri":["http://zotero.org/users/2112239/items/XYAPA36R"],"itemData":{"id":628,"type":"article-journal","abstract":"I investigated a recent infectious haematopoietic necrosis disease (IHN. We used an epidemiological simulation model to evaluate a hypothetical, waterborne spread of IHN virus among a susceptible population of farmed salmon in BC, after initial introduction into a single farm site. The purpose of the modelling was to evaluate existing viral management practices and to inform decision-making of regulatory agencies about spatial planning of future marine sites.

Material and methods

The simulation model (Romero et al. 2021) is spatially-explicit and allows modelling of 3 components of waterborne spread of IHNv in marine sites: within net-pens (a SEIR model as used for COVID-19 in humans), among net-pens (based on infection prevalence and a user-specified probability of transmission), and spread to distant sites, the probability of which decreases with increasing seaway distance. For distances of >15km, there is almost zero probability of waterborne IHNv spread between sites (Foreman et al. 2015). Data used to inform the model were production and location details of all 84 active farm sites in 2019, distributed across 10 MZs in BC. Expert opinion was used for number of net-pens per site, number of fish per net-pen, baseline daily fish mortality at the net-pen level, and mitigation measures for IHN. For each of the 10 MZs, different scenarios were explored to evaluate the combined effectiveness of disease surveillance and detection, depopulation procedures and vaccine use in reducing IHNv transmission. For every scenario, a single net-pen was selected as the infection source ("index") for the simulated IHNv incursion. This index pen was randomly sampled from the farm site with the lowest average seaway distance to other sites in the same MZ.

Results

The simulated waterborne IHN transmission is highly influenced by the mitigation measures implemented and the spatial distribution of farms within a MZ. IHN spreads mostly to farms closer to the "index", as lower seaway distances between farms corresponds to a higher probability of waterborne transmission (Figure 1). IHN spread varies between different MZs, as a consequence of the spatial distribution of farm sites within the MZ (Figure 2); the between-farm spread of IHN increases with the level of spatial clustering of farm sites within the MZ.

Discussion

The IHNv model demonstrated the ability to describe spatio-temporal patterns of IHN epidemics and assess the effectiveness of different mitigation strategies. This can be adapted to other aquatic-farmed species and pathogen spread in seawater by adjusting the open-source modelling framework (https://github.com/upei-aqua/DTU-DADS-Aqua). Particle tracking data from numerical circulation models (e.g., FVCOM) can be incorporated in the model to provide a stronger basis for assessing hydroconnectivity between sites.

(Continued on next page)

References

Foreman MG et al. (2015). Modelling Infectious Hematopoietic Necrosis Virus dispersion from marine salmon farms in the Discovery Islands, British Columbia, Canada. *PLoS ONE* 10(6): e0130951.

Garver, K., and Wade, J. (2017). Characterization of Infectious Hematopoietic Necrosis Virus (IHNV). *DFO Can. Sci. Advis. Sec. Res. Doc*. 2017/073. vi + 32 p.

Romero, J.F. et al. (2021). DTU-DADS-Aqua: a simulation framework for modelling waterborne spread of highly infectious pathogens in marine aquaculture. *Transbound. Emerg. Dis*.(in press).

Saksida, S.M. (2006). Infectious haematopoietic necrosis epidemic (2001 to 2003) in farmed Atlantic salmon in British Columbia. *Dis. Aquat. Org*, *72*(3), 213–223.

St-Hilaire, S. (2002). Epidemiological investigation of infectious hematopoietic necrosis virus in salt water net-pen reared Atlantic salmon in BC, Canada. *Aquaculture*, *212*(1), 49–67.

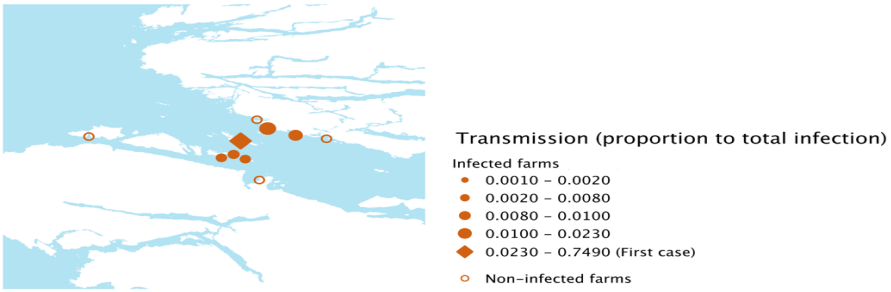


Figure 1. Spatial distribution of a simulated waterborne IHN epidemic among marine salmon farms in the coast of British Columbia. Diamond shape represents the “index” farm site. Shape size quantifies IHN transmission.

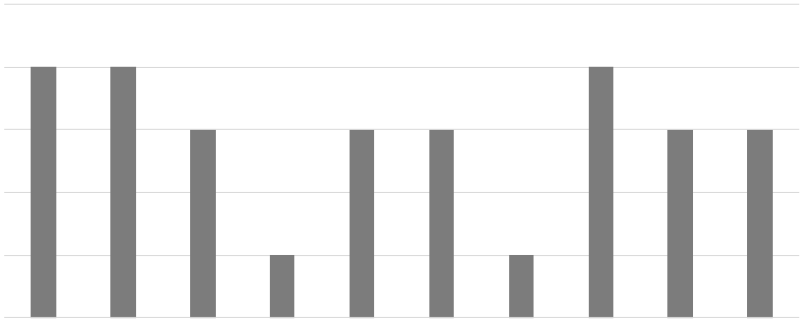


Figure 2. Total number of infected marine farms in simulated IHN epidemics in 10 Management Zones in the coast of British Columbia.

USE OF DIFFERENT SUSPENDED COLLECTORS FOR EUROPEAN FLAT OYSTERS (*Ostrea edulis*), CONTROL OF RECRUITMENT AND PRODUCT QUALITY IN A LONGLINE FARM OF THE MIDDLE ADRIATIC

Roncarati A., Mosconi G., Palermo F.A., Cocci P.

URDIS - School of Biosciences and Veterinary Medicine, Camerino University, Italy

Introduction

The flat oyster juveniles from the wild has always represented an important source of income for fishermen of the Marche region. Unfortunately, in recent years, there has been a sharp decline in the recruitment of spat which is arousing serious concern among operators and public administrators. The main causes include excessive exploitation of the flat oyster banks of natural areas, the decrease of benthic ecosystems and the lack of efficient management of marine areas. A limited amount of food available to bivalve specimens could affect the energy reserves able to guarantee a normal reproductive rhythm as well as changed conditions of the marine environment. In this situation, *Ostrea edulis* juveniles could have serious health risks coming from parasites such as *Bonamia* and *Marteilia* (Cocci et al., 2020; Colsoul et al., 2021). In Italy, flat oyster farming generally begins with a half-size product, taken from natural banks, not submitted to grading. The main technique is based on growing the oysters, introduced in trays in the suspended system, with attachment to long lines, from 20–25 mm until they reach market size.

In this study, the efficiency of different collectors suspended in the water column of the longline farm was evaluated and the collected specimens were reared in trays.

Materials and Methods

The trial was performed at a long-line shellfish plant located 5.5 km from the coast of Porto Recanati (43°26'42.76" N–13°43'45.33" E). Sea depth ranged between 12 and 13 m, and the sea floor was mostly sandy. The main long-line rope was 4 m below the surface of the water. The sea current was quite strong (9–12 cm/s) and continuous.

Three different types of collectors were used: Chinese hats; lantern nets with white plastic strips; lantern nets with cupped oyster shells. In July, all the collectors were attached to the longline system at a depth of 3 m under the sea level. At different times, the devices were periodically monitored until the harvest occurred in March.

Results and discussion

The best performances were obtained by the lanterns filled with white plastic trips followed by the lanterns with shells and the Chinese hats. As concerns the substrate composed by the white strips, this type of collector showed the easiness to remove the oysters in agreement with other papers reported (van der Brink et al., 2020).

The activities carried out promoted the enhancement of the bivalve farming; the need to respect the environment, thanks to the application of good practices for the rearing of bivalve molluscs which were focused on the correct management of the seabed in correspondence of longline shellfish farm, were in a perspective of sustainable farming.

Acknowledgements

Research supported by Flag Marche Centro. The authors thank the BIVI Shellfish Farm for the practical assistance and the Lamantino Brothers for videos that provided.

References

Cocci P., Roncarati A., Capriotti M., Mosconi G., Palermo F.A. 2020. Transcriptional Alteration of Gene Biomarkers in Hemocytes of Wild *Ostrea edulis* with Molecular Evidence of Infections with *Bonamia* spp. and/or *Marteilia refringens* Parasites. Pathogens 9:323.

Colsoul B., Boudry P., Pérez-Parallé M.L., Bratos Cetinic A., Hugh-Jones T., Arzul I., Merou N., Wegner K.M., Peter C., Merk V., Pogoda B. 2021. Sustainable large-scale production of European flat oyster (*Ostrea edulis*) seed for ecological restoration and aquaculture: a review. Reviews in Aquaculture 1-46.

van den Brink A.M., Maathuis M.A.M., Kamermans P. 2020. Optimization of off-bottom spat collectors for restoration and production of the European flat oyster (*Ostrea edulis*) in Dutch coastal waters. Aquatic Conserv Mar Freshw Ecosyst 30:2087-2100.

Wijsmann J.W.M., Troost K., Fang J., Roncarati A. 2018. Global Production of Marine Bivalves. Trends and Challenges. In: Smaal A., Ferreira J.G., Grant J., Petersen J.K., Strand Ø. (Eds.) Goods and Services of Marine Bivalves. Springer Nature.

OPTIMIZING A RAINBOW TROUT BREEDING PROGRAM WITH GENOMIC SELECTION

Chantal Roozeboom[1]*, Antti Kause[2] , Mark Camara[1], Hans Komen[1], John W.M. Bastiaansen[1]

[1] Department of Animal Breeding and Genomics, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen (The Netherlands)
[2] Natural Resources Institute Finland (Luke), Genomics and Breeding, Myllytie 1, FI-31600 Jokioinen, Finland
Email: chantal.roozeboom@wur.nl

Introduction

Traditional breeding programs use pedigree-based estimated breeding values for selective breeding to improve the performance of farmed fish species. However genomic selection can increase genetic gain. Genomic selection is routine in Atlantic Salmon, Tilapia and shrimp (Ødegård et al., 2014; Yoshida et al., 2019; Zenger et al., 2019), but has not been routinely implemented in breeding programs for important European cultured species such as rainbow trout, Gilthead seabream and European seabass. Breeding companies that are interested in implementing genomic selection benefit from optimizing the structure of breeding programs to maximize genetic gain (Sonesson & Ødegard 2016). Parameters to be optimized include selection intensity, mating strategy and the number and ratio of fish used for sib-testing and as selection candidates in the nucleus. Using stochastic simulations, we optimize these parameters and explore the selective genotyping strategy for a rainbow trout breeding program under restricted inbreeding to assess the potential impact of implementing genomic selection. .

Materials and Methods

A breeding program for rainbow trout was designed based on answers to questionnaires send to breeders. We used this pedigree-based breeding program design as the reference design. With this reference design, 10 generations of selection were implemented using stochastic simulation in R software. The simulated traits were tagging weight, weight in nucleus, gutted weight at sea, visceral percentage, fillet percentage and survival at sea. The traits gutted weight at sea, visceral percentage, fillet percentage and survival at sea were measured in the production environment on sibs of the selection candidates held in the nucleus. The reference design consisted of 200 full-sib families of 100 individuals produced each generation from 100 male and 100 female parents in a 2:2 mating design. Twenty five fish per full-sib family were pre-selected for the nucleus and 15 fish for the production environment. Selection was based on a multi-trait selection index of traditional, pedigree-based estimated breeding values (EBVs). We also simulated this reference design with genomically estimated EBVs (GEBVs). To optimize the reference design with genomic selection, we simulated scenarios with varying 1) numbers of selected sires and dams, 2) mating ratios, and 3) ratios of pre-selected fish assigned to the nucleus and production environment. Additionally, we simulated different genotyping strategies, namely genotyping with lower density SNP panels and genotyping only a part of the selection candidates. Twenty replicates were simulated for each scenario.

Results

Compared to traditional, pedigree based selection, genomic selection resulted in higher genetic gains for all traits and a lower rate of inbreeding (Table 1). Selection response is improved by decreasing the selected proportion in genomic selection to 0.02 from 0.05 in the reference design, for the same level of inbreeding. Changing the mating ratio did influence the rate of inbreeding, but the effect on genetic gain was small. The optimal mating ratio was the 2:2 mating ratio from the reference design. The reference design for the ratio of fish preselected for the nucleus or sea produced the highest genetic gain. The optimized design (reference design with genomic selection and a selected proportion of 0.02) resulted in an increase of 14.6%, 15.2%, 5.5%, 11.1% and 30.0% of genetic gain for the traits weight in nucleus, gutted weight at sea, visceral percentage, fillet percentage and survival compared to the reference design with genomic selection.

Table 1 Results of the simulations

	ΔG and ΔF in reference design	**ΔG and ΔF in reference design with GS**	**ΔG and ΔF in optimized design with GS**
Weight in nucleus	95.5 grams	121.7 grams	139.5 grams
Gutted weight at sea	136.9 grams	203.6 grams	234.6 grams
Visceral percentage	-0.23 points	-0.36 points	-0.38 grams
Fillet percentage	0.28 points	0.45 points	0.50 points
Survival at sea	0.026 points	0.05 points	0.06 points
Rate of inbreeding	0.75%	0.47%	0.72%

(Continued on next page)

Genotyping with a lower density SNP panel resulted in decreased genetic gains compared to genotyping with a high density SNP panel. Genetic gains from using a low density SNP panel in the optimized GS design were still higher than from the pedigree-based selection in the reference design. Selectively genotyping 50% or less of the selection candidates resulted in comparable or lower genetic gains, respectively, compared to the genetic gains in the reference design.

Discussion and Conclusion

Applying genomic selection while keeping the design the same resulted in higher genetic gains because genomic selection exploited both the between and within family variance. Pedigree-based selection does not exploit within-family Mendelian sampling for traits measured on sibs only. The rate of inbreeding was reduced due to implementing genomic selection in the reference design, since genomic selection results in higher differentiation between sibs in the nucleus.

The optimal design for genomic selection should balance a high genetic gain with an acceptable rate of inbreeding. Due to the decreased rate of inbreeding, the selected proportion could decrease substantially without the rate of inbreeding increasing above 1%. The effect of the mating ratio on inbreeding was found to be more important than the effect on genetic gain. Additionally, optimizing the genotyping strategy significantly reduced the cost of genotyping while the cost in genetic progress was limited.

Acknowledgement
This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 818367 - AquaIMPACT.

References

Ødegård, J., Moen, T., Santi, N., Korsvoll, S. A., Kjøglum, S., & Meuwissen, T. H. (2014). Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*). Frontiers in genetics, 5, 402.

Sonesson, A. K., & Ødegård, J. (2016). Mating structures for genomic selection breeding programs in aquaculture. Genetics Selection Evolution, 48(1), 46.

Yoshida, G. M., Lhorente, J. P., Correa, K., Soto, J., Salas, D., & Yáñez, J. M. (2019). Genome-wide association study and cost-efficient genomic predictions for growth and fillet yield in Nile tilapia (*Oreochromis niloticus*). G3: Genes, Genomes, Genetics, 9(8), 2597-2607.

Zenger, K. R., Khatkar, M. S., Jones, D. B., Khalilisamani, N., Jerry, D. R., & Raadsma, H. W. (2019). Genomic selection in aquaculture: application, limitations and opportunities with special reference to marine shrimp and pearl oysters. Frontiers in genetics, 9, 693.

PROPOSAL FOR A GILTHEAD SEABREAM CERTIFICATION SCHEME

A. Roque*[1], M. F. Castanheira[2], A. Toffan[3], P. Arechavala-Lopez[4], E. Brun[5], M. Vilarroel[6], E. Gisbert[1], C. Mylonas[7], A. Muniesa[8], A. Estevez[1], A. Dalmau[1], B. Basurco[8]

1. IRTA- 43540 Sant Carles de la Ràpita Spain; 17121 Monells Spain
*Ana.Roque@irta.cat
2.Wefare Consultant, Barcelona Spain
3. IZSVe 35020 Legnaro-Padova, Italy
4. CCMAR-8005 139 Faro Portugal
5. NVI-0454 Oslo, Norway
6. UPM- 28040 Madrid, Spain
7. HCMR-19013 Anavyssos Attiki Greece
8. IAMZ-CIHEAM- 50059 Zaragoza Spain

Introduction

There is a trend towards increased concern for the welfare of animals under human care, and this concern has expanded to include the welfare of farmed fish. However, at present, the necessary operational welfare indicators (OWI) and implementation protocols required to monitor and safeguard the welfare of farmed fish are lacking. Operational Welfare Indicators (OWI) in aquaculture are measures that can be used to assess welfare status in individual animals or groups of animals, made practical and operational on commercial aquaculture facilities (Martins *et al*. 2012).

The objective of this procedure was to develop a scheme to certify the welfare of Gilthead seabream (*Sparus aurata*) and to perform a pilot run to verify its feasibility.

Methodology

The approach taken is based on the five freedoms in an attempt to follow a methodology already developed to certify farmed terrestrial animals including mammals and birds. This methodology was selected for two reasons; firstly, it has been developed over the years by experts in the field of animal welfare from all over Europe and it has been validated for different farmed animal species, secondly it can be easily applied in a farmed fish welfare certification scheme.

To garantee animal welfare, different principles need to be covered. These principles include good feeding, good housing, good health, and appropriate behaviour and must be valid throughout the animal's entire life (Veissier and Evans 2007). Butterworth *et al*. (2009) conducted a literature review and identified a set of 12 welfare criteria (11 in the case of seabream). These criteria were then grouped under the four principles mentioned above. The welfare criteria must be applicable to all farmed species (in our case all farmed fish) and they should be grouped under the four wider principles. Measures to assess these criteria corresponded to 21 potential OWIs. Each OWI was then plotted on a small chart and a final calculation method was proposed for each OWI, then given specific weight to get a criterion and finally a principle punctuation. All 4 principles are assumed the same specific weight (0.25) in the final calculation (Figure 1).

Results

A test run was performed using 2 tanks with 180g -200 g seabream to verify the feasibility of the scheme. In general, the pilot testing went well, although some parameters have to be reviewed. The list of OWIs to be reviewed include novel object test for a group of fish and swimming behaviour. Score analysis is undergoing.

References

Butterworth *et al*. 2009- Welfare quality assessment for poultry (broilers, laying hens). Pp111.
Martins *et al* 2012- Fish Physiol Biochem 38, 17–41
Veissier and Evans 2007- Proceedings of the Second Welfare Quality R Stakeholder Conference, pp. 33–36.
Welfare Quality 2009- Welfare quality assessment for poultry (broilers, laying hens). Welfare Quality Consortium, Lelystad, Netherlands.

(Continued on next page)

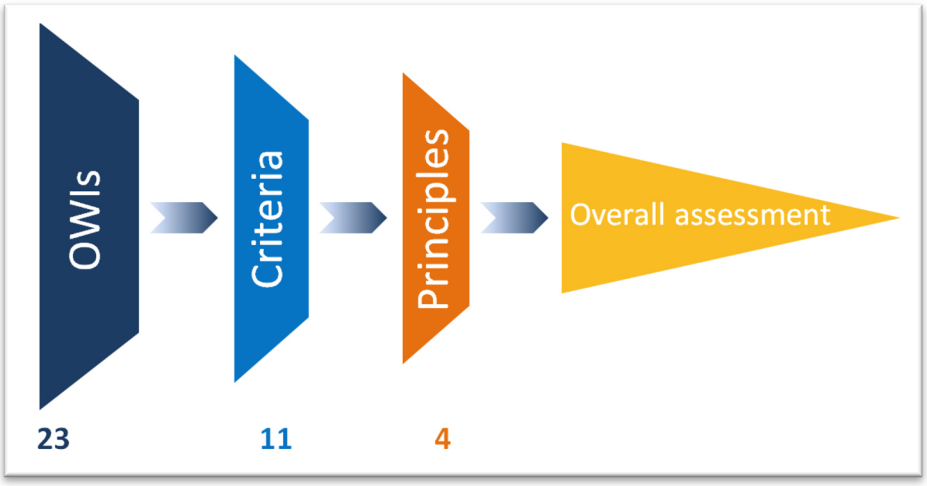


Figure 1. Approach for integrating the data of the different measures to an overall welfare assessment in seabream (modified from Welfare Quality 2009).

DESCRIPTION OF THE FEEDING BEHAVIOUR OF MULLET (*Mugil cephalus*) WITH FEED DEMAND IN RAS

Roque A*. and Duncan N.

IRTA 43540 Spain
*email: Ana.Roque@irta.cat

Introduction

In addition to the sustainability of the diet formulation, feed is the most expensive part in the culture of finfish species and careful feed management will greatly increase sustainability and reduce polluting environmental effects and waste. In the wild, different species of fish feed on prey such as phytoplanckton or zooplankton (Wassef and Eisawy, 1985; Andrade et al., 1996).

Demand feeding has been demonstrated to improve growth rates, improve food conversion ratio (FCR), reduce variation in size and reduce aggression in Atlantic salmon (Noble et al., 2007, 2008). In seabass improved growth and lower FCR was observed in both studies conducted in cages (Azzaydi et al., 1998) and tanks (Azzaydi et al., 2000), whilst in salmon results in different cages all improved growth and FCR. Some benefits of self demand system include ability to test the individual s capacity for differentiating between types of feed. Indeed, a feed demand system allows to study fish demand feeding behaviour and motivation (levels, rhythms, alteration) and to correlate it with physiological variables, individual growth and SGR. Present study investigated the the levels and rhytms of mullet (*Mugil cephalus*) maintained in a RAS with freshwater.

Material and methods

Experiment ran from October to January in 4 tanks, tanks were connected in pairs to a recirculation system (IRTAmar®). All tanks were fitted with a dark net to avoid stress and fish jumping from the tank. The system ran in freshwater at 21ºC and the photoperiod was 12h L:12h D. Each tank contained 80 mullet, 45,4 ± 2.9 g wet weight. Each tank had 2 automatic feeders in oposed positions. For the first fortnight, feeders were programmed to deliver a 3% of the biomass divided in 8 feeds per day so that fish knew where the food would appear. After this period the programme was changed to autodemand where 0.95 g would fall with each demand. The food fell from the feeder within 0-5 seconds after the demand when the pendulum was triggered. Tanks were left like this for 8 weeks to ensure fish knew how to use the feeders and the description of feeding behaviour corresponds to a further 4 weeks.
Fish were weighed and sampled at the beginning and end of the experiment.

Results and discussion

Results showed fish consummed an average of 0.27g per day and fed mainly during the day hours. Total average growth was for 28 days was 7.55g. The demand on the tanks varied and the demand on one tank was lower than the others. On average mullet demanded food 52.8 ± 3 times per day. The majority of the demands occurred when there was day light (between 6 am and 7 pm). A total of 2681,25 g was demanded during the night period and a total of 3998.5 g was demanded during the day period. One tank made its demands preferably during the night. No left over food was observed in the tanks. The estimated FCR for this period was 2.76 which was higher than what should be expected to omnivorous fish.

Performance in the tanks was different and there were preferences towards using one or the other feeder of tank.

In conclusion, fish learnt how to use the feeders, wasted no food but the growth performance was not very efficient.

References

Andrade etal. 1996. Aquaculture International, 4: 129– 141.
Azzaydi et al. 1998. Aquaculture, 163: 285-296
Azzaydi et al. 2000. Aquaculture, 182: 329-338.
Noble et al. 2007. Aquaculture Research, 38: 1686–1691.
Noble et al. 2008. Aquaculture, 275: 163-168.
Wassef and Eisawy 1985. Cybium 9, 233– 242.

MICROALGAE-SUPPLEMENTED DIET IMPROVES THE SKELETAL HEALTH OF GILTHEAD SEABREAM *Sparus aurata* JUVENILES

J. T. Rosa[1,2,*], A. Carletti[1,2], C. L. Marques[3], H. Ringeard[2,3], I. Borges[1,2], M. Barata[3], P. Pousão-Ferreira[3], P. J. Gavaia[1,2], M. L. Cancela[1,2,4], V. Laizé[1]

[1]Centro de Ciências do Mar (CCMAR)
[2]Universidade do Algarve (UALG)
[3]Instituto Português do Mar e da Atmosfera (IPMA)
[4]Algarve Biomedical Center (ABC)
*E-mail: jtrosa@ualg.pt

Introduction

Fish cultured in intensive farming conditions may develop severe skeletal deformities that affect both their external morphology and welfare. In the case of commercially important fish species, such as the gilthead seabream (*Sparus aurata*), this is a matter of great concern and a major factor that affects production costs, downgrades hatcheries' production and fish market value. Among the solutions proposed to improve the skeletal deformities of aquaculture fish, the supplementation of their diet with natural compounds or extracts that stimulate skeletogenesis is increasingly seen as an economically sound approach to improve the competitiveness of the aquaculture industry and fish health. Extracts from marine origin are already being used as fish diet supplements, as they are a valuable source of important nutrients, but recently, some were also found to contain osteogenic and mineralogenic compounds. To explore their potential to improve farmed fish skeletal status, we supplemented a commercial fish diet with ethanol extracts of microalgae strains commercially available in Portugal and shown to be rich in bone anabolic compounds, and fed gilthead seabream juveniles with these experimental diets. Morphological and hematological parameters, and skeletal status were then evaluated.

Material and Methods

Gilthead seabream (*Sparus aurata*) juveniles of 4.8±0.7 g were fed *at libitum* 3 to 4 times a day with a commercial diet (Sparos, Lda.) supplement with ethanolic extracts of *Skeletonema* sp. (at 1%) or *Tetraselmis* sp. (at 0.5%) until they tripled their weight. Fish were housed at the Estação Piloto de Piscicultura de Olhão (EPPO/IPMA) in 250-L rectangular tanks with water renewal of approximately 350 L per hour and a temperature of 25.3±1.0ºC. At the end of the trial, growth parameters such as total length and weight, and welfare/stress parameters such as cortisol levels and blood analytes (including gases such as oxygen and carbon dioxide, Na+, K+, Ca++, Cl-, glucose, lactate and creatinine) were determined to assess the innocuity of the algal supplement. The occurrence of skeletal deformities was evaluated through X-ray imaging, while bone mineral composition was determined from ashed vertebrae by microwave plasma atomic emission spectroscopy. To better understand the putative effect of the supplementation in the mechanisms underlying bone formation and status, several well-established bone markers were selected, and their expression determined by qPCR in fish vertebrae.

Results

Regarding fish growth performance indicators, dietary treatments had no impact on feed conversion rate (FCR), although a slight increase was observed for fish fed with the diet supplemented with *Skeletonema* sp. extract. The total length was not significantly altered in fish fed supplemented versus control diets, but a positive effect was observed on the weight of the fish fed the diet supplemented with *Tetraselmis* sp., which consequently show a moderate increased condition factor. The analysis of several haematological parameters revealed that food supplementation with microalgae extracts does not influence fish physiological status or stress levels, except for the increased in pCO_2 levels, which may indicate a higher cellular activity. In terms of bone parameters, the vertebrae of fish fed with the diet containing *Skeletonema* sp. extract showed a higher phosphorous content, although the optimal balance between calcium and phosphorous was maintained in the vertebrae of fish fed supplemented diets. The analysis of the type and severity of the skeletal deformities revealed that most of them are associated with the caudal fin complex, independently of the treatment, but that the incidence is reduced when fish are fed with the diets containing extract of *Skeletonema* sp. (deformity charge (df) = 1) or *Tetraselmis* sp. (df = 1.17) when compared to the control diet (df = 1.36). The expression of several bone marker genes was also altered upon dietary treatment, as it is the case of the osteoblast markers *sp7* and *col1a1*, that are, respectively, up and downregulated for fish treated with *Skeletonema* sp., and osteoclast marker *acp5*, that is upregulated for fish fed with *Tetraselmis* sp. Interestingly, the expression of the glutathione peroxidase *gpx1* was increased in the vertebrae of fish treated with both extracts, suggesting an activation of antioxidant mechanisms by both experimental diets. Overall, the data gathered in this study points out to a general improvement of fish skeletal status upon dietary treatment with microalgae extracts containing bone anabolic compounds, without compromising, and possibly improving, fish growth performance indicators.

Funding

This study was funded by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020 and by the European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 and project OSTEOMAR MAR-02.01.01-FEAMP-0057.

EFFECTS AND AMELIORATION OF NATURALLY OCCURRING AND EXPERIMENTALLY INDUCED MYCOTOXICOSES IN JUVENILE NILE TILAPIA *Oreochromis niloticus*

Rosen, R.[1*], Doupovec, B.[1], Kovalsky, P.[2], Gruber, C.[2], Bui Chau Truc, D.[3], Schatzmayr, D.[1]

[1]BIOMIN Research Center, Tulln, Austria
[2]BIOMIN Holding GmbH, Getzersdorf, Austria
[3]BIOMIN Aquaculture Centre for Applied Nutrition, Ho Chi Minh City, Vietnam,

Roy.Rosen@biomin.net

Introduction

Toxigenic filamentous fungi are common contaminates of agricultural crops. These fungi produce low molecular weight secondary metabolites commonly known as mycotoxins. Although contamination by these fungi frequently occurs in areas with a hot and humid climate (i.e. conditions favorable for fungal growth), they can also be found in temperate conditions.[1] Climate changes events are likely to influence toxigenic fungal spatial growth and the inherent risk of crop contamination.[2]

Mycotoxins are nearly ubiquitous in a variety of plant origin commodities, animal feeds and human foods causing serious health conditions to man and animals with severe economic consequences to the related industries.

Scientific literature describes mycotoxins-related pathologies in aquatic species since the 1960's and acute outbreaks are documented globally for the different species by common toxins throughout the years. Recently, similar to other livestock industries, more awareness is given to the chronic and sub-clinical effects of mycotoxins in aquaculture. Chronic mycotoxicoses are often due to moderately naturally contaminated raw materials used in untreated aquaculture feeds that do not exhibit signs of molding themselves and upon consumption do not evoke clear and specific clinical signs in the animals.

Ochratoxin A (OTA) is the most important member of the ochratoxins group concerning both human and animal health. It is produced by different species of *aspergillus* and *penicillium* fungi.[3] Beyond its direct effect on the consuming animal it also shows high levels of carry-over through the food chain from the contaminated animal feed through the animal's tissue to the consumer.[4] Maximum levels for OTA both for animal feeds and human foods are set in the European union[5] and regulatory efforts are also being applied by various authorities such as the FDA and other national bodies.

Several commercial products to counteract the negative effects of mycotoxins on livestock have been developed and described. A major limitation to a successful application of detoxifying products are their selectivity towards the mycotoxin while maintaining required nutritional components such as vitamins and minerals. Additionally, data on the product suitability and efficacy for aquatic organisms at their various developmental stages is often missing.

In two *in vivo* trials, the effects of naturally occurring mycotoxins and experimentally OTA-contaminated diets were evaluated in Nile tilapia (*Oreochromis niloticus*) in addition to the efficacy of detoxification and bioprotection solution using proprietary mitigation product was assessed.

Materials and methods

The experiment composed two trials lasting eight weeks long each. In both, juvenile, male-monosex population of Nile tilapia were housed in 24 recirculating aquaculture system (RAS) tanks.

In the first trial, the fish were divided in two groups, one receiving non-supplemented and naturally contaminated tilapia diet formulated according to industrial standards, and the other group received the same diet with addition of the mycotoxin detoxifying product at inclusion rate of 2.5 mg/kg.

In the second trial, a new group of fish was divided into 3 groups receiving control diet, artificially contaminated with OTA at 500 μg/kg inclusion rate and the same OTA contaminated diet with supplementation of 2.5mg/kg of the detoxifying product.

(Continued on next page)

Performance (zootechnical) parameters were evaluated before during and after each of the feeding trial periods. Additionally, immune and health markers were evaluated at the end of each trial and fish survival rate and response to additional immersion bacterial challenge using *Streptococcus agalactiae* was also included in the second trial where the OTA supplemented diet was used.

Results and discussion

In the experiment, significant weight and FCR differences were observed. Survival rate was also negatively affected by the natural presence of mycotoxins(Table 1). Furthermore, the experimental inclusion of OTA 500$\mu g*kg^{-1}$ in the diet exacerbated the negative effects and significantly reduced the specific growth rate and survival before and following the bacterial challenge. Supplementation of the detoxifying product with it's bioprotective component improved the performance parameters.

Toxin	**Value** $\mu g*kg^{-1}$
Total Fumonisins	121.03
Zearalenone	75.82
Type B trichothecenes	98.57
Type A trichothecenes	2.88
Ochratoxin	1.24
Ergot alkaloids	0.63

Table 1 Mean mycotoxin level of the naturally contaminated diet in the first experiment

The objective of the study was to evaluate the effects and offer an efficient and economical solution for mycotoxicosis both as chronic, naturally-occurring contamination and as acute intoxication in Nile tilapia. The different combinations of mycotoxin challenges proved to have detrimental effects in certain evaluated parameters and at the same time, the mycotoxin detoxifying product demonstrated to counteract those effects.

The problem of mycotoxins in aqua feeds is expected to increase in coming years due to environmental concerns such as climate changes, overfishing and sustainability concerns. These already bring advances in feed formulation such as the use of alternative ingredients and increase of plant material to replace proteins and oils from marine sources. Awareness, surveillance and detoxification strategy are the key factors for successful outcome of this challenge in aquaculture.

1. Bhatnagar, D., J. Yu, and K.C. Ehrlich, Toxins of filamentous fungi. Chem Immunol, 2002. 81: p. 167-206.
2. Wu, F. and N.J. Mitchell, How climate change and regulations can affect the economics of mycotoxins. World Mycotoxin Journal, 2016. 9(5): p. 653-663.
3. Matejova, I., et al., Impact of Mycotoxins on Aquaculture Fish Species: A Review. Journal of the World Aquaculture Society, 2017. 48(2): p. 186-200.
4. Battacone, G., A. Nudda, and G. Pulina, Effects of Ochratoxin A on Livestock Production. Toxins, 2010. 2(7): p. 1796-1824.
5. Schrenk, D., et al., Risk assessment of ochratoxin A in food. EFSA Journal, 2020. 18(5).

A BLEND OF ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS BOOSTED THE ANTIOXIDANT RESPONSE DURING AN INFLAMMATORY CHALLENGE IN ATLANTIC SALMON (*Salmo salar* L.)

B. Rossi*, A. Piva, and E. Grilli

*Vetagro S.p.A., via Porro 2 – 42124 Reggio Emilia (Italy)
barbara.rossi@vetagro.com

Introduction

Aquaculture is a very fast-growing food-producing sector and due to intensive fish farming practices, infectious disease can cause heavy loss to farmers. Fish have considerably higher exposure to pathogens than non-aquatic vertebrates and the outbreak of diseases is not merely related to the causing agents (Martin and Król, 2017). In fact, outbreaks of fish diseases commonly occur when fish are stressed due to a variety of factors associated with the aquaculture environment and management procedures such as high stocking densities, transport or handling. For this reason, an optimal immune function of farmed fish is important to protect them from infections and, in this context, there is also an increasing consumer demand for environmentally-friendly and animal-friendly alternative to the extensive preventive use of antibiotics.

Organic acids (OA) and botanicals (or phytogenic compounds) are widely used in terrestrial animals and they gained an increasing interest also in aquaculture. OA are well known for their antimicrobial power, as well as phytogenic compounds. Some botanicals have also been proven to exert antioxidant and anti-inflammatory properties, strengthening the immune-response of fish.

The aim of the study was to determine the effect of AviPlus®Aqua (Vetagro SpA), a microencapsulated blend of OA (sorbic and citric acid) and nature-identical compounds (NIC, thymol and vanillin), on Atlantic salmon parr (*Salmo salar* L.) immune-response, during an inflammatory challenge.

Materials and methods

Three hundred Atlantic salmon specimens of an average weight of 5.4 g were randomly distributed into 6 tanks (50 fish per tank) at SPAROS Lda (Portugal). The following diets were tested in triplicate tanks: 1) control (CTR), 2) AviPlus®Aqua 1500 ppm (D1500). Fish were fed for 92 days and then subjected to an inflammatory challenge by injecting them intraperitoneally (i.p.) with either 100 μl of UV-killed Yersinia ruckeri (Yr) (CTR+, D1500+) or Hanks' Balanced Salt Solution (HBSS) (CTR-, D1500-). Upon injection, fish were redistributed into new tanks according to diet and injection stimuli. Four- and 24-hours post-injection, fish injected with UV-inactivated Yr (n=3 per replicate tanks, n=9 per diet) or HBSS (n=3 per replicate tank, n=9 per diet) were subjected to moderate anesthesia and a sample of blood was collected by puncture of the caudal vein with a heparinized syringe. Serum was collected to study humoral parameters such as reactive oxygen species (ROS), total peroxidase activity (PA), and nitric oxide (NO). Moreover, plasma bactericidal activity against *Vibrio harveyi* was assessed according to Graham et al. (1998) with some modifications (Machado et al., 2015). Data were analyzed with a two-way analysis of variance, with diet inclusion and injection type (Yr and HBSS) as variables.

Results

At a basal state (prior to inflammatory challenge), in comparison to the non-supplemented control treatment, fish fed diets supplemented with 1500 mg/kg AviPlus® Aqua showed a higher plasma antibacterial activity ($P<0.05$).

Four- and 24 hours after injection, fish fed the D1500 diet continued to show a significantly higher plasma bactericidal activity (Fig. 1). No differences were found about plasma ROS concentration but plasma levels of NO and PA were significantly higher in fish fed the D1500 diet than those fed the CTRL diet, at both time points (Fig. 1).

Conclusion

In conclusion, Atlantic salmon parr fed a diet supplemented with 1500 mg/kg AviPlus® Aqua for 92 days showed an enhanced capacity to cope with an inflammatory condition.

(Continued on next page)

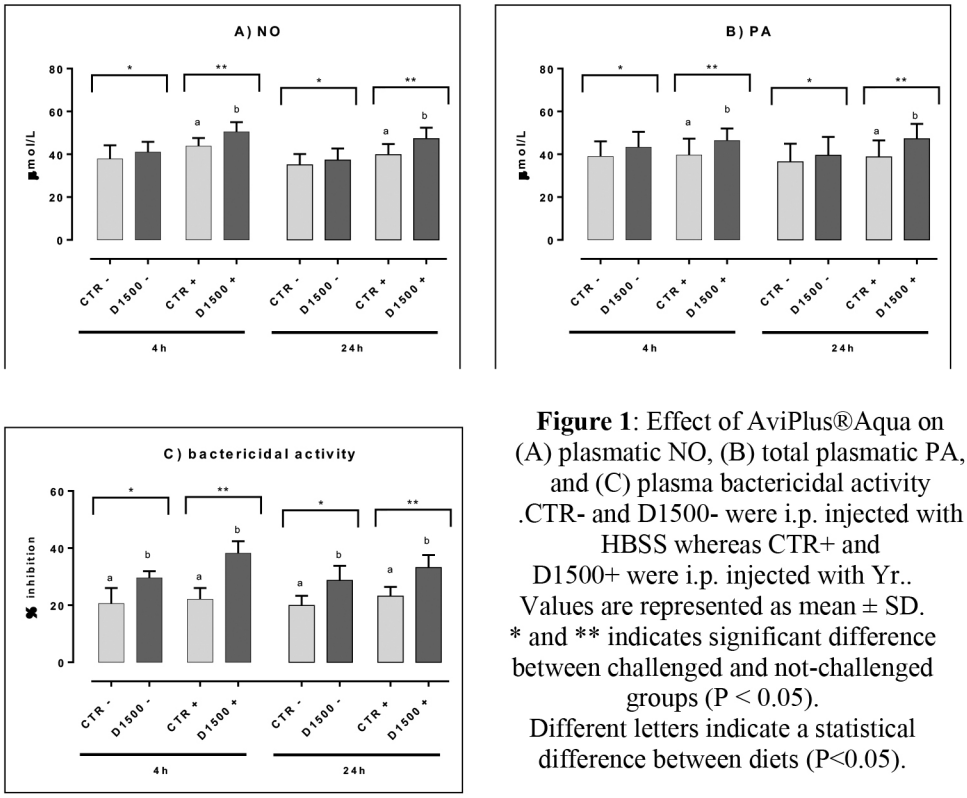


Figure 1: Effect of AviPlus®Aqua on (A) plasmatic NO, (B) total plasmatic PA, and (C) plasma bactericidal activity .CTR- and D1500- were i.p. injected with HBSS whereas CTR+ and D1500+ were i.p. injected with Yr.. Values are represented as mean ± SD. * and ** indicates significant difference between challenged and not-challenged groups ($P < 0.05$). Different letters indicate a statistical difference between diets ($P<0.05$).

Acknowledgement

The trial has been conducted at SPAROS Lda (Portugal) facilities by Dr. Jorge Dias.

Bibliography:

Martin and Król, 2017. doi: 0.1016/j.dci.2017.02.024
Graham et al. 1998. https://doi.org/10.1111/j.1365-2761.1988.tb00734.x
Machado et al., 2015. doi: 10.1016/j.fsi.2014.11.024

HOW FISH COLOR THEIR SKIN? MC2R A NEW ACTOR IN PIGMENTATION DEVELOPMENT

P. Soni[*1], L. Guerrero[1], L. Mendez[1], J.M. Cerda-Reverter[2] and J. Rotllant[1].

[1]Acuabiotec Lab, Department of Biotechnology & Aquaculture. Institute of Marine Research, (IIM-CSIC), 36208 Vigo, Spain;
[2]Department of Fish Physiology and Biotechnology, Institute of Aquaculture from Torre la Sal (IATS-CSIC), Castellon, Spain. 12595, Spain.

Email: rotllant@iim.csic.es

The melanocortin system is a complex neuroendocrine signaling mechanism involved in numerous physiological processes in vertebrates, including pigmentation, steroidogenesis and metabolic control. In humans, five melanocortin receptors (MCR) have been cloned, identified and shown to have a wide distribution throughout the body and likely many diverse functions. It has also been shown that a high degree of identity and conservation in structural characteristics and pharmacology exists between Mcrs from fish and mammals. However, in fishes, the number, affinity, specificity, tissue distribution and physiological roles are far from defined and appear to be species-specific. A clear example is the Mcr2 subtype. In humans, it is well known that Mc2r is expressed in the adrenal gland and controls steroidogenesis and in fact the same function has also been described in fish. However, the fact that Acth (a melanocortin agonist) may have a role in regulating fish pigmentation and that Acth acts on Mc2r, the involvement of Mc2r in fish pigmentation might be a possibility. Using CRISPR/Cas9 genome engineering tools we have generated “loss-of-function” mc2r mutant fish. We demonstrate that Mc2r , apart from controlling steroidogenesis, also has a direct role in regulating fish pigmentation.

ADVANCES AND OPPORTUNITIES IN SCIENCE COMMUNICATION: TIPS, TRICKS AND LESSONS LEARNED FROM PARTICIPATION IN GOJELLY H2020 PROJECT

A. Rotter[1]*, K. Klun[1], E. Grigalionyte-Bembič[1], R. Tiller[2]

[1]National Institute of Biology, Marine Biology Station Piran, Fornače 41, 6330 Piran, Slovenia
[2]SINTEF Ocean AS, Department, Box 4760 Torgarden, NO-7465 Trondheim, Norway
E-mail: ana.rotter@nib.si

Introduction

Applied sciences directly address societal challenges by forming collaborative networks. Consequently, scientists that are involved in these collaborations need specific skills and expertise (Rotter et al., 2021). In addition, a lot of top-down structures have been introduced in recent years, such as science communication, quadruple helix approach and the concept of the responsible research and innovation. There is a visible gap between the academic training and exercising science in practice, where these overarching skills that are needed in tackling scientific hypotheses, entering new research areas, obtaining scientific funding and collaborating are self-trained. A field which has been gaining increasing importance is science communication, which, especially in top-priority societal challenges (in recent years especially impacted by (micro)plastics pollution and recently also with the COVID-19 pandemics), needs special consideration. The talk will focus on (micro)plastics pollution and the unexpected interest for communicating the progress and results of one of the projects that tries to provide a solution to microplastic pollution, GoJelly (https://gojelly.eu/). The main aim of the project is to use one nuissance (increasing jellyfish blooms and the presence of non-indigenous jellyfish species) to solve another emerging problem, microplastic solution. The potential of wild harvesting or jellyfish aquaculture were also assessed during the project, thus addressing important environmental protection and sustainability bottlenecks.

Concepts and terminology

Collaborative networks are dynamic structures that are assembled based on the funding opportunity or emergence of a specific topic (Rotter et al., 2021). They typically have a limited duration, e.g. a predetermined time goal for providing concrete results. The most common collaborative networks are organized to target specific funding calls, promising innovative solutions with a realistic market-entry potential in future. One such example, the Blue Growth Horizon 2020 call (BG-07-2017: Blue green innovation for clean coasts and seas) identified chemical pollution as well as increasing jellyfish blooms as increasing problems in the oceans, seas and coasts. To address this problem, an international transdisciplinary consortium was formed with the aim of addressing one problem (microplastic pollution) using an unlikely source for a solution (jellyfish mucus). The idea behind the proposed solution was so novel and innovative, that it was convincingly financed. But, surprisingly, the solution was convincing and innovative also for an unexpected stakeholder category: the media, which typically represent a proxy for the general public. Indeed, since the formal approval of the project, the consortium members have been featured in many national, regional and global media.

The issue of microplastic pollution has been in the recent years recognized as a top priority area by the general public, the policy makers, the media as well as the scientific world. However, while scientists are still trying to evaluate the adverse effects of microplastics pollution on the environment, food and humans, the public and policy makers are eager to finally use the potential solutions.

In this perspective, the issue of microplastic pollution is an excellent example of transdisciplinary scientific collaboration, science communication, involvement of public and NGOs, efforts for legislation change, including the close collaboration with policy makers and finally offering approaches that can be used in practice and in future commercialized by small & medium enterprises as well as the industry.

The talk will present some introduction to the terminology and what scientific communication has done to catalyse this area of research. As the project is entering its final stage, the timing is right to deliver some basic concepts, project impact, lessons learned and provide tips and tricks that will enable to better shape the future generation of innovators and provide them with know-how to become efficient science communicators.

(Continued on next page)

Conclusion

The case of microplastic pollution and the GoJelly project is a great example of how the scientific community should operate. It is not self-sufficient, and industry, policy makers and the general public should all be involved to maximize the outputs. This is the concept of quadruple helix, which will also be presented.

Overall, we feel that there is a lack of »behind the scenes capacity building« presentations such as ours which we believe it will be well appreciated within the scientific and industrial community.

Rotter, A., Gaudêncio, S.P., Klun, K., Macher, J.N., Thomas, O.P., Deniz, I., et al. 2021. A new tool for faster construction of marine biotechnology collaborative networks. Front. Mar. Sci. 8:685164. https://doi.org/10.3389/fmars.2021.685164.

DATA ASSIMILATION APPROACH TO SUPPORT DAY-TO-DAY DECISION PROCESS: FORECASTING FISH WEIGHT DISTRIBUTION IN A LAND-BASED RAINBOW TROUT (*Oncorhynchus mykiss*) FARM

Edouard Royer [(1)*], Damiano Pasetto[(1)], Roberto Pastres [(1),(2)]

1) Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Via Torino, 155, 30170 Mestre, Venezia (Italy)
2) Bluefarm, s.r.l., Venice, Italy
E-mail: edouard.royer@unive.it

Introduction

Ecological intensification of aquaculture sector aims at improving productivity of the aquaculture farms while reducing environmental impact and ensuring fish welfare. In order to overcome the various challenges of this paradigm shift the GAIN H2020 project dedicated a non-negligible part of its work to the Precision Fish Farming (PFF) framework (Fore et. al., 2018), testing among other things some real-time biomass monitoring systems in farming conditions. One of the case studies was led in a rainbow trout farm in Preore (Trento, Northern Italy), where several cohorts of fish were monitored during several months using a weight monitoring system. Here we focus on the development of a reliable forecast system of population growth where model results and parameters are updated in real-time through the assimilation of the weight measurements. The data assimilation procedure improves then the short-term forecasts of the population distribution which is of huge interests for farm management.

The research leading to these results has received funding from the European Union's H2020 Framework Programme for Research and Innovation, under Grant Agreement No. 773330.

Methodology

Data Assimilation (DA) algorithms are being currently used in many scientific fields, e.g. mechanics, oceanography, meteorology, as they allow one to combine model output and field data as long as they are collected, avoiding the full re-calibration of the model. The purpose of DA is two-fold: i) to correct the prediction of output variables, based on the information provided by new data; ii) to improve the estimation of the un-observed state variables, which may include also model parameters. Most DA procedures describe the system state variables as stochastic variables which distribution changes in time in accordance with the differential equations governing the system; the system measurements are crucial to reduce the uncertainty on this distribution and possibly correct its mean value.

In the last decade, several fish biomass monitoring system were designed and commercialized, mainly destinated to salmon production as a consequence of high turnovers, and thus investments margins of this market. To our knowledge, no similar system was designed for land-based flow-through farming, and we then decided to use the Biomass Daily (BD) commercialized by Vaki Ltd in a trout farm in Northern Italy. In parallel of the installation and test of the device, we build a growth model able to forecast growth performance of individuals, based on temperature and feed ration. Trying to best take benefit of both elements, we implemented a DA approach that integrates daily measurements of the population into the model, in order to improve the reliability of daily population weight distribution forecast. The individual-by-individual representation of the whole population was replaced by a cohort approach based on the super-individual concept (Scheffer et al., 1995). The initial weights of this super-individuals, and the number of fish each one of them represents, where defined from the initial distribution of the population measured by BD. Daily feed ration, food composition, and water temperature were daily downloaded from the farmer's management system.

Results and discussion

Results show that the system allows to correctly predict daily fish weight distributions, providing thus an interesting additional level of information to the farmer that used until now only daily average weight predictions. On one side daily average weight is fully in line with the farmer's predictions and with the BD measurements. On the other side, the daily weight distribution fits with the BD observations, and is in line with the intermittent samples made by the farmer. This method could be of high relevance for farmer's days-to-day decision process as its results allow one to more accurately quantify some key parameters for farm management as feed quantity, oxygen supply, economical value of the biomass, but also the need for cohorts to be divided or moved to a larger raceway.

(Continued on next page)

References

Føre M., K. Frank, T. Norton, E, Svendsen, J. A. Alfredsen, T. Dempster, H. Eguiraun, W. Watson, A. Stahl, L. M. Sunde, C. Schellewald, K. R. Skøien, M. O. Alver, D. Berckmans, 2018. Precision fish farming: A new framework to improve production in aquaculture. Biosystems engineering 173 (20 18): 176 -193.

Pasetto, D, Arenas-Castro, S, Bustamante, J, et al. Integration of satellite remote sensing data in ecosystem modelling at local scales: Practices and trends. Methods Ecol Evol. 2018; 9: 1810– 1821.

Scheffer, M., Baveco, J.M., DeAngelis, D.L., Rose, K.A., van Nes, E.H., 1995. Super-individuals as a simple solution for modelling large populations on an individual basis. Ecological Modelling 80 (2-3): 161:170.

MONITORING THE DEVELOPMENT AND MITIGATION OF SOYBEAN MEAL INDUCED ENTERITIS (SBMIE) IN TWO DIFFERENT STRAINS OF RAINBOW TROUT *Oncorhynchus mykiss* OVER A LONG-TERM FEEDING TRIAL

Marina M. Rubio Benito*, Nicholas Romano, Kenneth Overturf, and Vikas Kumar

Aquaculture Research Institute, Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, ID, 83844, USA
mrubiobenito@uidaho.edu

Introduction

Plant protein sources have been largely studied for their potential in replacing fish meal (FM) as the main source of protein in aquafeeds. Specifically, soybean meal (SBM) has been widely used at high inclusion levels to improve sustainability and affordability of fish feeds. However, carnivorous species like rainbow trout (*Oncorhynchus mykiss*) are susceptible to feeds formulated with high inclusions of SBM, exhibiting reduced growth rates and distal intestinal inflammation or enteritis. Mitigation of SBM-induced enteritis (SBMIE) may be done by dietary supplementation of L-glutamine (Gln) as it has shown encouraging results in species like turbot, sturgeon, and carp in terms of improved intestine morphology, decreased pro-inflammatory gene expression and improved antioxidant enzymatic activity.

The goal of this study was to evaluate the potential mechanisms by which Gln might exert a protective effect on reducing inflammation and restore barrier function in two different commercial strains (A and B) of rainbow trout over a 30-week long-term experimental period.

Materials and methods

Diets: three experimental diets (isonitrogenous: 40% crude protein and isolipidic: 20% lipid) were formulated including a FM diet (control), a SBM diet (30% inclusion level), and a SBM-Gln diet (1.5% L-Alanyl-Gln supplemented).

Animals: 2,250 fish from two different commercial strains of rainbow trout (strain A = 1,125 and strain B = 1,125) initially weighing 24.3 ± 1.0 g, were randomly distributed into 18 350-L tanks (125 fish/tank) in a recirculating aquaculture system (RAS). During the trial, fish were fed to apparent satiation for 30 weeks. Fish were sampled five times at 6, 12, 18, 24 and 30 weeks for growth performance and intestinal tissue samples from three fish per tank were taken for histology and SBMIE molecular markers analysis, and for glutamate quantification.

Histology: distal intestine tissue samples from three fish per tank were fixed in Bouin's solution and dehydrated using a graded series of alcohol baths and infiltrated with paraffin wax. A total of four cross sections from each sample were cut at 5 μm. Sections were then stained with hematoxylin/eosin (H/E).

Statistical analyses: data was assessed for normality using the Shapiro-Wilk normality test and variance homogeneity using the Bartlett test of homogeneity of variances. Significant differences were analyzed using one-way and two-way ANOVA followed by Tukey's multiple comparison test.

Results

Weight gain at four out of five sampling points is presented in Table 1. Differences in weight gain of strain A were detected at 18 weeks by one-way ANOVA analysis, being significantly higher in fish fed the SBM diet ($p = 0.02$) as compared to FM. However, two-way ANOVA revealed no impact of diet, strain, or their interaction on weight gain and FCR at any sampling point up to 24 weeks. Histology analyses of distal intestine after 6 and 12 weeks of dietary exposure showed significant differences ($p < 0.05$) in terms of villi length and width at all dietary treatments, and inflammation was reduced in the SBM-Gln group in both strains (Fig. 1). Distal intestine gene expression analyses are underway for the inflammatory markers: TNF-α, NF-ϰB, IL-8, IL-10; barrier function markers: MLCK, ZO-1, occludin; as well as brush border transporters: FABP2, and SLC1A5. Distal intestine samples were also collected to evaluate glutathione peroxidase enzymatic activity and for glutamate quantification.

(Continued on next page)

Strain	Diet	IW (g)	WG (g)			
	Week	0	6	12	18	24
A	FM	25.0 ± 0.04	72.9 ± 1.7	156.1 ± 7.9	254.9^{a} ± 12.6	487.7 ± 27.2
	SBM	25.1 ± 0.2	69.5 ± 6.0	172.7 ± 25.6	356.0^{b} ± 26.8	583.6 ± 38.7
	SBM-Gln	24.3 ± 1.2	65.4 ± 1.9	172.6 ± 12.1	328.0^{ab} ± 18.4	526.5 ± 27.5
B	FM	23.2 ± 0.8	72.4 ± 3.2	165.7 ± 1.6	307.7 ± 13.9	531.0 ± 42.5
	SBM	24.1 ± 0.7	65.6 ± 1.0	154.5 ± 13.0	292.4 ± 22.7	508.3 ± 32.1
	SBM-Gln	23.9 ± 1.4	65.9 ±2.6	177.4 ± 16.0	345.3 ± 32.1	511.4 ± 35.0

Table 1. Values are presented as mean ± S.E. for initial weight and weight gain (g) at 0, 6, 12, 18 and 24 weeks of the 30-week feeding trial. Different letters indicate one-way ANOVA significant statistical difference ($p < 0.05$). IW = initial weight; WG = weight gain; S.E. = standard error.

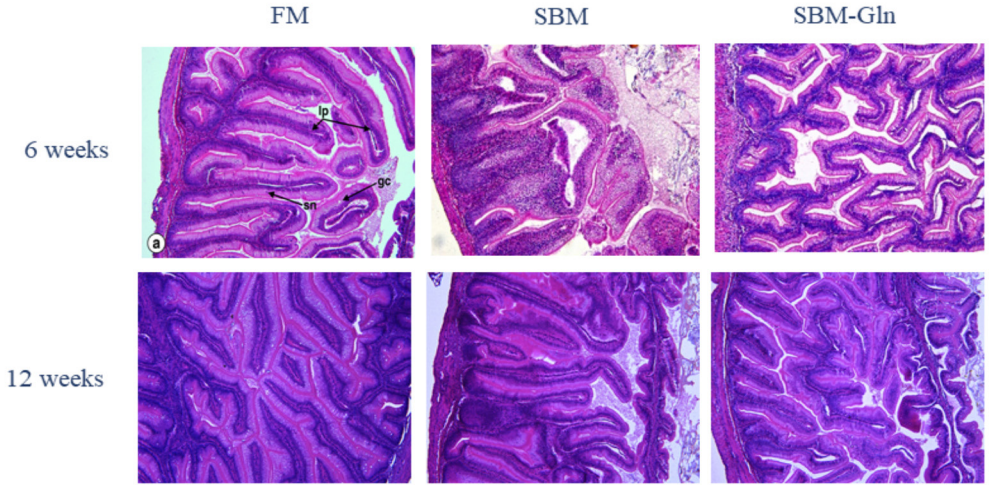


Fig. 1. Histopathology of distal intestine of trout A strain after 6 and 12 weeks. 200X magnification.

Conclusion

Growth performance in terms of weight gain showed no differences across treatments and strains; moreover, weight gain was not negatively impacted in fish fed the SBM diet as was expected, with strain A showing slightly higher values in this dietary group overall throughout the trial and especially at 18 weeks. Nonetheless, histology results show morphological alterations of the distal intestine such as widened and shorter villi in both strains. Interestingly, it apppears that SBMIE was lessed at 12 weeks in both strains, possibly indicating the fish are adapting to dietary SBM. Final growth performance results from 30-week sampling point as well as gene expression analyses and glutamate quantification in the distal intestine will help elucidate the molecular mechanism underlying Gln driven SBMIE mitigation.

HETEROTROPHIC VS AUTOTROPHIC PRODUCTION OF MICROALGAE. BRINGING SOME LIGHT INTO THE EVERLASTING COST CONTROVERSY

Jesús Ruiz [1]*, Rene H. Wijffels [2,3], Manuel Dominguez [1], Maria J. Barbosa [2]

[1]Algades – Alga Development, Engineering and Services, S.L.; El Puerto de Santa María, Spain
[2]Wageningen University, Bioprocess Engineering, AlgaePARC; Wageningen, The Netherlands
[3]Nord University, faculty of Biosciences and Aquaculture; Bodø, Norway.
E-mail: jesus.ruiz@algades.com

Introduction
Heterotrophic or autotrophic? This is the continuous question industry faces when microalgae production is the endeavor. Without a doubt, production cost may be among the reasons to come to a decision. Surprisingly, still today specialists have not reached a consensus on which is the most economical option.

To shed some light on the issue, the current work thoroughly analyses costs for heterotrophic and autotrophic cultivation of microalgae at industrial scale. Our work follows a similar procedure as the one we previously developed to explore costs of autotrophic production (Ruiz et al., 2016). These cost studies on heterotrophic and autotrophic production follow analogous methodologies, and they both share some of the fundamental points, such as location or biomass capacity. These facts indicate that a reliable comparison can be performed.

Methods
We developed a model to perform a Techno-economic assessment of microalgae production in fermenters under heterotrophic conditions. Biomass capacity of the projected facility is 6.094 ton·year^{-1} measured as dry weight. It is identical to the autotrophic production in our Article Towards industrial products from microalgae for Flat Panels in Spain (Ruiz et al., 2016), which enables comparisons.

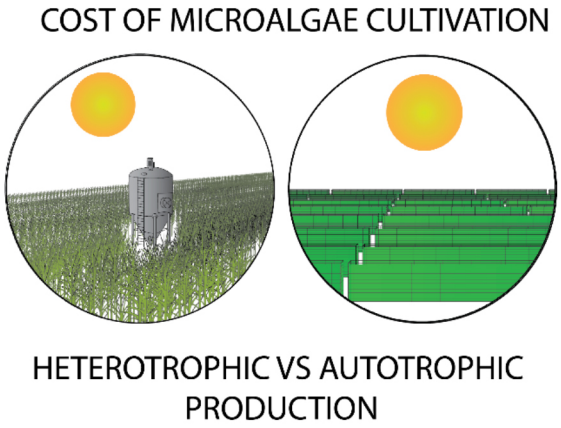
Studied species: Chlorella sp. is used as cell factory. This is the most studied heterotrophic microalga and its performance exceeds most of other microalgae under these conditions. The cultivation is performed in batch process with a biomass productivity of 4.81 g·l^{-1}·d^{-1} (this is an average from 23 tests on Chlorella sp. in glucose, which ranged between 31.86 and 0.15 g·l^{-1}·d^{-1}).

Results

After comparing the value from our heterotrophic base case (4.00 €·kg^{-1}) and the base case of autotrophic production in flat panels (3.50 €·kg^{-1} (Ruiz et al., 2016)) we can sustain that heterotrophic carbon nutrition of microalgae is costlier (Figure 1). However, it depends on the production system, as our previous study also reveals that photosynthetic production in tubular photobioreactors or open ponds can reach a cost above 5.20 €·kg-1 (Ruiz et al., 2016). Thus, although we can claim that autotrophic culture in flat panels would be cheaper than heterotrophic production, the latter is not always the most expensive option.

Conclusions
The results show a comparable cost for heterotrophic and autotrophic production of microalgae at industrial scale. Nevertheless, although large-scale cultivation in fermenters and open ponds came true, existing facilities based on photobioreactors are still relatively small. Microalgae need a leap forward in production scale to become a competitive novel feedstock for biobased products. A facility producing thousands of tons of biomass per year could benefit from the economy of scale, overcoming most cost restraints.

(Continued on next page)



Results

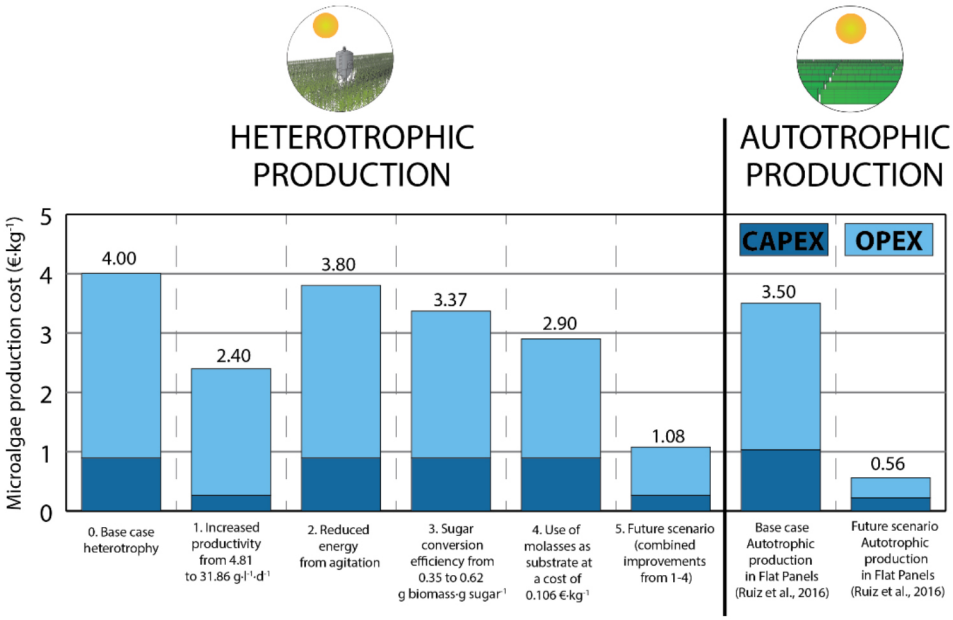


Figure 1: Projected biomass production costs (cultivation and harvesting) for current scenarios and the future projection for south of Spain. Costs as the sum of CAPEX and OPEX.

References

Ruiz, J., Olivieri, G., de Vree, J., Bosma, R., Willems, P., Reith, J.H., Eppink, M.H.M., Kleinegris, D.M.M., Wijffels, R.H., Barbosa, M.J., 2016. Towards industrial products from microalgae. Energy Environ. Sci. 24, 405–413. https://doi.org/10.1039/C6EE01493C

DIETS SUPPLEMEnTED WITH BILE SALTS REDUCED BODY ADIPOSITY AND IMPROVED HEALTH CONDITION IN JUVENILE GILTHEAD SEABREAM

Alberto Ruiz Hernández*, Karl B. Andree, Dolors Furones, Enric Gisbert [1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
* Email: alberto.ruiz@irta.cat

Introduction

Commercial aquafeeds are usually high in lipids, since this ingredient is the main source of dietary energy and essential fatty acids for fish. However, an excess of fat can generate an increase in the amount of lipid bodies and adipose tissue, which has a negative impact on animal welfare, as well as in terms of the fillet quality (Salmerón et al., 2018). Since bile salts (BS) act as lipid emulsifiers and contribute to the digestion and absorption of fat (Swann et al., 2011), they have been tested as additives in fish diets, showing an improvement in growth performance, in efficiency of feed utilization and in the oxidative state (Ding et al., 2020; Mansour et al., 2020). Their supplementation in diets has also contributed to elucidate their regulatory role on lipid digestibility, lipase activity, adiposity and, cholesterol and bile acid (BA) levels in fish (Gu, Bai & Kortner, 2017). Likewise, in a nutritional trial conducted by Xiong et al. (2018), grass carp were fed diets supplemented with different BS and, as a result, it was observed that, especially the ones containing primary BS (i. e., sodium taurocholate), could modify the BA profiles in the gall bladder and change gut microbiome. These variations in the BA profile were attributed to a change in the intestinal epithelium permeability induced by gut microbiota, which would lead to a higher reabsorption (Xiong et al., 2018). Previous studies have revealed that BA can act as regulators of gut microbiome community structure, but also that the intestinal microbiota would be responsible for the regulation of the BA pool size and composition through mechanisms such as BS deconjugation (Ridlon et al., 2014). Based on this information, the objective of this work was to test whether by adding bile salts in diets with a high saturated fats content it was possible to improve the health and quality of gilthead sea bream (*Sparus aurata*), and its regulating effect on growth performance, feed utilization, oxidative stress, adiposity, fatty acids profile, and gut microbiome.

Materials and Methods

A 90-day feeding trial was carried out in which juvenile *S. aurata* (initial body weight: 44.05 ± 4.08 g) were fed with three experimental isoproteic (44 %), isolipidic (22.6 %) and isoenergetic (21.4 MJ/kg) diets: a "control", with a high content of saturated fats, and two others with a composition similar to the previous one but supplemented with a blend of BS (sodium taurocholate and deoxycholic acid) at different levels of inclusion (0.06 % and 0.12 %). During the trial, monthly sampling to monitor fish growth in terms of weight (g) and standard length (SL) was performed. At the end, the specific growth rate (SGR) and the feed conversion ratio (FCR) were calculated, as well as the hepatosomatic index and the perivisceral fat index. Oxidative stress in liver was measured through its total antioxidant capacity (TAC), lipid peroxidation (LPO) (through the concentration of MDA), and enzymatic activity of catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD). Microscopic observation of histological sections of the liver and foregut allowed to evaluate the overall condition and accumulation of fat in these tissues. In addition, the *villi* height, thickness of the musculature, height of enterocytes and density of goblet cells in the intestinal mucosa was evaluated, as well as its level of inflammation. The serum levels of cholesterol, triglycerides, alkaline phosphatase, GTP transaminase, GOT transaminase, albumin, globulins, and total proteins of the fish were measured. Fatty acid profiles of muscle and liver were analysed using gas chromatography. From DNA extracted from scraping the interior walls of the intestine, Next-Generation Sequencing (Illumina - MiSeq platform) using specific primers for the V3-V4 hypervariable regions of 16S rRNA genes was performed and RDP database was used for classifying the OTUs, identifying the composition of the gut microbial communities.

Results and Discussion

At the end of the nutritional trial, growth results showed that fish fed the diets with a BS inclusion of 0.06 % and 0.12 % were respectively 2.4 % and 2.1 % heavier than those fed the control diet ($P < 0.05$). In addition, a trend towards an increase in SGR was observed in both groups with respect to the control, obtaining significant differences in those fed the diet with 0.06 % BS ($P < 0.05$). This is in accordance with the results obtained by Ding et al. (2020). Furthermore, the inclusion of BS in diets reduced the level of perivisceral fat. In particular, PVI was 3.01 ± 0.28 % in the control group and 2.58 ± 0.19 %

(Continued on next page)

and 2.67 ± 0.19 % in fish fed the diets with 0.06 and 0.12 % BS, respectively ($P < 0.05$). On the other hand, the addition of BS in the diet did not affect the hepatosomatic index and FCR values ($P > 0.05$). Although *a priori* the antioxidant status of the liver did not vary between the groups, a lower CAT activity was detected in the diet with an inclusion of 0.06 % of BS (63.76 ± 15.87 nmol min^{-1} mg $protein^{-1}$) with respect to the control group (96.88 ± 5.62 nmol min^{-1} mg $protein^{-1}$) ($P < 0.05$). This could be part of a response to less oxidative stress in fish fed these diets. Histological results showed lower levels of hepatic and intestinal fat accumulation in fish fed with the 0.06 % BS diet. These results are in accordance with data from the perivisceral fat index; both results showing that the addition of BS reduces adiposity, possibly thanks to their role as fat emulsifier and in lipid metabolism (Swann et al., 2011). There were no differences in the rest of the histological and serum parameters, which remained within the range of the normal values for the species, indicating an optimal health condition. In conclusion, the addition of BS in diets with high saturated fats content is capable of modulating growth efficiency, accumulation of perivisceral, hepatic and intestinal fat and, in part, oxidative stress in *Sparus aurata*, which would lead to an improvement in the health and quality of the fish. Results of fatty acid profiles, BA profiles and gut microbiota will also be discussed.

References

Ding, T., Xu, N., Liu, Y., Du, J., Xiang, X., Xu, D., ... & Ai, Q. (2020). Effect of dietary bile acid (BA) on the growth performance, body composition, antioxidant responses and expression of lipid metabolism-related genes of juvenile large yellow croaker (*Larimichthys crocea*) fed high-lipid diets. *Aquaculture, 518*, 734768.

Gu, M., Bai, N., & Kortner, T. M. (2017). Taurocholate supplementation attenuates the changes in growth performance, feed utilization, lipid digestion, liver abnormality and sterol metabolism in turbot (*Scophthalmus maximus*) fed high level of plant protein. *Aquaculture*, *468*, 597-604.

Mansour, A. T., El-Feky, M. M., El-Beltagi, H. S., & Sallam, A. E. (2020). Synergism of Dietary Co-Supplementation with Lutein and Bile Salts Improved the Growth Performance, Carotenoid Content, Antioxidant Capacity, Lipid Metabolism, and Lipase Activity of the Marbled Spinefoot Rabbitfish, *Siganus rivulatus*. *Animals*, *10*(9), 1643.

Ridlon, J. M., Kang, D. J., Hylemon, P. B., & Bajaj, J. S. (2014). Bile acids and the gut microbiome. *Current opinion in gastroenterology*, *30*(3), 332.

Salmerón, C (2018). Adipogenesis in fish. *Journal of Experimental Biology*, *221*(Suppl_1), jeb161588.

Swann, J. R., Want, E. J., Geier, F. M., Spagou, K., Wilson, I. D., Sidaway, J. E., ... & Holmes, E. (2011). Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proceedings of the National Academy of Sciences*, *108*(Suppl_1), 4523-4530.

Xiong, F., Wu, S. G., Zhang, J., Jakovlić, I., Li, W. X., Zou, H., ... & Wang, G. T. (2018). Dietary bile salt types influence the composition of biliary bile acids and gut microbiota in grass carp. *Frontiers in microbiology*, 9, 2209

Acknowledgments: This project has been funded by the Ministry of Science, Innovation and Universities (with reference RTI2018-095653).

EFFECT OF AN ESSENTIAL OIL DERIVED FROM *Capsicum sp.* AS A DIETARY SUPPLEMENT ON BODY ADIPOSITY, HEALTH AND QUALITY OF GILTHEAD SEABREAM

Alberto Ruiz Hernández*, Karl B. Andree, Dolors Furones, Enric Gisbert [1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
* Email: alberto.ruiz@irta.cat

Introduction

In aquaculture, lipids are frequently used as the main source of energy and essential fatty acids for fish. However, high-fat diets can generate an increase in the amount of lipid bodies and adipose tissue, which generates a negative impact on animal welfare and on production due to its effect on the half-life and organoleptic properties of the product (Salmerón et al., 2018). In this context, according to recent studies feed additives can modulate fish body adiposity (Zhou et al., 2018), being essential oils (EOs) a good candidate, given the existing antecedents on the modulating role of some of them in lipid metabolism (Firmino et al., 2021). Some studies have suggested that these compounds can influence the kind and amount of secretion produced by the intestinal mucosa, which allows an improvement in digestion and absorption of some nutrients, such as lipids, by the microbiota (Sutili et al., 2018). The use of EOs as animal feed additives is booming due to its innumerable properties, including their capacity to promote growth performance, to modulate the gut microbiota, to inhibit bacterial resistance and virulence factors and to induce the immune response, as well as the anti-inflammatory and antioxidant activities (Sutili et al., 2018).

Capsicum is as a genus of angiosperm plants belonging to the Solanaceae family, native to America, cultivated worldwide for their chili pepper fruit, whose natural extractives, oleoresins and EOs are generally recognized as safe (GRAS) for their intended use in food (Rogers et al., 2018). In mammals, *Capsicum* has been shown to increase thermogenesis and whole-body energy expenditure, to boost fat oxidation, to decrease triglyceride storage and to reduce body fat, to a great extent thanks to the action of capsaicinoids, which also have been shown to prevent inflammation and associated diseases, like cancer and heart disease (Varghese et al., 2017). However, there are not many studies using *Capsicum* as aquafeed supplement (Abdalla and Agouz, 2014; Parrino et al., 2020), so its effects on fish health and production remain still partly unknown. Based on the above, the objective of this work is to test whether by adding an EO based on *Capsicum sp*. in diets with a high saturated fats content it is possible to regulate the adiposity of gilthead sea bream (*Sparus aurata*) and to test its consequences in terms of health and quality.

Materials and Methods

Juvenile *S. aurata* (initial body weight: 44.07 ± 4.24 g) were fed during 90 days with three isoproteic (44 %), isolipidic (22.6 %) and isoenergetic (21.4 MJ/kg) diets with a high content of saturated fats and increasing concentrations of a based-on *Capsicum sp*. essential oil: 0 % ("control"), 0.1 and 0.15 %. During the trial, fish growth in terms of body weight (BW, g) and standard length (SL, cm) was monthly monitored. At the end, the specific growth rate (SGR), the feed conversion ratio (FCR), the hepatosomatic index (HSI) and the perivisceral fat index (PVFI) were measured. To determine oxidative stress in liver, the activity of glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were measured, as well as lipid peroxidation (LPO) (through concentration of MDA) and total antioxidant capacity (TAC). Hepatic metabolism was also monitored through the enzymatic activity of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) in liver. Histological sections of the liver and gut allowed to evaluate fat accumulation, and other physiology parameters in gut (*villi* height, thickness of the musculature, density of goblet cells, height of enterocytes and presence or absence of inflammation). Furthermore, total proteins, globulins, albumin, triglycerides, cholesterol, GOT, GPT and alkaline phosphatase in serum were measured.

(Continued on next page)

Results and Discussion

Fish fed the diets with an inclusion of 0.1 and 0.15 % of based on *Capsicum* EO were respectively 2.78 % and 3.24 % heavier than those fed the control diet ($P < 0.05$). In addition, an increase in SGR with respect to the control was observed in both groups fed the diets supplemented with EO ($P < 0.05$), which is in accordance with the results obtained by Parrino et al. (2020) in rainbow trout. Although addition of *Capsicum* EO did not affect the FCR and HSI, PVFI decreased from 3.01 ± 0.28 % (a) in the control group to 2.32 ± 0.37 % ($P < 0.05$; b) and 2.68 ± 0.28 % ($P > 0.05$; ab) in fish fed the diets with 0.1 and 0.15 % EO, respectively. Furthermore, in histological observation, less fat accumulation was appreciated in the groups fed with the EO diets than in the control. This would be indicative that the EO derived from *Capsicum sp*. can reduce the adiposity of gilthead sea bream. Having into account the fact that a reduction of hepatic fat accumulation was only observed in the diet with the lower inclusion of EO, together with the PVFI results, everything seems to indicate that the most effective dose of *Capsicum* EO to meet the purpose of reducing body fat in *S. aurata* would be 0.1%. No differences were observed in the rest of measured parameters, indicating that the EO had no harmful effect for the health and quality of the fish, although it has been proven in freshwater fish that, under certain conditions, dietary supplementation with *Capsicum* can ameliorate blood parameters (Parrino et al., 2020) and even their reproductive performance (Abdalla and Agouz, 2014).

References

Abdalla, A., & Agouz, H. M. (2014). Effect of hot pepper as feed additive on reproductive and productive performance of Nile tilapia (*Oreochromis niloticus*). *Abbassa Int. J. Aqua., 7*(1), 196-209.

Firmino, J. P., Galindo-Villegas, J., Reyes-López, F. E., & Gisbert, E. (2021). Phytogenic bioactive compounds shape fish mucosal immunity. *Frontiers in Immunology*, *12*, 695973.

Parrino, V., Kesbiç, O. S., Acar, Ü., & Fazio, F. (2020). Hot pepper (Capsicum sp.) oil and its effects on growth performance and blood parameters in rainbow trout (Oncorhynchus mykiss). *Natural product research*, *34*(22), 3226-3230.

Rogers, J., Urbina, S. L., Taylor, L. W., Wilborn, C. D., Purpura, M., Jäger, R., & Juturu, V. (2018). Capsaicinoids supplementation decreases percent body fat and fat mass: adjustment using covariates in a post hoc analysis. *BMC obesity*, *5*(1), 1-10.

Salmerón, C (2018). Adipogenesis in fish. *Journal of Experimental Biology*, *221*(Suppl_1), jeb161588.

Sutili, F. J., Gatlin III, D. M., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture*, *10*(3), 716-726.

Varghese, S., Kubatka, P., Rodrigo, L., Gazdikova, K., Caprnda, M., Fedotova, J., ... & Büsselberg, D. (2017). Chili pepper as a body weight-loss food. *International journal of food sciences and nutrition*, *68*(4), 392-401.

Zhou, J. S., Chen, H. J., Ji, H., Shi, X. C., Li, X. X., Chen, L. Q., ... & Yu, H. B. (2018). Effect of dietary bile acids on growth, body composition, lipid metabolism and microbiota in grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutrition, 24*(2), 802-813.

Acknowledgments:

This project has been funded by the Ministry of Science, Innovation and Universities (with reference RTI2018-095653).

PLANT-BASED DIETS WITH LOW METHIONINE SUPPLEMENTATION LEAD TO A REDUCTION ON MEAGRE, *Argyrosomus regius,* JUVENILES PERFORMANCE AND TO AN INCREASE IN MUSCLE FIBRE RECRUITMENT

Margarida Saavedra[1]*, Teresa Gama Pereira[1], Marisa Barata[2], Cláudia Aragão[3], Bárbara Requeijo[2], Luís Conceição[4] and Pedro Pousão-Ferreira[2]

[1]Portuguese Institute for the Sea and Atmosphere**,** Rua Alfredo Magalhães Ramalho, nº6, 1495-006 Lisbon, Portugal
[2]Aquaculture Research Station of IPMA (EPPO), Av. 5 de Outubro, 8700-305 Olhão, Portugal
[3]Centre of Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, building 7, 8005-139 Faro, Portugal
[4]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
*margarida.saavedra@gmail.com

Reduction of fish meal is an important goal of the aquaculture industry but its replacement by plant-proteins have often a negative impact on the content of amino acids such as methionine and taurine. The aim of this study was to evaluate meagre juvenile response to different levels of dietary methionine and taurine. To achieve this, three diets were formulated: D1 diet had low methionine supplementation and a deficiency of this AA estimated in 25 % and a supplement of 1 % taurine; the D2 and D3 diets had a methionine content 18 % above the estimated requirement and were supplemented with 1 % and 2 % taurine, respectively. The diets were tested in juvenile meagre (initial weight: 12.0 ± 1.6 g) for eight weeks. Fish were held in 1500 L fibre tanks at a density of 0.08 fish /L. Survival varied between 95 and 100 %. The results showed that meagre fed the D1 diet had lower specific growth rate (2.2 to 2.5), lower feed efficiency (0.9 to 1.2) and higher FCR (1.1 to 0.8) as well as a lower activity of the ALAT enzyme. Furthermore, a higher recruitment of muscle fibres was observed (46 compared to 36 %) as well as a higher fibre density (1019 compared to 870 fibres/mm^2). In conclusion, this study shows that methionine supplementation in plant-based formulations is crucial to overcome the reduction of fish performance and growth associated to fish meal replacement of meagre diets. Moreover, taurine supplementation was not able to mitigate the effects of methionine deficiency.

Aknowledgements: This work was supported by Diversiaqua, Mar 2020 (16-02-01-FEAM-66). CA was supported by the Portuguese Foundation for Science and Technology (FCT): UID/Multi/04326/2019 and DL57/2016/CP1361/CT0033.

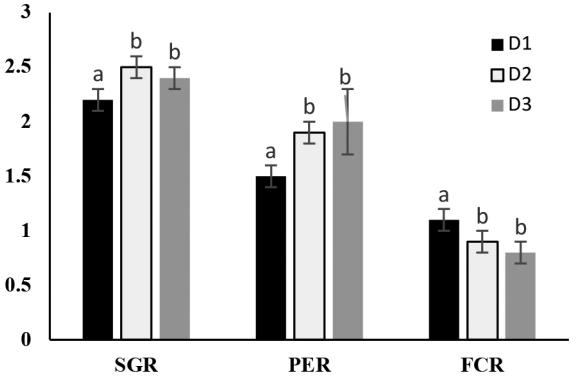


Fig. 1. Meagre juvenile specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR) at the end of the experimental trial which tested three diets: D1- diet with 0.2% methionine and 1 % taurine supplementation; D2- diet with 0.6 % methionine and 1 % taurine supplementation; D3- diet with 0.6 % methionine and 2 % taurine supplementation. Values are mean and standard deviation. Different letters represent significant differences for $p<0.05$.

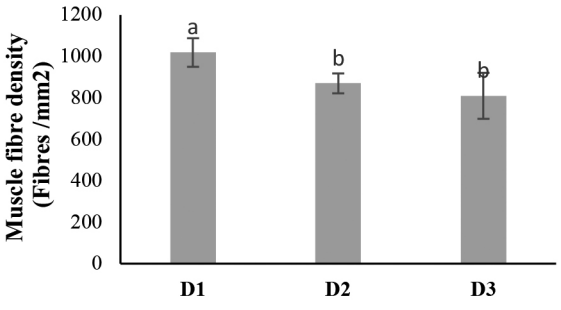


Fig. 2. Muscle fibre density of meagre juveniles fed a diet with 0.2 % methionine and 1 % taurine supplementation (D1); a diet with 0.6 % methionine and 1 % taurine supplementation (D2); a diet with 0.6 % methionine and 2 % taurine supplementation (D3). Values are mean and standard deviation. Different letters represent significant differences for $p<0.05$.

EFFECT OF PARTIAL FISH MEAL REPLACEMENT WITH INSECT MEAL ON MEAGRE, *Argyrosomus regius*, JUVENILES GROWTH, MUSCLE CELLULARITY, PROTEOLYTIC AND DIGESTIVE ENZYME ACTIVITY AND INTESTINE HISTOMORPHOLOGY

Margarida Saavedra[1]*, Marisa Barata[2], Ana Catarina Matias[2], Ana Couto[3], Laura Ribeiro[2], Ahmed Md. Salem[4], Teresa Gama Pereira[1], Margarida Gamboa[2], Cátia Marques[2], Florbela Soares[2], Jorge Dias[5] and Pedro Pousão-Ferreira[2]

[1]Portuguese Institute for the Sea and Atmosphere, Rua Alfredo Magalhães Ramalho, nº6, 1495-006 Lisbon, Portugal
[2]Aquaculture Research Station of IPMA (EPPO), Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
[3]CIIMAR, Av. General Norton de Matos, S/N, 289; 4450-208- Matosinhos, Portugal
[4]National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt
[5]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
*margarida.saavedra@gmail.com

There is a continuous effort to find an alternative to fish meal (FM), not only from a sustainability perspective but, as well, because of the high cost it represents. Considerable research has been carried using plant-feedstuffs, but the results have been far from ideal. In recent years, insect meal (IM) has been tested as an alternative ingredient to partially replace FM. IM shows several advantages, such as its antimicrobial properties and its more sustainable and economic production. In this experimental trial, three diets were tested in meagre juveniles of approximately 10 g. In the first treatment, FM was replaced by 20 % of IM (INS20), in the second treatment, FM was replaced by 10 % (INS10) and the control (C) treatment had no IM. The results showed that the inclusion of 20 % IM had a negative impact on fish final weight, specific growth rate and food conversion ratio (Fig. 1). However, these differences in growth were not reflected in changes in the mean fibre area and density or in several markers of protein degradation. In fact, the inclusion of 10% IM in the diets, rather than 20%, seems to induce a higher proteolytic activity in the hepatic and muscle tissues of meagre juveniles. The overall digestive enzyme activity in meagre was not significantly affected by the level of incorporation of the IM in the diets. Nevertheless, differences were found for the total activity of aminopeptidase (lower in INS20) and for alkaline phosphatase intestinal maturation index (higher in the control group). In relation to the intestine histomorphology, fish fed the C and INS10 diet showed changes in the enterocytes with hyper vacuolization and nucleus misplacement whereas fish fed the INS20 diet showed a more regular and normal sized vacuolization.

Acknowledgements: This work was supported by DIVERSIAQUAII (Mar2020-P02M01-0656P).

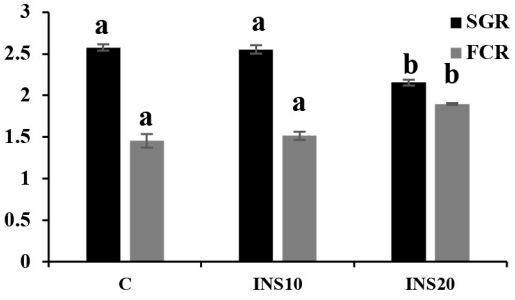


Fig. 1 Specific growth rate (SGR) and food conversion ratio (FCR) of meagre fed a diet with 10 % insect meal (INS10), 20 % insect meal (20%) and a control diet (C). Values are mean and standard deviation. Significant letters represent significant differences for $p \leq 0.05$.

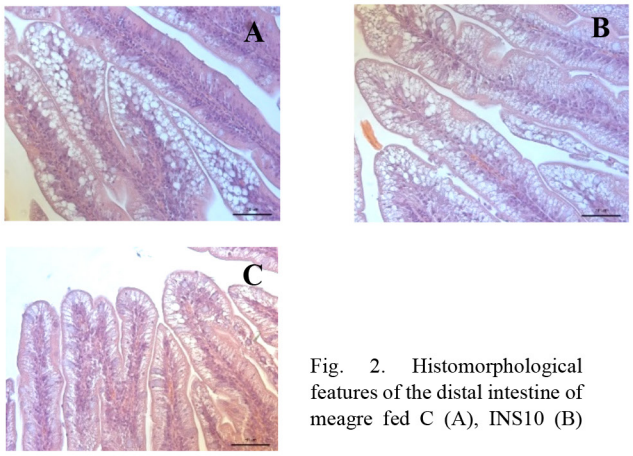


Fig. 2. Histomorphological features of the distal intestine of meagre fed C (A), INS10 (B)

PARENTAL CONTRIBUTIONS AND GROWTH RATE IN AN ATLANTIC X MEDITERRANEAN CROSS OF THE CLAM *Ruditapes decussatus*

C. Saavedra[1*], I. Gairín[2], D. Cordero[1], I. Ibarrola[3], I. Urrutxurtu[3], E. Navarro[3], M. B. Urrutia[3]

[1] Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes (Castellón), Spain
[2] IRTA , Sant Carles de la Ràpita (Tarragona), Spain
[3] Departamento de Genética, Antropología Física y Fisiología Animal, Universidad del País Vasco, Leioa (Vizcaya), Spain
*Email: saavedra@iats.csic.es

Introduction and objectives

The interest of the native European clam *Ruditapes decussatus* for aquaculture is increasing (da Costa et al., 2020). Spat of this species is usually utilized to restocking harvested natural populations (Borrell et al., 2014). A detailed knowledge of the performance in hatcheries and an adequate management of the species' genetic diversity are essential to develop a science-based management of clam fisheries and aquaculture. Adequate levels of genetic diversity in the hatchery stock depends on the contribution of a large number of parents, but this often is not the case in bivalves (Borrell et al., 2014). Moreover, *R. decussatus* is characterized by the existence of three races, which could be maintained by endogenous barriers to gene flow (Cordero et al, 2014; Arias-Pérez et al., 2016). Applying microsatellites as genetic markers to the progeny of a cross between clams of Atlantic and West Mediterranean races, we have studied three topics which are important for clam production and conservation of genetic resources: 1) male and female contributions to offspring after conditioning and spawning induction in the hatchery; 2) testing for genetic barriers between the two races; and 3) growth rate differences among families and populations.

Materials and Methods

Sixty wild clams were sampled in Cambados (NW Spain, Atlantic Ocean), and Mar Menor (SE Spain, Mediterranean Sea) in spring 2016. Hatchery methods were as described (Markaide et al., 2021). Equal numbers of good-quality eggs and sperm from four males and four females from each locality were chosen for performing mass fertilization at the IRTA facilities. In the winter of 2017, a subset of 250 offspring was transferred to the IATS facilities, where they were individually tagged and measured for shell dimensions. Three months later (June 2017) clams were again measured and then sacrificed. Individual growth rate was assessed as $GR = (X_f - X_0)/ X_f$, where X_0 stands for initial shell length or height, and X_f stands for final shell length or height. DNA was extracted from a piece of gill from parents and offspring by standard minicolumns. The genotypes of the parents and each offspring were scored for 6 microsatellites (Borrell et al., 2014). Parentage assignment was carried out using the software Cervus 3.0 (Kalinowski et al., 2007).

Results & Discussion (I) : parental contributions and race genetic barriers

Parentage analysis allowed ascription of 249 offspring to their parents at 80% confidence, including 134 at 95% confidence. All parents contributed to the offspring but at very different proportions. Females mothered an average of 31 ± 10 offspring (mean ± S.E.)(range 4-90), and three females alone mothered 78% of all progeny. Males fathered an average of 31 ± 7 offspring (range 7-72), and the two most fertile males fathered 51% of the progeny. These results indicate that individual reproductive success of *R. decussatus* is low in the hatchery for both males and females, and a very large broodstock would be necessary to keep the genetic variability at the level of the wild stock.

Results & Discussion (II) : genetic barriers

Atlantic females mothered 54% of the progeny, and Atlantic males fathered 42%. The proportion of offspring of hybrid (Atlantic x Mediterranean) origin was 51%. These results are indicative of equal fertilization and survival rates of pure and hybrid crosses (P=0.90, two-tailed Fisher exact test), and demonstrate an absence of important reproductive barriers between the two races.

Results & Discussion (III): growth rate.

Shell length and shell height varied widely among sibs, as is usual in bivalves (Markaide et al., 2021). Growth rate (GR) could be measured in 170 animals. GR in length varied between 0.00 and 0.47 among individuals, and GR in height varied between 0.00 and 0.36. In both cases GR was normally distributed. Differences in growth rate across families were nonsignificant (ANOVA, $P > 0.05$). Average GR of hybrid clams was slightly higher than that of pure-bred clams for both shell length and shell height, but variances were very large and, therefore, the observed differences were non-significant (ANOVA, $P< 0.05$). These results suggest the occurrence of heterosis for growth in interracial crosses of *R. decussatus*.

References

Arias-Pérez, A., Cordero, D., Borrell, Y., Sánchez, J.A., Blanco, G., Freire, R., Insua, A., Saavedra, C. 2016. Assessing the geographic scale of genetic population management with microsatellites and introns in the clam *Ruditapes decussatus*. Ecology and Evolution, 6: 3380-3404.

Borrell, Y.J., Arias-Pérez, A., Freire, R., Valdés, A., Sánchez, J.A., Méndez, J., Martínez, D., López, J., Carleos, C., Blanco, G., Insua A.M. 2014. Microsatellites and multiplex PCRs for assessing aquaculture practices of the grooved carpet shell *Ruditapes decussatus* in Spain. Aquaculture, 426-427: 49-59.

Cordero, D., Peña, J.B., Saavedra, C. 2014. Phylogeographic analysis of introns and mitochondrial DNA in the clam *Ruditapes decussatus* uncovers the effects of Pleistocene glaciations and endogenous barriers to gene flow. Molecular Phylogenetics and Evolution, 71: 274–287.

da Costa, F., Cerviño-Otero, A., Iglesias, Ó., Cruz, A., Guévélou, E. 2020. Hatchery culture of European clam species (family Veneridae). Aquaculture International, 28: 1675-1708.

Kalinowski, S.T., Taper, M.L., Marshall, T.C.2007.Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16: 1099-1106.

Markaide, P., Gairín, I., Cordero, D., Ibarrola, I., Saavedra, C.2021.No hybridization and marked interspecific differences in individual growth rate in mixed cultures of Manila clam (*Ruditapes philippinarum*) and grooved carpet-shell clam (*R. decussatus*). Aquaculture, 541, art. no. 736824.

Acknowledgements

Thanks to Dr. José Carlos Mariño, the Cofradía San Antonio (Cambados) and Dr. Benjamín García (IMIDA, Murcia) for providing the clam broodstock. This work was financed through grants AGL2017-87745-C2-1-R and AGL2017-87745-C2-2-R.

FISH TRANQUILIZATION, ANESTHESIA AND EUTHANASIA IN AQUACULTURE

Nick Saint-Erne, DVM, Certified Aquatic Veterinarian

World Aquatic Veterinary Medical Association - Phoenix, Arizona USA
Email: nsainterne@gmail.com

Introduction

Tranquilization aids in shipping live fish and in handling fish during physical examination, for biopsy sampling or for breeding purposes such as egg and milt stripping during artificial spawning. Anesthesia and analgesia are required for surgical or invasive procedures. Surgery can be performed on anesthetized fish to implant transponders or for research purposes, and to repair wounds, remove skin and fin tumors, or to remove abdominal masses. Sometimes euthanasia is needed to perform diagnostic testing and necropsies, to end the suffering of a sick or injured fish, or for research or other purposes. Each of these techniques can be accomplished with fish by adding anesthetic medications to the water, and sometimes by injection or oral administration of anesthetics. Food fish have specific limitations to medications that can be used with them, and withdrawal times for approved medications must be observed.

Tranquilization and Anesthesia Techniques

Many chemicals have been used to induce tranquilization (sedation) or anesthesia (unconsciousness) in fish. All have some element of risk, but when used carefully they have successfully induced sedation or anesthesia. Anesthetic agents used in lower doses produce tranquilization, and at higher doses they are used for anesthesia purposes. Care must be taken not to overdose the fish, or leave them anesthetized too deeply for too long of time. It is recommended to start with a lower dose and add more as needed if using a new drug or working with an unfamiliar species of fish. Monitor the heart rate, blood oxygen concentration, and operculum (gill cover) motion during anesthesia to ensure fish is not too deeply anesthetized.

Most fish anesthetics are added to clean, well-oxygenated water in a suitable glass or plastic container. The water is thoroughly mixed to ensure all the chemical is dissolved and dispersed evenly. The anesthetic solution should be the same temperature and pH as the water from which the fish was taken. Use a thermometer to monitor the water temperature during anesthesia, and if an oxygen meter is available, also monitor the dissolved oxygen concentration of the anesthetic solution. An aquarium air pump with an air stone should be placed into the water to circulate it to maintain adequate oxygen level, especially with a large fish. The water should be tested to ensure all the water quality parameters are in the correct range for the fish species.

A pulse oximeter can be clipped onto the caudal fin of large fish, near the tail base, to monitor the pulse and blood oxygen concentration. Electrocardiogram (ECG) monitors can also be used in large fish by attaching the monitor clips to hypodermic needles placed into the muscles on either side of the body by the pectoral fins. This will create a 2-lead ECG that will show the heart rate of the fish. It is important to get a baseline heart rate and monitor for slowing, rather than to see if the heart stops, as the heart in fish can continue to beat after the fish is dead!

When placed into the container with the anesthetic in the water, the fish will gradually begin to lie on its side and the respiratory rate will slow as the chemical induces anesthesia. In some cases, there may be an excitatory stage, so the anesthetic chamber may need to be covered to prevent fish from jumping out. After the fish is anesthetized in the anesthetic bath, it can be removed from the water for short-term examination or diagnostic procedures. If the fish is removed for longer procedures, anesthetic solution can be dripped across the gills through an IV bag and drip line, by hand with a syringe, or with a recirculating water pump or aquarium filter powerhead. Have oxygenated fresh water on hand to syringe across the gills if the plane of anesthesia becomes too deep. Keep the body moist if out of the water for examination or surgery. Use ophthalmic ointment on the eyes to keep them from drying. Monitor the respiration rate (operculum movements) to assess the depth of anesthesia.

(Continued on next page)

Stages of Anesthesia in Fishes

Stage	Plane	Description	Signs
0	0	Normal	Swimming actively, equilibrium normal
I	1	Light sedation	Reduced motion, ventilation decreased
I	2	Deeper sedation	Motionless unless stimulated
II	1	Light anesthesia	Partial loss of equilibrium
II	2	Deep anesthesia	Total loss of equilibrium
III	1	Surgical anesthesia	Total loss of reactivity when stimulated
III	2	Deep surgical anesthesia	Decrease in respiratory and heart rates
IV	1	Medullary collapse	Cessation of respiratory movements
IV	2	Cardiac arrest	Death

Recuperation after anesthesia is accomplished by transferring the fish into a container of fresh, well-aerated water without any anesthetic. It is helpful to move the air pump and air stone to the recovery container to continue to aerate the water. Never leave a fish unattended while it is under anesthesia. Some large fish tend to jump during induction or recovery from anesthesia. Moving the fish gently in a forward direction will aid the flow of fresh water across the gills, hastening anesthesia release from the gills. Do not slosh the fish back and forth in the water. Once there are steady operculum movements let the fish rest and gradually recover in a quiet, dim environment. The longer a fish is under anesthesia, the longer it will take to recover from the anesthetic. Monitor the fish until it has regained its equilibrium and is swimming normally and can be transferred back into the aquarium or pond.

Euthanasia

Humane death can be induced in fish by immersing them in an anesthetic solution, usually ten times the normal anesthetic concentration is used, and leaving them in the solution until respiration and heart beats cease. It is recommended to wait at least an hour after respiration has stopped to confirm death. Secondary methods of euthanasia, such as pithing, can be performed after the fish is anesthetized to ensure death.

References

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, American Veterinary Medical Association, Schaumburg, IL, 2013. https://www.avma.org/kb/policies/documents/euthanasia.pdf

Bowser, P.R., Anesthetic options for fish. In: *Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals*. Gleed, R.D. and Ludders, J.W. (eds.), International Veterinary Service, Ithaca, NY; 2001. www.ivis.org/advances/Anesthesia_Gleed/bowser/chapter_frm.asp?LA=1

Loh, **R.** *Fish Vetting Medicines*, Richmond Loh Publishing, Perth, 2012.

Neiffer, D. L. and **M. A. Stamper**, Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs, *ILAR Journal,* 2009. http://vpresearch.louisiana.edu/sites/research/files/NeifferFishSedation.pdf

Saint-Erne, **N.** *Advanced Koi Care*, 2nd edition, Erne Enterprises, Glendale, AZ, 2010.

ENHANCED SYSTEMIC IMMUNE RESPONSE IN ATLANTIC SALMON (*Salmo salar*) FED WITH OLIVE FRUIT EXTRACT-SUPPLEMENTED DIET

Ricardo Salomón*[1], Felipe E. Reyes-López[3], Lluis Tort[4], Joana P. Firmino, José C. Quintela[5], José M. Pinilla-Rosas[5], M. Angeles Esteban, Cristóbal Espinoza Ruíz, M. Dolors Furones[1], Eva Vallejos-Vidal[2], Enric Gisbert[1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
[2] Centro de Biotecnología Acuícola, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile
[3] Consorcio Tecnológico de Sanidad Acuícola, Ictio Biotechnologies S.A., Santiago, Chile
[4] Dept. Cell Biol., Physiol. and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain
[5] Natac Biotech, Calle Electrónica 7, 28923 Alcorcón, Madrid, Spain
* Email: ricardo.salomon@irta.cat

Introduction

Aquaculture is the fastest growing animal food-producing industry. Intensified production systems and climate change, facilitate the occurrence of disease outbreaks due to the favoring of stressed and immuno-compromised animals. Therefore, a suitable environmentally friendly solution is the use of feed additives with immunomodulatory properties that may be used as functional feeds to improve disease resistance (Dawood et al., 2021). A wide spectrum of phytogenics have been studied in aquafeeds due to their growth promoting, antimicrobial, immunostimulant, antioxidant and anti-inflammatory properties (Reverter et al. 2021). In this study, we have evaluated a feed additive rich in triterpenic compounds and polyphenols (AQUOLIVE®, NATAC Biotech SL, Spain) on the systemic immune response and disease resistance of Atlantic salmon (*Salmo salar* L.) smolts.

Materials and Methods

Atlantic salmon parrs were kept in tanks of 450 L connected to a water recirculation system (IRTAmar®) at an initial density of 2 kg m^{-3}. Water temperature, pH and oxygen levels during the trial were 12 ± 0.1 °C, 7.4 ± 0.3 and 9.6 ± 0.2 mg/L. After 47 days, fish were smoltified during 10 days and transferred to 35 ppt, and kept at similar water temperatures than the parr phase during 76 days. Photoperiod was 24 h light and 0 h darkness. Two experimental diets were tested: D1, control diet (40% protein; 22.15% fat; 21.60 energy MJ/kg); D2, control diet supplemented with the feed additive AQUOLIVE® at 0.15% inclusion. This feed additive was obtained from olive tree containing 10% of olive bioactive compounds (8.0% triterpenic acid and 2% polyphenols). At the end of the trial, in order to investigate the immunomodulatory properties of the phytogenic tested against a bacterial infection, an *in vivo* challenge with *Aeromonas salmonicida* was performed (4 tank replicates; 8 fish per tank). In addition, total RNA was extracted from the head kidney (HK) of individual fish (n = 18 fish per dietary treatment) and its transcriptomic profiling analyzed by means of a microarray platform (Krasnov et al., 2011).

Results and Discussion

At the end of the trial (133 days), no differences in body weight were observed between fish fed the diet containing 0.15% AQUOLIVE® (252.3 ± 9.2 g) and the control group (240.2 ± 19.3 g). The transcriptomic profiling of the HK from smolts fed the control and AQUOLIVE® diets revealed a total of 1,027 differential expressed genes (DEGs). Among them, 805 DEGs were up-regulated (UP) and 222 genes were significantly down-regulated (DOWN). Furthermore, the ClueGO platform (Bindea et al., 2009) was used to identify the biological processes (BPs) linked to the above-mentioned DEGs. The enriched BPs were mainly associated with immune pathways. Particularly, "leukocyte activation" (34 UP and 14 DOWN genes), "granulocyte activation" (26 UP and 11 DOWN genes) and "neutrophil degranulation" (25 UP and 11 DOWN genes), indicating that the BPs identified were the primary actors of the innate immune response promoted by the tested functional additive in this lymphoid organ. In the case of granulocyte, neutrophils are one of the three types of granulocytes identified in fish, whereas neutrophilic granulocytes are the most abundant in salmonids (Rønneseth et al., 2006). As their main function is arriving first at the site of the infection and having a central role in host tissue protection by killing and degradation of microorganisms; therefore, neutrophils are an essential part of the innate immune system whose main activities are to phagocyte pathogens and stimulate lymphocytes and other immune cells (Secombes and Wang, 2012). Thus, the results of our functional analysis regarding leukocyte activation, granulocytes and neutrophils, in particular, might suggest and increased specific immune capacity promoted by the tested functional diet.

(Continued on next page)

Among others BPs identified, the the "i-kappaB kinase/NF-kappaB signaling" pathway was evidenced. Nuclear factor kappa B (NF-κB) pathway is a nuclear transcription factor involved in the regulation of several cytokines, chemokines, antimicrobial peptides, and interferon-stimulated genes, being crucial for a multitude of important immnulogical transcriptional programs, including inflammatory responses to antigens by innate immune cells (Dorrington et al., 2019).

Results from the transcriptomic profiling of the HK were validated in an *in vivo* pathogenic challenge with *A. salmonicida* (intraperitoneal injection of 10^7 CFU/mL). The bacterial challenge lasted 15 days and at the end of the assay, cumulative survival was higher in fish fed the AQUAOLIVE diet (96.9 ± 6.4%) than in fish from the control group (60.7 ± 13.5%).

Conclusions

These results indicate that the dietary supplementation of the functional feed additive AQUOLIVE® at level of 0.15% enhanced the systemic immune response in Atlantic salmon smolts as the combined analysis of the HK transcriptomic profiling and results from the *A. salmonicida* challenge indicated.

Acknowledgements: this project has received funding from the European Union's Horizon 2020 Research and Innovation Programme (H2020, SME Instrument) under grant agreement No. 830202.

References

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–3.

Dawood, M.A.O. (2021). Nutritional immunity of fish intestines: important insights for sustainable aquaculture. *Reviews in Aquaculture* 13, 642–663.

Dorrington, M.G., Fraser, I.D.C. (2019). NF-κB signaling in Macrophages: Dynamics, Crosstalk, and Signal Integration. *Frontiers in Immunology* 10, 705.

Krasnov, A., Timmerhaus, G., Afanasyev, S., Jørgensen, S.M. (2011). Development and assessmentof oligonucleotide microarrays for Atlantic salmon (*Salmo salar* L.). Comparative *Biochemestry and Physiology. Part D*. 6, 31-38.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D. (2021). Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture* 13, 537-555.

Rønneseth, A., Eirin, F.P., Heidrun, I.W. (2006). Neutrophils and B-cells in blood and head kidney of Atlantic salmon (*Salmo salar* L.) challenged with infectious pancreatic necrosis virus (IPNV). *Fish & Shellfish Immunology* 20, 610-620.

Secombes, C.J., and Wang, T. (2012). "The innate and adaptive immune system of fish" in Infectious Disease in Aquaculture: Prevention and Control, ed. Austin, B. (Woodhead Publishing), 3-68.

PHYTOGENICS FROM SAGE AND LEMON VERBENA PROMOTE GROWTH, SYSTEMIC IMMUNITY AND DISEASE RESISTANCE TO *Aeromonas salmonicida* INFECTION IN ATLANTIC SALMON

Ricardo Salomón*[1], Eva Vallejos-Vidal[2,3], Felipe E. Reyes-López[3], Lluis Tort[3], José C. Quintela[4], José M. Pinilla-Rosas[4], M. Dolors Furones[1], Enric Gisbert[1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
[2] Centro de Biotecnología Acuícola, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile
[3] Dept. Cell Biol., Physiol. and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain
[4] Natac Biotech, Calle Electrónica 7, 28923 Alcorcón, Madrid, Spain
* Email: ricardo.salomon@irta.cat

Introduction

Aquaculture intensification and sustainability has led to new industrial challenges, among them the need of reducing the use of antimicrobials. Phytogenics are good candidates for this purpose, as they may enhance fish growth, health and welfare due to their antimicrobial, immunostimulant, antioxidant and anti-inflammatory properties (Reverter et al. 2021). In this study, we have evaluated a feed additive rich in verbascoside and triterpenic compounds like ursolic acid on the systemic immune response and disease resistance of Atlantic salmon (*Salmo salar*) smolts.

Materials and Methods

Atlantic salmon parrs were kept in tanks of 450 L connected to a water recirculation system (IRTAmar®) at an initial density of 2 kg m^{-3}. Water temperature, pH and oxygen levels during the trial were 12 ± 0.1 °C, 7.4 ± 0.3 and 9.6 ± 0.2 mg/L. After 47 days fish smoltified during 10 days and were then transferred to 35 ppt during 76 days. Water temperature was maintained similar than the parr phase and photoperiod was 24 h light and 0 h darkness. Two experimental diets were tested: D1, control diet (40% protein; 22.15% fat; 21.60 energy MJ/kg); D2, control diet supplemented with a medicinal plant leaf extract (MPLE) at 0.1% inclusion. This feed additive was obtained from sage (*Salvia officinalis*) and lemon verbena (*Lippia citriodora*) containing 10% ursolic, 3% triterpenic compounds, 2% verbascoside and <1% polyphenols (NATAC Biotech SL, Spain). At the end of the trial, in order to investigate the immunomodulatory properties of the phytogenics tested against a bacterial infection, an *in vivo* challenge with *Aeromonas salmonicida* was performed (4 tank replicates; 8 fish per tank). Total RNA was extracted from the head kidney (HK) of individual fish (n = 18 fish per dietary treatment) and its transcriptomic profiling analyzed by means of a microarray platform (Krasnov et al., 2011). Differences in somatic growth between both diets (control; 0.1% MPLE) were evaluated by means of a *t*-test.

Results and Discussion

At the end of the trial (133 days), significant differences in body weight were observed between fish fed the diet containing 0.1% MPLE and the control group (271.5 ± 9.2 g vs. 240.2 ± 19.3 g, respectively; $P < 0.05$). This weight increase is in agreement with previous studies evaluating the same phytogenic in seabream (Salomón et al., 2020) and they might be attributed to the potential growth-promoting effects of polyphenolic compounds like verbascoside. In addition, FCR values were lower in fish fed the MPLE diet (1.09 ± 0.05) than in smolts fed the control diet (1.25 ± 0.08) ($P < 0.05$). The transcriptomic profiling of the HK from smolts fed the control and MPLE diets revealed a total of 1,178 differential expressed genes (DEGs). Among them, 802 DEGs were up-regulated and 376 genes were significantly down-regulated. Furthermore, the ClueGO platform (Bindea et al., 2009) was used to identify the biological processes (BPs) linked to the above-mentioned DEGs. The enrichment analysis indicated that the BPs were mainly associated with immune pathways. Particularly, "leukocyte activation", "neutrophil mediated immunity", "lymphocyte activation involved in immune response", among others, indicating that the identified BPs were the primary actors of the innate immune response promoted by the tested functional additive in this lymphoid organ.

These results support those obtained from an *in vivo* pathogenic challenge with *A. salmonicida* (intraperitoneal injection of 10^7 CFU/mL). The bacterial challenge lasted 15 days, and at the end of the assay, the cumulative survival was higher in fish fed the MPLE diet (90.6 ± 6.4%) than in the control group (60.7 ± 13.5%). This is relevant, since the additives tested might be used as sustainable and environmental-friendly treatment strategy for fighting fish diseases, through an increased systemic immune capacity. Moreover, the tested additive regulated other BPs like "stem cell differentiation" and "antigen processing and presentation of antigen via MHC class II". These results are important since leucocytes develop

(Continued on next page)

from a common cell origin, the multipotent hematopoietic stem cell (Øverland et al., 2010). Besides, MHC class II in fish granulocytes has been suggested to play a key role in the ability to present antigens (Øverland et al., 2010). Collectively, these results led to an increased capacity in terms adaptive immunity, thus capable to generate immunological memory.

Furthermore, the "iNF-κB signaling" and "exocytosis" pathways were evidenced. This is of special relevance since the nuclear factor kappa B (NF-κB) pathway is involved in the regulation of several cytokines, antimicrobial peptides, and interferon-stimulated genes, being crucial for a multitude of important immunological transcriptional programs, including inflammatory responses to antigens by innate immune cells (Dorrington et al., 2019). In addition, exocytosis is recognized by its important role in the immune response participating in neutrophil function, in the cell-mediated cytotoxicity and finally, in the immunological synapses among cells (Faurschou and Borregaard, 2003).

Conclusions

The present results from the combined analysis of the HK transcriptomic profiling and from the *A. salmonicida* challenge indicate that the dietary supplementation of the functional feed additive MPLE at the level of 0.1% promoted somatic growth, improved feed efficiency and enhanced the systemic immune response in Atlantic salmon smolts.

Acknowledgements: this project has received funding from the European Union's Horizon 2020 Research and Innovation Programme (H2020, SME Instrument) under grant agreement No. 830202.

References

Bindea, G., Mlecnik, B., Hackl, H., et al. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–3.

Dorrington, M.G., Fraser, I.D.C. (2019). NF-κB signaling in Macrophages: Dynamics, Crosstalk, and Signal Integration. *Frontiers in Immunology* 10, 705.

Faurschou, M., and Borregaard, N. (2003). Neutrophil granules and secretory vesicles in inflammation. *Microbes and Infection* 5, 1317–1327.

Krasnov, A., Timmerhaus, G., Afanasyev, S., et al. (2011). Development and assessmentof oligonucleotide microarrays for Atlantic salmon (*Salmo salar* L.). Comparative *Biochemestry and Physiology. Part D*. 6, 31-38.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D. (2021). Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture* 13, 537-555.

Salomón, R., Firmino, J.P., Reyes-López, F.E., et al. (2020). The growth promoting and immunomodulatory effects of a medicinal plant leaf extract obtained from *Salvia officinalis* and *Lippia citriodora* in gilthead seabream. *Aquaculture* 524, 735291.

Øverland, H.S., Pettersen, E.F., Rønneseth, A., Wergeland, H.I. (2010). Phagocytosis by B-cells and neutrophils in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.). *Fish & Shellfish Immunology* 28, 193-204.

DIETARY OLIVE FRUIT (*Olea europaea* L.) EXTRACT PROMOTES INTESTINAL IMMUNITY IN ATLANTIC SALMON (*Salmo salar* L.)

Ricardo Salomón*[1], Eva Vallejos-Vidal[2,3], Felipe E. Reyes-López[3], Lluis Tort[3], José C. Quintela[4], José M. Pinilla-Rosas[4], M. Dolors Furones[1], Enric Gisbert[1]

[1] IRTA, Aquaculture Program, Sant Carles de la Ràpita, Spain
[2] Centro de Biotecnología Acuícola, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile
[3] Dept. Cell Biol., Physiol. and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain
[5] Natac Biotech, Calle Electrónica 7, 28923 Alcorcón, Madrid, Spain
* Email: ricardo.salomon@irta.cat

Introduction

Aquaculture will supply the majority of aquatic dietary protein by 2050, playing a relevant role in food security and supply, and poverty alleviation (Stentiford et al., 2020). In this context, Atlantic salmon (*Salmo salar* L.) production has increased strongly in recent decades thanks to the expansion in the northern Europe and in North and South America with Norway and Chile as the mains world producers (FAO, 2020). This fast and continued growing in the salmon industry has side effects. Under intensive culture conditions, fish may be exposed to several environmental and husbandry related stimuli that may have a potential noxious or stressful effect. All these factors have negative impacts on fish welfare and overall performance. In addition, a higher susceptibility to disease has been observed since reduced immune response allows pathogens to act with greater efficiency with an impact in the industry causing sanitary crisis and economic losses (Tort, 2011). Therefore, the development of functional feeds focused on their growth promoting, antimicrobial, immunostimulant, antioxidant, anti-inflammatory and sedative properties has been encouraged during the last decade (Reverter et al., 2021). In the current study we evaluated the transcriptomic profile of the intestine in Atlantic salmon smolts fed a functional diet rich in triterpenic compounds and polyphenols (AQUOLIVE®, NATAC Biotech SL, Spain) obtained from olive fruit (*Olea europaea* L.) extract on the local immune response of smolts.

Materials and Methods

Atlantic salmon parrs were kept in tanks of 450 L connected to a water recirculation system (IRTAmar®) at an initial density of 2 kg m^{-3}. Water temperature, pH and oxygen levels during the trial were 12 ± 0.1 °C, 7.4 ± 0.3 and 9.6 ± 0.2 mg/L. After 47 days, fish were smoltified during 10 days and transferred to 35 ppt, and kept at similar water temperatures than the parr phase during 76 days. Photoperiod was 24 h light and 0 h darkness. Two experimental diets were tested: D1, control diet (40% protein; 22.15% fat; 21.60 energy MJ/kg); D2, control diet supplemented with the feed additive AQUOLIVE® at 0.15% inclusion. This feed additive was obtained from the fruit olive containing 10% of olive bioactive compounds (8.0% triterpenic acid and 2% polyphenols). In addition, total RNA was extracted from the intestine of individual fish (n = 18 fish per dietary treatment) and its transcriptomic profiling analyzed by means of a microarray platform (Krasnov et al., 2011).

Results and Discussion

At the end of the trial (133 days), no differences in body weight were observed between fish fed the diet containing 0.15% AQUOLIVE® (252.3 ± 9.2 g) and the control group (240.2 ± 19.3 g). The transcriptomic profiling of the gut from smolts fed the control and AQUOLIVE® diets revealed a total of 2,200 differential expressed genes (DEGs). Among them, 1564 DEGs were up-regulated (UP) and 636 genes were significantly down-regulated (DOWN). Furthermore, the ClueGO platform (Bindea et al., 2009) was used to identify the biological processes (BPs) linked to the above-mentioned DEGs. Considering the close relationship between diet and gut condition and the consequences on the organism and overall health, evaluating the interactions between dietary ingredients and the intestine is of special relevance due to the wide array of functions that have been associated to the gastrointestinal tract (Calduch-Giner et al., 2016). This is of special relevance when evaluating functional feed additives that are supposed to promote health and nutrition in farmed animals (Dawood et al., 2021). Among the most representative BPs enriched obtained from the intestine of the Atlantic salmon were the following: myeloid leukocyte activation (7.95%), response to organic substance (10.23%), regulation of metabolic process (12.5%), cellular response to cytokine stimulus (17.05%), protein localization to endoplasmic reticulum (17.05%) and establishment of protein localization to endoplasmic reticulum (22.73%). Therefore, this positive regulation of the aforementioned BPs might suggest to a more metabolically active intestine due to greater protein synthesis, which could lead to a stimulation not only of the innate immune response, but also adaptive, although they are needed further research to confirm this hypothesis.

(Continued on next page)

Conclusions

These preliminary results indicate that the dietary supplementation of the functional feed additive AQUOLIVE® at level of 0.15% promoted transcriptional innate and adaptive immune responses in gut, especially through the modulation of those processes involved in leukocyte activation, cellular response to cytokines, among others.

Acknowledgements: this project has received funding from the European Union's Horizon 2020 Research and Innovation Programme (H2020, SME Instrument) under grant agreement No. 830202.

References

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–3.

Calduch-Giner, J.A., Sitjà-Bobadilla, A., Pérez-Sánchez, J. (2016). Gene expression profiling reveals functional specialization along the intestinal tract of a carnivorous teleostean fish (*Dicentrarchus labrax*). *Frontiers in Physiology* 7, 359.

Dawood, M.A.O. (2021). Nutritional immunity of fish intestines: important insights for sustainable aquaculture. *Reviews in Aquaculture* 13, 642–663.

FAO (2020). The State of World Fisheries and Aquaculture 2020. FAO Fisheries and Aquaculture Department. The Food and Agriculture Organization of the United Nations, Rome, Italy.

Krasnov, A., Timmerhaus, G., Afanasyev, S., Jørgensen, S.M. (2011). Development and assessmentof oligonucleotide microarrays for Atlantic salmon (*Salmo salar* L.). Comparative *Biochemestry and Physiology. Part D*. 6, 31-38.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D. (2021). Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture* 13, 537-555.

REPRODUCTION AND LARVICULTURE OF THE SOUTHERN BLACK DRUM *Pogonias courbina*, A NEW SPECIES FOR AQUACULTURE IN SOUTH AMERICA

L.A. Sampaio*, J. Sgandella, L. Maltez, M.H. Okamoto, O. Menossi, T.P.A.P. Oliveira, and R.V. Rodrigues

Universidade Federal do Rio Grande – FURG, Instituto de Oceanografia, Laboratório de Piscicultura Estuarina e Marinha (LAPEM). Rio Grande (Brazil).
E-mail: luisandresampaio@gmail.com

Introduction
Marine fish culture is an emerging industry in the Western South Atlantic coast. Two species are currently commercially produced in Brazil, the cobia *Rachycentron canadum* (Sampaio et al 2010) and the grouper *Epinephelus* (Mello et al, 2018). Nevertheless, there is a continuous search for prospective species.

Sciaenidae fish are produced in North America and Europe, aquaculture production for red drum (*Sciaenops ocellatus*) reachs 77,000 ton, while meagre (*Argyrosomus regius*) 37,500 ton (FAO 2021), thus suggesting other large Sciaenidae species, such as endangered Southern black drum (*Pogonias courbina*) would also have good aquaculture potential.
This is the first report of successful mass production of juvenile Southern black drum *P. courbina*.

Materials and Methods
Twenty adult fish were captured during the winter 2019 (from July to September) at Cassino Beach, in Southern Brazil (32°S-52°W), and immediately transferred to the Laboratory of Marine Fish Culture at FURG. They were measured, tagged, and stocked in a 15 ton tank, attached to a RAS. Water temperature and photoperiod at the hatchery mimicked the conditions in the wild. Broodstock were hand fed daily on frozen fish, squid, and shrimp.

Live feed was provided to the larvae, including microalgae (*Nannochloropsis oceanica*), rotifer (*Brachionus plicatilis*), and *Artemia sp* (nauplii and metanauplii). Rotifer and *Artemia* metanauplii were enriched using commercially available lipid emulsions rich in HUFA (Red pepper – Bernaqua). During cofeeding, up to weaning, larvae were offered commercial diets (Win Fast – Sparos and NRD – Inve).

Larvae were reared in 300 L tanks attached to a RAS. Continuous light was provided during larviculture, temperature was kept at 26°C and salinity was set to 30‰.

Results
Four months after the last broodstock were stocked in the hatchery, during January 2020, naturally fertilized eggs of *P. courbina* were obtained for the first time. At the time of spawning water temperature was 26°C and photoperiod was equal to 14 h light and 10 h darkness. The precise moment of spawning was not determined, but in the morning, embryos often had already reached the 180° stage, hatching took place between 9 and 12PM. Newly hatched larvae measured 1.9 mm.

Exogenous feeding started 48 h after hatching, larvae were fed exclusively on rotifers for 5 days, when Artemia nauplii was introduced. Rotifers were discontinued 11 dah and Artemia nauplii 13 dah, when larvae were fed only enriched Artemia (Figure 1). Co-feeding with dry diets began 15 dah and larvae were fully weaned 22 dah. One month after hatching larvae reached 29 mm and weighed 270 mg. Survival up to metamorphosis was between 75 - 90%.

Discussion and conclusion
One of the first steps towards the introduction of a new species for aquaculture is to accomplish its controlled reproduction in captivity (Mylonas et al 2010). Wild caught broodstock of Southern black drum readily adapt to captivity and were conditioned to spawn volitionally.

Larviculture of teleost fish is dependent of live food, but their larvae should be readily be weaned into dry diets, therefore reducing the need for live prey production and the high costs associated to production of rotifers and purchase of *Artemia* cysts (Campoverde et al, 2017).

Larviculture of Southern black drum larvae is faster than most species. Larvae were fully weaned as soon as 22 dah. Survival was also high, up to 90% upon completion of metamorphosis.

The successful mass production of striped drum juveniles suggests it may be a prospective species for aquaculture in South America.

(Continued on next page)

Figure 1 – Newly hatched larvae of *Pogonia courbina*, and 10 days old larvae feeding actively on *Artemia* nauplii.

References

Campoverde, C.; Rodriguez, C.2 | Perez. J.; Gisbert, E.; Estevez, A. 2017. Early weaning in meagre *Argyrosomus regius*: Effects on growth, survival, digestion and skeletal deformities. Aquaculture Research, 48: 5289-5299.

FAO. 2021 Aquaculture production 1950–2019. FISHSTATJ – Software for Fishery and Aquaculture Statistical Time Series.

Mello, P.H., Araújo, B.C., Campos, M.F., Rodrigues-Filho, J.A., Garcia, C.E.O., Moreira, R.G. 2018. Embryonic and larval development and fatty-acid profile of *Epinephelus marginatus* spawned in captivity: Tools applied to captive rearing. Journal of Fish Biology, 92, 1126-1148.

Mylonas, C.C., Fostier, A., Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. General and Comparative Endocrinology, 165: 516-534.

Sampaio, L.A., Moreira, C.B., Miranda-Filho, K.C., Rombenso, A.N. 2011. Culture of cobia *Rachycentron canadum* (L) in near-shore cages off the Brazilian coast. Aquaculture Research 42: 832-834.

Acknowledgements

The authors would like to thank Brazilian CNPq-MCTI, CAPES, and FAPERGS. The support of European Community (AquaVitae Project – H2020 SC2) is also appreciated. L.A. Sampaio is a research fellow of CNPq.

INFLUENCE OF EXTREME CLIMATIC EVENT ON LOCAL FISH LANDING DUE TO MASSIVE ESCAPES IN WESTERN MEDITERRANEAN SEA

P. Sanchez-Jerez*[1], D. Fernandez-Jover[1], J.M. Valero-Rodriguez[1], Jose L. SanchezLizaso[1], I. Sola-Macia[1], D. Izquierdo-Gomez[2], P. Arechavala-Lopez[3], F. Romero[1], and K. Toledo-Guedes[1]

[1]Department of Marine Science and Applied Biology. University of Alicante. Spain [2]Université de Pau et des Pays de l'Adour, Collège Sciences et Technologies de l'Énergie et de l'Environnement. France.
[3]Fish Ethology and Welfare Group. Centro de Ciencias do Mar. Faro, Portugal.
E-mail: psanchez@ua.es

Introduction

The acceleration of climate change has exacerbated existing environmental problems in the Mediterranean Basin that are caused by the combination of changes in land use, increasing pollution, and declining biodiversity (MedECC, 2018). One of the connections between extreme events due to climate change and the effects on marine ecosystems are fish escapes from aquaculture facilities. As it is complicated to determine the extent and duration of the presence of escapes in coastal areas, local fisheries can be a proxy to assess the relevance of this impact at the mesoscale level. The objective of the work was that to assess if the magnitude of the storms should be reflected in the local fishermen's landings in the fish market. The duration of landings above average landings was also assessed in relation to the distance to the aquaculture facilities and cultivated species.

Material and Methods

We analyzed the temporal trends of daily fishing landings from 30 fish markets along the Spanish Mediterranean coast using official data from the Ministry of Fisheries of the Government of Spain of meagre (*Argyrosomus regius*), seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) from 2018 to 2021. On the other hand, the events of storms with waves higher than 1 m in the areas where aquaculture is most intensively developed were analyzed. The SIMAR simulation model based on oceanographic data from the oceanographic buoy measurements of *Puertos del Estado* (Government of Spain) and atmospheric data was used to obtain the significant wave height for the positions of the most significant aquaculture facilities. The data were filtered to obtain representations of storms with waves higher than 1 m.

Results and Conclusions

During the studied period, four storms with waves greater than three meters were identified between 2019 and 2021. The most significant event was storm Gloria in January 2020, with waves greater than 4 m (Figure 1). The landings from the fish farms were clearly correlated with the intensity and duration of the storms, increasing between 10 and 100 times the average catches of each species. A clear relationship was also detected between the distance to the facilities and regional and temporal differences in the cultivation of each species. For example, in La Villa Joisa there was an important peak in meagre catches (Figure 2), followed by seabass, the main species cultivated in the area. Of course, the effect of each fleet in its area of influence is related to its fishing effort capacity and the type of fishing gear used. The relevance of Gloria is reflected in the magnitude of the catches and their duration over time. Therefore, catches in local fisheries may be a suitable proxy for estimating the impact of escapes from aquaculture in coastal environments, which are clearly related to extreme oceanographic events. Strategic plans for aquaculture development should be able to forecast and take into account the future climatic projections and local oceanographic conditions. It should not be forgotten that other extreme events can act negatively synergistically with global warming as strong storms or torrential rains, which in turn should be taken into account in spatial planning plans for aquaculture. On the other hand, fish escaping from net pens have always been considered a major source of ecological issues and local artisanal fisheries have proved the ability to mitigate escape events by recapturing escapees (Izquierdo-Gomez and Sanchez-Jerez 2016).

(Continued on next page)

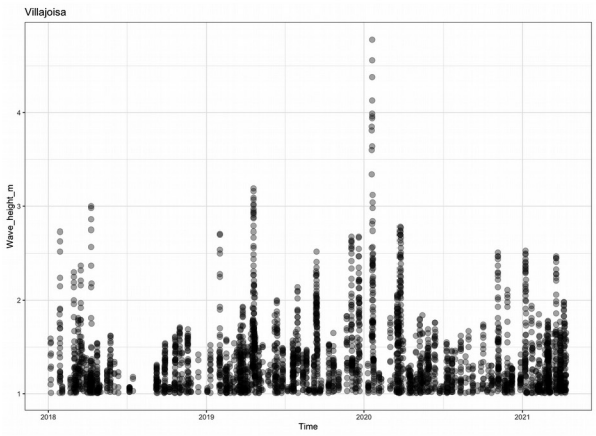


Figure 1. Significant wave height estimate using SIMAR model from *Puertos del Estado* (Spanish Government) for waves bigger than 1 m.

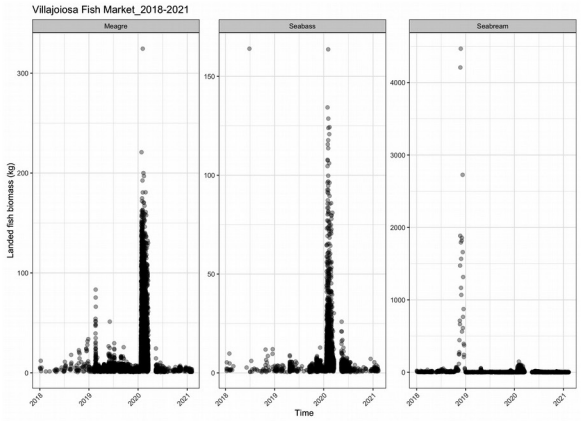


Figure 2. Daily fish landing (kg) in La Villa Joiosa fish market (Alicante, Spain) for meagre, seabass and seabream.

References

Izquierdo-Gomez, D. & Sanchez-Jerez, P (2016) Management of fish escapes from Mediterranean Sea cage aquaculture through artisanal fisheries. Ocean & Coastal Management 122:57-63. dx.doi.org/10.1016/j.ocecoaman.2016.01.003

MedECC W. Cramer, J. Guiot & K. Marini (2018) Risks associated to climate and environmental changes in the Mediterranean region

Acknowledgements

Thanks to *Puertos del Estado* and Ministry of Agriculture, Fisheries and Food for data supply. Funded by Project GLORiA, supported by the Biodiversity Foundation of the Spanish Ministry for the Ecological Transition and Demographic Challenge, through the Pleamar Programme and cofinanced by the European Maritime and Fisheries Fund (EMFF). It is also part of the LIFE IP INTEMARES project "Integrated, innovative and participatory management of the Natura 2000 Network in the Spanish marine environment".

This work is part of project GLORiA. GLORiA is supported by the Biodiversity
Foundation of the Spanish Ministry for the Ecological Transition and Demographic Challenge, through the Pleamar programme and co-financed by the European Maritime and Fisheries Fund (EMFF). It is also part of the LIFE IP INTEMARES project "Integrated, innovative and participatory management of the Natura 2000 Network in the Spanish marine environment".

CHARACTERISATION OF GENES ESSENTIAL FOR PATHOGENESIS OF FISH ADAPTED *S. Agalactiae*

Morena Santi*, Adam Blanchard, James Leigh and Sharon A. Egan

School of Veterinary Medicine and Science, University of Nottingham
Email:morena.santi@nottingham.ac.uk

Introduction

Streptococcus agalactiae or Group B Streptococcus (GBS), is a highly infectious zoonotic pathogen, responsible for streptococcosis in fish, meningitis and septicaemia in humans and mastitis in cattle. In fish, it manifests as septicaemia, resulting in high morbidity and mortality in a number of wild and farmed species, in particular tilapia, sea bass and rainbow trout. Next-generation sequencing (NGS) associated with transposon mutagenesis of a comprehensive mutant library allows the genome-wide measurement of individual genes involved in the fitness of a bacteria growing in different conditions, enabling the identification of conditionally essential genes by negative selection consisting of an estimated 1.1 million transposon mutants and we have used genomic DNA from this mutant pool, and Illumina nucleotide sequencing to prime from the transposon and sequence into the adjacent target DNA. With this method, which we have called TraDIS (transposon directed insertion-site sequencing. This can provide important information to determine bacterial pathogenesis, key genes linked to virulence and host adaptation and to discover potential drug targets or vaccine candidates.

Materials and methods

The temperature sensitive plasmid, pGh9:IS*S1*, was used to generate a mutant population of *S. agalactiae* 01173 by random integration. This bacterial mutant population was grown in 3 separate growth conditions related to pathogenesis in fish tilapia serum, bacterial media containing either hydrogen peroxide or antimicrobial peptides, and bacterial media used as a comparative control. Harvested DNA was processed by inverse PCR, NGS sequenced and processed through PIMMS a bioinformatic pipeline to identify and quantitate transposon insertions present in the bacterial genome.

Results

Comparative sequence analysis was performed on approximately 100,000 individual mutants for each condition using the PIMMS transposon analysis platform. Among the conditionally essential genes, 3 were shared between growth conditions, *atpB*, *atpC* and *dltX*. A*tpB* and *atpC* are part of ATP synthase or ATPase operon, involved in the regulation of internal pH and the production of ATP, while *dltx* is involved in d-alanylation of teichoic acids important for charge-based regulation of the cell envelope and resistance to the effects of positively charged host immune factors. Other conditionally essential genes were shared only between two of the three conditions including the heat shock protein *dnaK* and protease *clpX*. DnaK is key for the regulation of accurate protein folding whilst clpX removes misfolded or damaged proteins.

This study highlights the potential use for transposon mutagenesis to rapidly identify potential vaccine targets for bacterial aquaculture related disease allowing this growing industry to become more sustainable for food production.

ACUTE AND SUBCHRONIC TOXIC EFFECTS OF MICROPLASTICS AND COPPER, ALONE OR COMBINED, IN BLACKSPOT SEABREAM (*Pagellus bogaraveo*) LARVAE

Dércia Santos[1a]*, Montse Perez[2], Evaristo Perez[2], Ana Luzio[1], Luís Félix[1,3,4], Sandra M. Monteiro[1], Juan Bellas[2]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences, CITAB, University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal
[2] Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, IEO, Subida a Radio Faro 50, 36390 Vigo, Spain
[3] Laboratory Animal Science, Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Rua Alfredo Allen, nº 208, 4200-135 Porto, Portugal
[4] Instituto de Investigação e Inovação em Saúde (i3s), Universidade do Porto, Rua Alfredo Allen, nº 208, 4200-135 Porto, Portugal

e-mail: dsantos@utad.pt

Introduction

Microplastics (MPs) comprise a major percentage of marine litter, being nowadays an environmental issue of global concern. It has been demonstrated that MPs can adsorb, for example, heavy metals, which can affect their bioavailability in aquatic biota and lead to biological adverse effects. Their presence in the marine ecosystems can impact commercial fisheries and aquaculture industries, posing serious risks for fish productivity and a potential constraint for food security. In this context, it is crucial to clarify the risks inherent to MPs, with or without other pollutants, to fisheries and aquaculture, to understand how species development may be affected and the aquatic organism's fitness compromised. Considering the above, the present study aimed to evaluate the effect of MPs, associated with copper (Cu), another contaminant of high concern, in the later larvae development of the economically and ecologically important species, the blackspot seabream (*Pagellus bogaraveo*). For this, in the present study, newly hatched blackspot seabream larvae were exposed to MPs, with or without Cu, for 10 days, to evaluate its effects on survival, growth, oxidative damage, antioxidant activity and expression of antioxidant and neurotoxicity-related genes.

Materials and Methods

The experiments were conducted with 120 newly hatched blackspot seabream larvae, randomly distributed through 500 mL glass beakers and incubated under semi-static conditions (16 ± 1 °C and a photoperiod of 12 /12 h light: darkness). Larvae were initially exposed, from 3 days post-fertilization (dpf) until 6 dpf, to a control (filtered natural seawater), MPs (0.3 mg/L of red fluorescent particles of 1-5 μm), five concentrations of Cu (Cu10, 10 μg/L; Cu30, 30 μg/L; Cu90, 90 μg/L; Cu270, 270 μg/L; and Cu810, 810 μg/L) and five binary mixtures of MPs and Cu (Cu10+MPs; Cu30+MPs; Cu90+MPs; Cu270+MPs; and Cu810+MPs). Three replicates were established for each group. To evaluate the acute effects of MPs and Cu, alone or combined, pools of larvae were sampled at 6 dpf, for gene expression and biochemical parameters analysis. The remaining larvae of the control, MPs and the two lowest concentrations of Cu and Cu+MPs groups, remained continuously exposed until 12 dpf, to evaluate the subchronic effects of sublethal doses of MPs and Cu on their development. At 12 dpf, pools of larvae were also sampled for gene expression and biochemical/metallothioneins analysis. Body length and the ingestion of MPs by larvae was also assessed at 12 dpf. For gene expression, RNA was isolated and the expression levels of the antioxidant enzymes (*cat* and *gst*) and neuronal (*pcna* and *ache*) genes were evaluated by quantitative real-time PCR (qPCR). The biochemical biomarkers, namely reactive oxygen species (ROS), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), reduced (GSH) and oxidized states (GSSG) of glutathione levels, lactate dehydrogenase (LDH), acetylcholinesterase (AChE) and metallothioneins (MT), were determined according to standardized protocols. The obtained data were then statistically analyzed.

(Continued on next page)

Results and Discussion

The acute exposure, induced a decreased survival and a time- and dose-dependent sensitivity to Cu, reaching a 72-h LC_{50} of 92 μg Cu/L. Interestingly higher mortality was observed in the Cu+MPs than in the Cu alone groups, although the differences were not statistically significant. At 6 dpf, ROS levels were not affected by the treatments, however, in the Cu270+MPs group, a significant increase in SOD activity was observed ($p<0.05$), indicating a cellular response against ROS. Nevertheless, the induction of oxidative damage in larvae exposed to high Cu concentrations was not prevented since an increase of LPO levels was observed in both Cu270 ($p=0.018$) and Cu270+MPs ($p<0.0001$) groups. Also, the CAT and GST activities decreased in all exposed groups ($p<0.001$), suggesting an imbalance of the antioxidant system, which may have contributed to the induction of oxidative damage and higher mortality, observed in the higher concentrations groups. However, the *cat* and *gst* genes did not support the cellular biomarkers, since the expression of these two genes was not affected by the treatments ($p>0.05$).

Blackspot seabream larvae ingested the plastic particles, along with the food. Indeed, after the subchronic exposure, an accumulation of plastic particles was observed, particularly, in the gastrointestinal tract. These observations point out the potential negative impacts on normal absorption and food digestion. Despite this, no significant effects of MPs, alone or combined with Cu, were observed in the body length of larvae. Considering the potential induction of oxidative stress by the treatments, it was observed an increase of the ROS levels in the MPs and Cu30 exposed larvae ($p<0.05$), followed by a decrease of CAT activity in all exposed groups ($p<0.0001$). In turn, the GST activity increased in the MPs, Cu30 and Cu30+MPs groups ($p<0.05$), suggesting that MPs and Cu, alone or combined, induce oxidative stress, with larvae responding with the subsequent activation of the antioxidant enzyme system to prevent oxidative damage. Indeed, no oxidative damage was observed in larvae, since LPO levels did not suffer significant changes. The results of the present study indicate the involvement of different cellular mechanisms and signalling pathways to prevent oxidative stress.

Overall, this study contributes to current research providing information on the mechanisms involved in the toxicological effects of MPs, as well their interaction with heavy metals in fish early life stages. In turn, it highlights that precautionary measures should be applied to reduce the entry of MPs in aquaculture systems to ensure aquatic organisms health and human food safety.

Acknowledgements
This work was co-financed by National Funds by FCT - Portuguese Foundation for Science and Technology, under the projects UIDB/04033/2020, project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF) and the FCT-PhD grant (PD/BD/127992/2016) attributed to Dércia Santos. The access to Aquaculture Facility IEO-Vigo (AquaCOV) was funded by the European Union‘s Horizon 2020 Programme under grant project AQUAEXCEL2020 (AE120003-COPPLAST).

SHORT-TERM IMMUNE RESPONSES OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES FOLLOWING *Photobacterium damselae* subsp. *piscicida* INFECTION

P. Santos[1,2 3*], D. Peixoto[1,2], I.A. Ferreira[1,2,4] R. Passos[3], P. Pires[3], T. Baptista[3], B. Costas[1,2]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto (Portugal)
[2]Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[3]MARE - Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Peniche, Portugal
[4]Fish Immunology and Vaccinology Group, IBMC-Instituto de Biologia Molecular e Celular, University of Porto, 4200-135 Porto, Portugal
*E-mail: paulo.santos@ciimar.up.pt

Introduction

The occurrence of fish bacterial diseases is one of the biggest challenges of world aquaculture intensification. Photobacteriosis is a septicemic disease caused by *Photobacterium damselae* subsp. *piscicida* (*Phdp*), affecting a wide range of marine species and leading to relevant economic losses. Although several studies have yet been performed regarding the pathogen features and virulence factors, few information about the host defence mechanisms activated after infection is available. The present study aimed to evaluate gilthead seabream (*Sparus aurata*) innate immune response to infection with *Phdp*.

Materials and methods

A time-course study was performed at CETEMARES (Politécnico de Leiria, Peniche, Portugal) facilities with 72 seabream juveniles (9.8 ± 2.2 g). After 2 weeks of acclimation, 12 fish were sampled before infection (time 0). The remaining animals were randomly selected and intraperitoneally (i.p.) injected with 100 μl PBS (placebo group) or 100 μl of exponentially growing *Phdp* (10^6 CFU mL^{-1}; infected group) and distributed as a complete randomized design in 6 recirculating seawater systems (i.e. triplicates *per* experimental condition). Two animals per tank (n = 6 *per* treatment) were sampled at 3, 6, 9, 24 and 48 h after i.p. injection. Fish were euthanized with 2-phenoxyethanol (0.5 mL L^{-1}) and blood samples were collected for haematological procedures. The remaining blood was centrifuged for plasma collection and innate humoral parameters (i.e. peroxidase, protease and antiprotease activities) were evaluated. Liver and head-kidney were also collected for oxidative stress (lipid peroxidation, total glutathione content, catalase, superoxide dismutase and glutathione-S-transferase activities) and gene expression analyses. Head-kidney total RNA isolation was performed with NZY Total RNA Isolation kit and first-strand cDNA synthesized with NZY First-Strand cDNA Synthesis Kit. Gene expression of innate immune selected genes was analysed by RT-qPCR and normalized by Pfaffl method using EF-1α as housekeeping gene. Although both placebo and infected groups were analysed, significant differences were mainly registered on infected groups against unstimulated animals and therefore are highlighted for a better understanding of host response to *Phdp* infection.

Results

Infection produced a host anaemic state since haemoglobin concentration and mean corpuscular haemoglobin concentration decreased in infected animals 48 h after bacterial challenge. Also total erythrocyte levels decreased 9 h after infection, while haematocrit presented lower values 48 h post-infection. In contrast, total peripheral leucocytes produced few differences throughout the time course. Circulating neutrophil and monocyte numbers augmented since the first hours of infection compared to non-infected animals. Regarding humoral responses, infected animals presented increased antiproteases activity 48 h post-infection. Hepatic oxidative stress biomarkers resulted on increased lipid peroxidase and glutathione-S-transferase 9 hours after pathogen inoculation, whereas catalase produced a later but similar variation. Moreover, hepatic total glutathione decreased 24 hours after bacterial challenge. Molecular findings go along with the cellular, humoral and oxidative stress results, with increased expression of interleukin1β, interleukin-34, interleukin-10 and major histocompatibility complex I, which are recognized genes related to inflammatory response and phagocytic processes. Also genes related to the iron metabolism such as haptoglobin and transferrin increased mRNA expression during infection, sugesting haemolysis and presenting a possible mechanism from host to avoid the bacterial uptake of iron.

(Continued on next page)

Discussion and conclusion

The outputs of this study include several pathways that contribute for a steady immune response. Cellular mechanisms are driven by peripheral monocytes and neutrophils that migrate for the infection site and release antioxidant and antibacterial substances trying to cope with the infection. Inflammation develops through a balanced production of anti and pro-inflammatory cytokines that result on a sustained but directed response. It is also remarkable the selective differentiation of macrophages through the interleukin-34/colony stimulating factor 1 receptor route and the increase of transferrin production that might be considered preferred routes to avoid bacterial survival and proliferation.
A broad view of the host mechanisms involved on other bacterial infections are essential for the definition of health biomarkers that can be suitable for an early disease detection.

Acknowledgements

This work was supported by project BE4AQUAHEALTH: RASTREIO NACIONAL DE PATOLOGIAS DE PEIXES DE AQUACULTURA: UMA APOSTA NA PREVENÇÃO (16- 02-05-FMP-0013), funded by Mar2020 Operational Programme and the European Union through FEDER, and by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. DP, IAF and BC were supported by FCT (UI/BD/150900/2021, SFRH/BD/147750/2019 and IF/00197/2015, respectively).

QUORUM-QUENCHING *Bacillus* SPP. PROTECT FISH FROM *Edwardsiella tarda* INFECTION AND MODULATE THE IMMUNE SYSTEM OF GILTHEAD SEABREAM (*Sparus aurata*)

Rafaela A. Santos[1,2,3,4]*, Nuno Mariz-Ponte[1], Nicole Martins[1,2], Russell Jerusik[5], Maria J. Saavedra[2,3,4,6], Aires Oliva-Teles[1,2], Helena Peres[1,2], António P. Carvalho[1,2], Conceição Santos[1], Cláudia R. Serra[1,2]

[1] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal.
[2] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal.
[3] CITAB - Centro de Investigação e Tecnologias Agroambientais e Biológicas, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.
[4] CECAV – Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, P.O. Box 1013, 5001-801 Vila Real, Portugal.
[5] Epicore Bionetworks Inc., 4 Lina Lane, Eastampton, New Jersey 08060, United States of America.
[6] Departamento de Ciências Veterinárias, ECAV, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.
*rafaela.santos@ciimar.up.pt

Introduction

Infectious bacterial fish diseases remain a major challenge for aquaculture development. Although antibiotics are an important tool for disease treatment, their damaging effects on the environment and public health have led to increased restrictions to their use in aquaculture. Quorum-Sensing (QS) is a regulatory mechanism closely related to the expression of virulence factors contributing to the pathogenicity of fish diseases. Hence, disruption of bacterial QS, known as Quorum-Quenching (QQ) is being proposed as a possibility to fight aquaculture diseases dissemination [1]. *Bacillus* spp. are sporeforming bacteria with recognizable biotechnology applications including as a source of natural antimicrobial molecules, such as QQ molecules. Indeed, we recently isolated from the gut of three marine fish species, *Bacillus* spp. capable of inhibiting the growth and biofilm formation of 14 different fish pathogens and with *in vitro* QQ capacity [2]. Alongside with other probiotic health-promoting characteristics, *Bacillus* spp. are also known for their immunostimulatory effects enhancing the host's innate and adaptive immunity against fish pathogens [3].

Here, we screened the QQ capacity of the extracellular components of our collection of *Bacillus* spp., previously isolated from the gut of different marine fish species and tested their ability to degrade AHLs QS-signals produced by important fish pathogens, their protective effects in an *in vivo* model when challenged with *E. tarda* and their putative modulation of the fish immune system.

Materials and Methods

We took advantage of 200 Fish isolates (FI) isolated from the gut of different fish species (*Sparus aurata*, *Dicentrarchus labrax*, and *Diplodus sargus*) [2, 4] to explore their QQ potential, by testing the ability to degrade AHLs QS molecules using CV026 biosensor. FI with extracellular QQ activity were tested for AHL enzymatic degradation of 30 μM of 3-Oxo-C6-HSL and evaluated for their QQ capacity against AHLs QS-signals produced by *Aeromonas* spp., *Vibrio* spp., *Photobacterium damselae*, *Tenacibaculum maritimum*, *Edwardsiela tarda,* and *Shigella sonnei* using a well-diffusion method and CV026 biosensor. The fish isolates' protection against *E. tarda* infection was performed using zebrafish larvae (*Danio rerio*). Larvae were treated once with the lyophilized extracts of FI314, FI436 and FI464 after mouth's opening (7dpf), for 2h at 28°C. The treated 10 dpf larvae were challenged by immersion with *E. tarda* at $1x10^8$ CFU mL^{-1}, and cumulative mortalities were registered between 16-24hours post-infection.

To elucidate the immune-modulatory properties of the QQ strains, head–kidney leucocytes from gilthead seabream were isolated and treated for 24h with extracts of FI314, FI436 and FI464 before stimulation with *E. tarda* ($5x10^6$CFU/mL). Different innate immune parameters – viability (propidium iodide), respiratory burst activity and phagocytosis – were evaluated using flow cytometry. Simultaneously, expression of immune-relevant genes (Hsp70, Cox-2, IL-1β, IL-6, IL-10 and Tnfα) were measured using real-time PCR.

(Continued on next page)

Results

We found out that ~12% of our fish-gut *Bacillus* spp. were able to interfere with synthetic AHLs signalling molecules. We further selected 10 isolates as producers of extracellular putative AHL-lactonase enzymes. Conjugating the QQ genomic profile, QQ bioactivities and molecular identification FI314, FI436 and FI464 were tested for their QQ capacity against natural AHLs produced by fish pathogens. We observed that *A. veronii* and *E. tarda* produce AHLs molecules detectable by *Chr. violaceum* biosensor, that were degraded when subjected to the extracellular extracts of fish-gut isolates FI314, FI436 and FI464. Moreover, when compared to the control (non-treated zebrafish larvae infected with *E. tarda*), FI extracellular compounds from FI314, increased the average survival rate of challenged larvae by 43% ($p<0.01$), and strains FI436 and FI464 increased the survival rate upon challenge by 50% ($p<0.001$).

Although, *Bacillus* spp. extracellular extracts did not stimulate the respiratory burst activity and cell viability, they remarkably increased pathogens' phagocytosis when the seabream leukocytes were exposed to *E. tarda*. Statistical analysis revealed that all extracts significantly increased ($p<0.001$) the engulfment of *E. tarda* 1h post-infection. In cells treated with the extracellular extracts, we observed an up-regulation of the immune genes associated with inflammation, including IL-1β, IL-6 and Cox-2, indicating a stimulus of the immune system. FI314 extracellular extract significantly increased the expression of IL-1β ($p=0.04$), IL-6 ($p=0.005$) and Cox-2 ($p=0.021$) and, FI436 and FI464 significantly increased IL-6 expression ($p=0.047$ and $p=0.009$, respectively).

Conclusions

Taken together our experiments revealed three promising fish-gut *Bacillus* spp. that produce extracellular molecules capable of quenching aquaculture pathogens' communication, protect fish from *Edwarsiella tarda* infection in an *in vivo* model and improve the immune system of gilthead seabream. Thus, the extracellular molecules from FI314 (*B. subtilis*), FI436 (*B. vezelensis*) and FI464 (*B. pumilus*) may be promising tools for disease control in aquaculture.

Funding: RAS, NP and NM are recipients of a PhD grant (SFRH/BD/131069/2017, SFRH/BD/138187/2018, SFRH/BD/137919/2018, respectively) from FCT; CRS has a scientific employment contract supported by national funds through FCT. This research was partially supported by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020, through national funds provided by FCT and ERDF.

References

1. Defoirdt, T., et al., *Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture*. Aquaculture, 2004. **240**(1): p. 69-88.
2. Santos, R.A., et al., *Isolation and Characterization of Fish-Gut Bacillus spp. as Source of Natural Antimicrobial Compounds to Fight Aquaculture Bacterial Diseases*. Mar Biotechnol (NY), 2021.
3. Kuebutornye, F.K.A., E.D. Abarike, and Y. Lu, *A review on the application of Bacillus as probiotics in aquaculture*. Fish & Shellfish Immunology, 2019. **87**: p. 820-828.
4. Serra, C.R., et al., *Selection of carbohydrate-active probiotics from the gut of carnivorous fish fed plant-based diets*. Scientific Reports, 2019. **9**(1): p. 6384.

EVALUATION OF THE NUTRITIONAL QUALITY AND BIOCHEMICAL PROFILE OF MEAGRE (*Argyrosomus regius*) PRODUCED IN RECIRCULATION AQUACULTURE SYSTEM (RAS)

D. Santos*[1], N. Leite [2], J. Rito [2], M. Neves [1], S. C. Marques [1], S. M. Leandro [1]

[1] MARE –Marine and Environmental Sciences Centre, Polytechnic of Leiria, 2520-630 Peniche, Portugal
Email: daniela.g.santos@ipleiria.pt
[2] SEAentia
Parque Tecnológico de Cantanhede, Núcleo 04, Lote 2, 3060-197 Cantanhede - Portugal

Introduction

Meagre (*Argyrosomus regius*) is a migratory marine species with widespread distribution in the Mediterranean and eastern Atlantic and receiving currently special attention due to the need of species diversification in Mediterranean mariculture (Fernández-Alacid *et al*, 2019, Duncan *et al,* 2013; Parisi *et al,* 2014). In what concerns to its captive rearing performance, demonstrates a low feed conversion ratio (FCR — 0.9 - 1.2) (Monfort, 2010), high fecundity index of 380 780 ± 167 577 eggs kg^{-1} (Mylonas *et al*, 2013) and high growth rates (1.45 ± 0.02 % day, at 26°C) (Kounna *et al*, 2021). Because of its meat quality and consumer acceptance (Grigorakis *et al,* 2011; Duncan *et al,* 2013), is considered a promising species with the potential to become one of the most cultivated species in the Mediterranean region. However, the characteristics of its meat and growth performance will depend on the farming conditions and the respective production system.

The present study aims to evaluate the nutritional quality and biochemical profile of meagre (*Argyrosomus regius*) reared on a recirculation aquaculture system (RAS).

Material and Methods

This study was conducted in a marine indoor RAS at SEAentia (Peniche, Portugal), which is highly optimized to offer the best growth conditions of meagre, and in MARE - Polytechnic of Leiria (Peniche, Portugal) where samples processing and analysis were performed. Juvenile meagre with a mean body weight of 57.12 ± 8.39 g were sampled after 20 days being fed with a tailor-formulated fishfeed (SPAROS, Olhão, Portugal). Muscle samples from both fillets of ten individuals (n=10) were collected and kept at -80°C. After their lyophilization, samples were analyzed for: (i) total protein by the Kjeldahl method (AOAC, 2016); (ii) lipid content (Folch, Lees & Stanley, 1957), (iii) ash (incineration at 500°C, for 12 hours) and (iv) fatty acids profile by gas chromatography (GC-FID).

Results and Discussion

The author is working on this section, to further present the obtained results.

References

Duncan, N. J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C. M., Roo, J., ... & Vallés, R. (2013). Aquaculture production of meagre (Argyrosomus regius): hatchery techniques, ongrowing and market. In *Advances in aquaculture hatchery technology* (pp. 519-541). Woodhead Publishing. https://doi.org/10.1533/9780857097460.3.519.

Fernández-Alacid, L., Sanahuja, I., Ordóñez-Grande, B., Sánchez-Nuño, S., Herrera, M., & Ibarz, A. (2019). Skin mucus metabolites and cortisol in meagre fed acute stress-attenuating diets: correlations between plasma and mucus. Aquaculture, 499, 185-194.
https://doi.org/10.1016/j.aquaculture.2018.09.039.

Folch, J., Lees, M., Stanley, G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, 226(1), 497-509.

Grigorakis, K., Fountoulaki, E., Vasilaki, A., Mittakos, I., & Nathanailides, C. (2011). Lipid quality and filleting yield of reared meagre (Argyrosomus regius). *International journal of food science & technology*, *46*(4), 711-716.
https://doi.org/10.1111/j.1365-2621.2010.02537.x

Kounna, C., Fountoulaki, E., Miliou, H., & Chatzifotis, S. (2021). Water temperature effects on growth performance, proximate body and tissue composition, morphometric characteristics and gastrointestinal evacuation processes of juvenile meagre, Argyrosomus regius (Asso 1801). Aquaculture, 540, 736683. https://doi.org/10.1016/j.aquaculture.2021.736683.

Monfort, M. C. (2010). Present market situation and prospects of meagre (Argyrosomus regius), as an emerging species in mediterranean aquaculture. *Studies and Reviews-General Fisheries Commission for the Mediterranean*, (89).

Mylonas, C. C., Mitrizakis, N., Castaldo, C. A., Cerviño, C. P., Papadaki, M., & Sigelaki, I. (2013). Reproduction of hatchery-produced meagre Argyrosomus regius in captivity II. Hormonal induction of spawning and monitoring of spawning kinetics, egg production and egg quality. *Aquaculture*, *414*, 318-327. https://doi.org/10.1016/j.aquaculture.2013.09.008.

Parisi, G., Terova, G., Gasco, L., Piccolo, G., Roncarati, A., Moretti, V. M., ... & Pais, A. (2014). Current status and future perspectives of Italian finfish aquaculture. *Reviews in fish biology and fisheries*, *24*(1), 15-73.
https://doi.org/10.1007/s11160-013-9317-7

EVALUATION OF THE NUTRITIONAL QUALITY AND BIOCHEMICAL PROFILE OF MEAGRE (*Argyrosomus regius*) PRODUCED IN RECIRCULATION AQUACULTURE SYSTEM (RAS)

D. Santos*[1], N. Leite [2], J. Rito [2], M. Neves [1], S. C. Marques [1], S. M. Leandro [1]

[1] MARE –Marine and Environmental Sciences Centre, Polytechnic of Leiria, 2520-630 Peniche, Portugal
Email: daniela.g.santos@ipleiria.pt

[2] SEAentia
Parque Tecnológico de Cantanhede, Núcleo 04, Lote 2, 3060-197 Cantanhede - Portugal

Introduction

Meagre (*Argyrosomus regius*) is a migratory marine species with widespread distribution in the Mediterranean and eastern Atlantic and receiving currently special attention due to the need of species diversification in Mediterranean mariculture (Fernández-Alacid *et al*, 2019, Duncan *et al,* 2013; Parisi *et al,* 2014). In what concerns to its captive rearing performance, demonstrates a low feed conversion ratio (FCR — 0.9 - 1.2) (Monfort, 2010), high fecundity index of 380 780 ± 167 577 eggs kg^{-1} (Mylonas *et al*, 2013) and high growth rates (1.45 ± 0.02 % day, at 26°C) (Kounna *et al*, 2021). Because of its meat quality and consumer acceptance (Grigorakis *et al,* 2011; Duncan *et al,* 2013), is considered a promising species with the potential to become one of the most cultivated species in the Mediterranean region. However, the characteristics of its meat and growth performance will depend on the farming conditions and the respective production system.

The present study aims to evaluate the nutritional quality and biochemical profile of meagre (*Argyrosomus regius*) reared on a recirculation aquaculture system (RAS).

Material and Methods

This study was conducted in a marine indoor RAS at SEAentia (Peniche, Portugal), which is highly optimized to offer the best growth conditions of meagre, and in MARE - Polytechnic of Leiria (Peniche, Portugal) where samples processing and analysis were performed. Juvenile meagre with a mean body weight of 57.12 ± 8.39 g were sampled after 20 days being fed with a tailor-formulated fishfeed (SPAROS, Olhão, Portugal). Muscle samples from both fillets of ten individuals (n=10) were collected and kept at -80°C. After their lyophilization, samples were analyzed for: (i) total protein by the Kjeldahl method (AOAC, 2016); (ii) lipid content (Folch, Lees & Stanley, 1957), (iii) ash (incineration at 500°C, for 12 hours) and (iv) fatty acids profile by gas chromatography (GC-FID).

Results

Muscle proximate composition showed: 19.8 ± 0.6% of protein; 3.5 ± 1.1% of total lipid content; 1.2 ± 0.1% of ash and 75.2 ± 1.3% of water content. Analysis of fatty acids showed the most abundant n-3 fatty acids were eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with a percentage of abundance of 8.21 ± 0.33% and 13.76 ± 0.79%, respectively. Linoleic acid was the most abundant n-6 fatty acid (8.77 ± 0,28%). The value of hypocholesterolemic and hypercholesterolemic fatty acids ratio was 2.3 ± 0.0. Both atherogenicity index (AI) and thrombogenicity index (TI) were also assessed showing respective values of 0.5 ± 0.0 and 0.3 ± 0.0. Muscle fillets composition presented a ratio n-3/n-6 of 2.4 ± 0.1.

Discussion and Conclusion

Our study showed that SEAentia's meagre has a high quality fillet due to a low fat and high protein profile. Moreover, it showed a very healthy fatty acids profile. This fact shows that meagre is a suitable species for RAS production, having been proven that SEAentia's RAS production system and operations are adequate for meagre growth, guaranteeing its flesh quality.

(Continued on next page)

References

Duncan, N. J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C. M., Roo, J., ... & Vallés, R. (2013). Aquaculture production of meagre (Argyrosomus regius): hatchery techniques, ongrowing and market. In *Advances in aquaculture hatchery technology* (pp. 519-541). Woodhead Publishing. https://doi.org/10.1533/9780857097460.3.519.

Fernández-Alacid, L., Sanahuja, I., Ordóñez-Grande, B., Sánchez-Nuño, S., Herrera, M., & Ibarz, A. (2019). Skin mucus metabolites and cortisol in meagre fed acute stress-attenuating diets: correlations between plasma and mucus. Aquaculture, 499, 185-194.
https://doi.org/10.1016/j.aquaculture.2018.09.039.

Folch, J., Lees, M., Stanley, G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, 226(1), 497-509.

Grigorakis, K., Fountoulaki, E., Vasilaki, A., Mittakos, I., & Nathanailides, C. (2011). Lipid quality and filleting yield of reared meagre (Argyrosomus regius). *International journal of food science & technology*, *46*(4), 711-716.
https://doi.org/10.1111/j.1365-2621.2010.02537.x

Kounna, C., Fountoulaki, E., Miliou, H., & Chatzifotis, S. (2021). Water temperature effects on growth performance, proximate body and tissue composition, morphometric characteristics and gastrointestinal evacuation processes of juvenile meagre, Argyrosomus regius (Asso 1801). Aquaculture, 540, 736683. https://doi.org/10.1016/j.aquaculture.2021.736683.

Monfort, M. C. (2010). Present market situation and prospects of meagre (Argyrosomus regius), as an emerging species in mediterranean aquaculture. *Studies and Reviews-General Fisheries Commission for the Mediterranean*, (89).

Mylonas, C. C., Mitrizakis, N., Castaldo, C. A., Cerviño, C. P., Papadaki, M., & Sigelaki, I. (2013). Reproduction of hatchery-produced meagre Argyrosomus regius in captivity II. Hormonal induction of spawning and monitoring of spawning kinetics, egg production and egg quality. *Aquaculture*, *414*, 318-327. https://doi.org/10.1016/j.aquaculture.2013.09.008.

Parisi, G., Terova, G., Gasco, L., Piccolo, G., Roncarati, A., Moretti, V. M., ... & Pais, A. (2014). Current status and future perspectives of Italian finfish aquaculture. *Reviews in fish biology and fisheries*, *24*(1), 15-73.
https://doi.org/10.1007/s11160-013-9317-7

INSIGHTS FROM HIGH THROUGHPUT NON-CODING RNA SEQUENCING DURING EUROPEAN SEA BASS DEVELOPMENT REARED AT DIFFERENT TEMPERATURES

Maria Papadaki[1,2], Elisavet Kaitetzdiou[1], Ioannis Papadakis[1], Dimitris Sfakianakis[2], Constantinos C. Mylonas[1], Elena Sarropoulou[1*]

[1]Institute for Marine Biology, Biotechnology, and Aquaculture, Hellenic Centre for Marine Research, Greece
[2]University of Crete, Department of Biology, P.O. Box 2208, Heraklion 71409, Crete, Greece

email: sarris@hcmr.gr

Introduction

The European seabass (*Dicentrarchus labrax*) is one of the most extensively cultured species in European aquaculture productions. Environmental effects on the well-being of organisms and especially in teleost have been demonstrated within a broad range of studies. In particular, the temperature is known to control every biological function in such organisms [1], especially during early larval development, and can have both direct effects, such as on the growth rate, the development of skeletal deformities, but also indirect effects, such as on sex differentiation and the final sex ratio of the populations in fish with temperature-dependent sex determination system (TSD). Previous studies in the European sea bass have shown the existence of TSD [2, 3]although suspected in several species, is thought to be evolutionarily unstable and has been proven in very few cases. In the European sea bass, temperature is known to influence the sex ratio. We set up a factorial mating, producing 5.893 individuals from 253 full-sib families, all reared in a single batch to avoid any between-families environmental effects. The proportion of females in the offspring was 18.3%, with a large variation between families. Interpreting sex as a threshold trait, the heritability estimate was 0.62 +/- 0.12. The observed distribution of family sex ratios was in accordance with a polygenic model or with a four-sex-factors system with environmental variance and could not be explained by any genetic model without environmental variance. We showed that there was a positive genetic correlation between weight and sex (r(A. Besides the significance of sex control in aquaculture influenced by environmental factors, embryonic and larval stages represent one of the most critical periods to ensure high performance and superior quality in the subsequent developmental phases of the life cycle. Key developmental events occur early in development and are influenced by external parameters such as stress, temperature, salinity, and photoperiod. Any failure may cause malformations, developmental delays, poor growth, and massive mortalities.

Here we report the influence on miRNAs at three different temperatures during the European sea bass development.

Material and methods

Ethical approval for the study was obtained by the relevant Greek authorities (National Veterinary Services) under the license No 255368 (ΑΔΑ: 6Λ4Σ7ΛΚ-ΩΜΥ). Samples of larvae were collected at the following developmental stages: mouth opening, flexion, all fins, and metamorphosis, and total RNA extraction was carried with the use of the Nucleospin miRNA kit (Macherey-Nagel, Duren, Germany) following the manufacturer's instructions. miRNA libraries were generated using the NEBnext multiplex Small RNA Library Preparation and sequenced over 4 lanes on the Illumina NextSeq sequencing platform at the Microchemistry laboratory of Forth, Crete, Greece. Samples at the same developmental stages were also collected for eye development evaluation and were preserved in 4% formaldehyde:1% glutaraldehyde until histological analysis. For skeletal deformities evaluation, during the samplings, which were conducted at the flexion and the metamorphosis stages, specimens were anesthetized with ethylene glycol-monophenylether (Merck, 0.2–0.5 ml l_ 1), fixed individually in phosphate-buffered 5% formalin, and stored in the dark at room temperature before staining.

Results

In total, 654 million sequencing reads were obtained, with an average of 13 million reads per sample. A correlation matrix based on read counts was generated (Fig.1a) was generated. Expression profiles of all stages at 15°C, 17°C, and 20°C were assessed (Fig.1b). Stages are ordered according to their respective developmental time course confirming correct staging during sampling.

Temperature differences were detected mainly between 15°C versus 20°C (203) and 17°C versus 20°C (145) in the metamorphosis stage. The flexion stage reveals to be of special interest since no differential expression was detected between the temperature 15°C and 20°C but between 15°C and 17°C (321) as well as between 17°C and 20°C (299). The same phenoma has been observed in the development of rod cells in the eye as well as in the lower jaw elongation.

(Continued on next page)

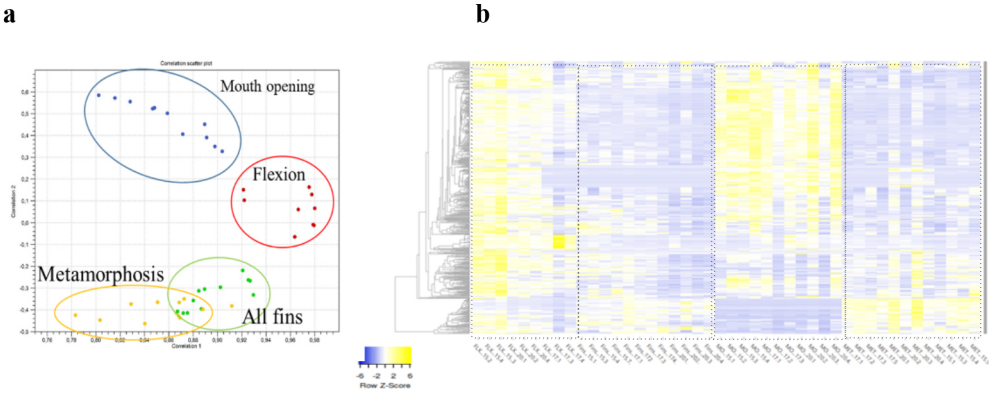


Fig. 1a Correlation matrix of obtained miRNA expression matrix. **1b** Heatmap showing the expression profile of all miRNAs of all stages at 15°C, 17°C and 20°C.

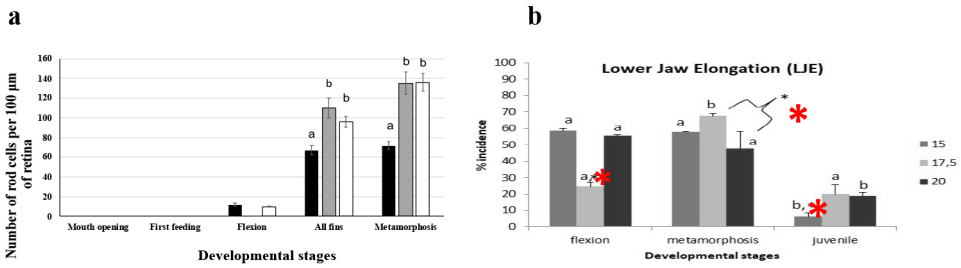


Fig.2a Mean (±SEM) number rod cells in the different developmental stages at the three different temperatures. **b** Mean (± SEM, n=2) incidence (%) of Lower Jaw Elongation deformity in response to rearing temperature. The existence of significant differences is indicated by * (=P<0.05). Different letters indicate significantly different means (between sampling times within temperature groups.

Discussion and conclusions

The present study showed clearly the importance of miRNA concerning temperature changes in early developmental stages. The main stages being influenced by temperature are the metamorphosis as well as the flexion stage. Identified miRNAs are found to be largely regulating genes belonging to the immune response. This finding may indicate that changes in temperature influences the immune system. The particularity of the flexion stage may comprise the first indications that during this stage the gender may be determined since identified miRNAs are found to regulated sex-determining genes. Future research will be focused on the evaluation by functional studies of expressed differentially miRNAs according to temperature changes and their putative target.

References

Anastasiadi D, Díaz N, Piferrer F. Small ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. Sci. Rep. 2017;7.

Vandeputte M, Dupont-Nivet M, Chavanne H, Chatain B. A polygenic hypothesis for sex determination in the European sea bass *Dicentrarchus labrax*. Genetics. 2007;176:1049–57.

Mylonas CC, Anezaki L, Divanach P, Zanuy S, Piferrer F, Ron B, et al. Influence of rearing temperature during the larval and nursery periods on growth and sex differentiation in two Mediterranean strains of *Dicentrarchus labrax*. J. Fish Biol. 2005;67:652–68.

Funding: This study was carried out within the MedAid project, funded by the European Union in the frame of Horizon 2020, grant agreement number 727315.

POPULATION STRUCTURE AND GENETIC VARIABILITY BASED ON GENOMIC INFORMATION IN GILTHEAD SEABREAM AND EUROPEAN SEABASS POPULATIONS

M. Saura[1*], A. Fernández[1], J. Fernández[1], R. Peiro-Pastor[1], C. Peñaloza[2], L. Bargelloni[3], CS Tsigenopoulos[4] and B. Villanueva[1]

[1] INIA (Spain)
[2] The Roslin Institute, University of Edinburgh (UK)
[3] University of Padova (Italy)
[4] HCMR (Greece)
saura.maria@inia.es

Introduction

Gilthead sea bream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) are the most important marine farmed fish in the Mediterranean. In recent years, different companies have initiated breeding programmes and the proportion of genetically improved stocks has increased.

Understanding population structure and genetic diversity within and between wild (*W*) and farmed (*F*) populations is of paramount importance to develop optimal strategies for the conservation of *W* populations and to achieve sustainable aquaculture production of Mediterranean seabream and seabass. In particular, the success of any breeding programme critically depends on the way in which the base population of breeders is built and on the control of inbreeding (or equivalently, the effective population size, N_e) once selection programmes are running. Also, although little is known on escapees from seabream and seabass farms, they pose an ecological risk of transferring diseases to wild fish and may cause undesirable genetic effects in native populations due to interbreeding.

Recently, a combined 60K SNP array for both species has resulted from the collaboration between the EU projects MedAID and PerformFISH and is an essential tool for genetic studies for estimating N_e and relationships between populations (Peñaloza et al. 2021). The objectives of this study were to i) identify the extent of *W* and *F* population stratification within species considering the geographic origin of individuals; and ii) assess the genetic status of *W* and *F* populations through the estimation of current N_e.

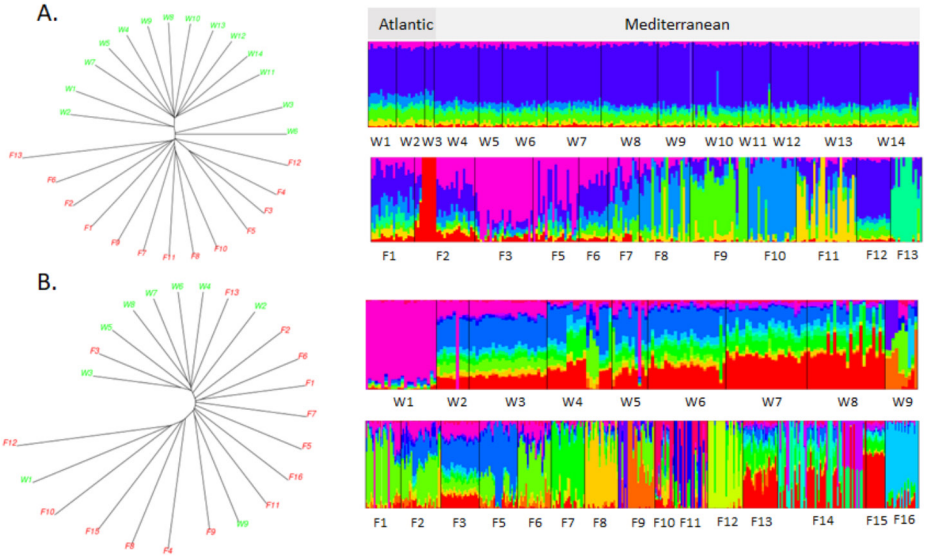


Fig.1. Evolutionary dendrograms (left) and admixture plots (right) for seabream (A) and seabass (B) populations analysed. Populations are numbered in accordance to the geographic proximity from West to East (*W*: in green in the dendrograms; *F*: in red in the dendrograms).

(Continued on next page)

Material and Methods

Samples were collected from *W* and *F* populations of both species from East to West Mediterranean. Three populations of seabream from the Atlantic were also sampled. For seabream, SNP genotypes were available for 462 individuals sampled from 14 *W* and 12 *F* populations. For seabass, SNP genotypes were available for 516 individuals collected from 9 *W* and 15 *F* populations. After quality filtering the total number of SNPs available for analysis was 25,319 (seabream) and 22,507 (seabass). Population structure was assessed through (i) Principal Component Analysis (PCA) using the software PLINK (Purcell et al. 2007) and a R script; (ii) distant-based clustering methods using an R-package; (iii) ancestry clustering using ADMIXTURE (Alexander and Lange, 2011); and (iv) pairwise F_{ST} using Metapop2 (López-Cortegano et al. 2019). Current estimates of N_e were obtained from linkage disequilibrium between independent SNPs as implemented in the software NeEstimator (Do et al. 2014).

Results

Results from PCA did not show a clear differentiation between *W* and *F* populations, and showed a considerably dispersion within and between populations. However, clustering methods and F_{ST} revealed a clear differentiation between *W* and *F* populations. Despite the little differentiation among *W* populations, Atlantic/Mediterranean (seabream) and West/East Mediterranean (seabream, seabass) patterns were detected (Fig. 1). In general, N_e was large (> 1000) for *W* and small (< 100) for *F* populations of both species, with some exceptions. Farmed populations were more heterogeneous and presented in many cases critical values of N_e. Average F_{ST} was lower between *W* (0.020 for seabream, 0.047 for seabass) than between *F* (0.033 for seabream and 0.051 for seabass) populations.

Discussion

In general, our results are in line with those obtained in the EU AquaTrace project (https://cordis.europa.eu/project/id/311920/reporting/es) that used a much more limited number of SNPs to explore population structure and genetic variability of wild and farmed populations of both species (about 1,200 for seabream and 2,700 for seabass). They also suggested that wild populations are more similar among them and present higher genetic variability than farmed populations, and that there are subtle levels of population structure in both species. The low differentiation between wild populations indicates that considerable gene flow exists, facilitating sampling decisions when base populations are established. These results also indicate that there are no specific requirements for conservation of wild populations. The low N_e estimated for some farmed populations highlight the need of applying measures to increase this size to ensure the sustainability of the breeding programmes. Finally, since farmed populations differ from wild populations, escapees from farms should be avoided and only wild broodstock should be used if fish stocking were done. In summary, this report evidence the high potential of the genomic tool developed by the MedAID project (i.e., the MedFish SNP array) for establishing and monitoring breeding programmes in aquaculture. This is the first time a population genetic analysis in these species has been carried out with such a high number of SNP markers.

References

Alexander DH, Lange K (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12:246.

Do C et al. (2014). NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Mol. Ecol. Resour. 14, 209-214.

López-Cortegano et al. (2019). Metapop2: Re□implementation of software for the analysis and management of subdivided populations using gene and allelic diversity. Mol. Ecol. Resour. 19(4):1095-1100.

Peñaloza C, et al. (2021). Development and testing of a combined species SNP array for the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). Genomics 113(4): 2096-2107.

Purcell et al (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses Am. J Hum. Genet. 81(3):559-75.

HATCHERY PRODUCTION OF FLAT OYSTER (*Ostrea edulis*) FOR CONSERVATION AND AQUACULTURE IN *Bonamia* INFESTED AREA: A COMBINATION OF VARIOUS TOOLS

Camille Saurel*[1], Pascal Barreau[1], Lone Madsen[2], Homère J. Alves Monteiro[3], Nicolas Araujo Piñeiro[1], Pernille Nielsen[1], Jens Kjerulf Petersen[1]

[1]Technical University of Denmark, National Institute of Aquatic Resources DTU Aqua, Section for Coastal Ecology, Øroddevej 80, 7900 Nykøbing Mors, Denmark
[2]DTU Aqua, Unit for Fish and Shellfish Diseases, Kgs. Lyngby, Denmark
[3]DTU Aqua, Section for Marine Living Resources, Silkeborg, Denmark

E-mail: csau@aqua.dtu.dk

Introduction

Until a couple of years ago, the flat oyster (*Ostrea edulis*) population was thriving in the Limfjorden (Denmark), with a sustainably regulated fishery with landings up to 320t (2018/2019) and the identification of new populations. Although, the pathogen *Bonamia ostrea* had been detected from national surveys in flat oyster individuals in 2014 in aquaculture sites and in 2017 in the wild, no mass mortality events had been observed. But in late 2019, fishermen reported the first event of mass mortality in some fishing areas. *Bonamia* outbreaks are known for causing up to 70-90% mortality of flat oyster populations. It was not the first time since the recolonization of the Limfjorden 170 years ago by flat oysters that *Bonamia* had been detected. During this period, millions of flat oysters from several places in Europe were imported to supplement the local population for exploitation. These imports potentially have led to genetically mixed populations, and were the cause of the first introduction of *Bonamia* in the Limfjorden in 1980 from an oyster batch from France. The infected oysters were removed, however, no surveys were conducted until 1996 when a national monitoring program started, lasting until 2018. During 1996-2014, no *Bonamia* was detected and the Limfjorden was declared *Bonamia sp*. and *Marteilia refringens*-free.

After the 2019 mass mortality event, we conducted a new sampling program and found that *Bonamia* was present and spreading to new areas. At the same time, specimens were sampled for genetic diversity. Simultaneously, the sole flat oyster hatchery in Denmark implemented a new strategy to produce *Bonamia*-free spat and preserve high genetic diversity in the offspring: i) implementation of new biosecurity measures, ii) establishment of broodstock with new tested specimens from areas where *Bonamia* had not been detected, iii) collaboration with fishermen for mass mortality monitoring, and iv) a genetic program for establishing the broodstock genetic diversity, effective population size (Ne) and effective number of breeders (Nb). In parallel, alongside the reliable spat production protocol already developed for aquaculture, new protocols for spat-on-shell production for restoration were developed in the hatchery.

Material and methods

Molecular methods were used for screening of the oysters samples from the wild and in the hatchery: a duplex Real Time PCR method for *Bonamia* sp and *Marteilia refringens* (Canier et al. 2020). Oysters from the broodstock were anesthetised (Suquet et al. 2010) for gill sampling for both genetic diversity, effective number of breeders and pathogen analysis prior entering the hatchery and post reproduction. The population structure of the Limfjorden's flat oyster along its distribution range will be studied using low-coverage whole genome sequencing data. Western Europe and Scandinavian populations are included in this DNA-sequencing dataset, which will permit "global-to-local" scales of comparison. Using seventeen microsatellite markers, we gathered genotypic information for the entire broodstock (of 2019, 2020, and 2021) and assigned offspring with high confidence.

Results and discussion

The presentation will describe the different tools put in place to produce *Bonamia*-free spats, ensure genetic diversity of the broodstock, monitor wild oyster population genetic diversity, mapping of wild population biomass fluctuation and *Bonamia* infestation, and consolidate and develop hatchery protocols. Ensuring harvesting of new *Bonamia*-free broodstock in the Limfjorden is challenging due to the rapid spread of the disease and the chronic nature of the pathogen. Biosecurity measures and repeated screenings are necessary to maintain disease-free hatchery compartments. The effective number of breeders (Nb) and effective population size (Ne) are important parameters for a sustainable spat production especially for restoration. The developed protocols for spat-on-shell production for restoration was successful and scalable to large production. Finally, with the new construction of a large-scale commercial hatchery including a quarantine, biosecurity measures and high water quality and treatment, the establishment of a secure *Bonamia* free compartment in the Limfjorden is tangible.

(Continued on next page)

References

Canier L, Dubreuil C, Noyer M, Serpin D, Chollet B, Garcia C, Arzul I. (2020) A new multiplex real-time PCR assay to improve the diagnosis of shellfish regulated parasites of the genus *Marteilia* and *Bonamia*. Prev Vet Med.183:105126. doi: 10.1016/j.prevetmed.2020.105126.

Suquet, M., Araya R.G., Lebrun L., Queau I., Mingant C., Robert R. (2010)
Anaesthesia and gonad sampling in the European flat oyster (*Ostrea edulis*)
Aquaculture 308:196-198

ASSESSMENT OF OPTIMAL TEMPERATURE AND SALINITY CONDITIONS TO CULTURE *Aurelia solida*

S. Schäfer[1,2*], S.K.M. Gueroun[1,3,4], C. Andrade[3,4,5] and J. Canning-Clode[1,6]

[1] MARE - Marine and Environmental Sciences Centre, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Madeira, Portugal
[2] GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany.
[3] Maricultura Centre of Calheta, Calheta, Madeira, Portugal
[4] OOM - Madeira Oceanic Observatory, Funchal, Madeira, Portugal
[5] CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal
[6] Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA.
* Email: sschaefer@mare-centre.pt

Introduction

Jellyfish blooms and their consequences have been of increasing interest over the last decades, often highlighting challenges for local economies (Purcell et al., 2015). More recently, studies started investigating new opportunities and the potential use of jellyfish for a great variety of purposes such as human consumption, aquaculture feed, cosmetics, or microplastic filter (Torri et al., 2020; Prieto et al., 2018). For example, *Aurelia aurita*, has been presented as a crispy product with a potential gastronomic interest (Pedersen et al., 2017). This new marketing of jellyfish drives the interest in jellyfish culture and the possibility to produce fast-growing biomass for economic purposes. Understanding jellyfish ecology is crucial to determine optimal culture conditions as studies have shown that there are differences between closely related species (Hubot et al., 2017). In this context, the present study tested the effect of different temperature and salinity regimes on the rearing success of *Aurelia solida*.

Material and Methods

Mesocosm experiments investigated the simultaneous effects of temperature and salinity on the rearing of the jellyfish *Aurelia solida*. Experiments were performed on two different life stages, polyps and ephyra, at the mesocosm system (MOSS) and laboratory facilities at the Madeira research unit of MARE – Marine and Environmental Research Centre, located at Quinta do Lorde Marina, Madeira, Portugal. Temperature and salinity (S) levels represented values typically found for the species across their distributional range and covered temperatures between 10 and 28°C and salinities of 20 up to 40. Survival, asexual reproduction of polyps and growth of ephyra were monitored for several weeks to determine successful rearing conditions for *A. solida*.

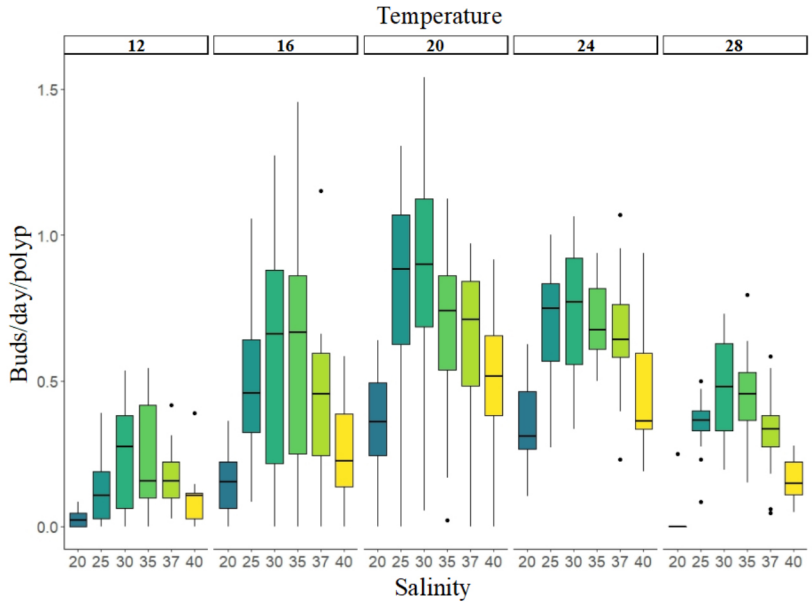


Fig. 1. Daily bud production of *A. solida* polyps kept at 30 different combinations of temperature (12, 16, 20, 24, and 28°C) and salinity (20, 25, 30, 35, 37, and 40).

(Continued on next page)

Results

Survival of the *A. solida* polyps was generally high (83-100%) in almost all treatments. Only the combination of the highest temperature (28°C) and lowest salinity (20), resulted in 0% survival. Polyps showed asexual reproduction in all combinations but produced the most at 20°C and 30S (Figure 1). Strobilation occurred mainly at 16°C and 35S.

Ephyrae kept at lower temperatures (10 and 15°C) showed the highest survival rates. However, preliminary results suggest that the highest growth rates were reached at 20°C in combination with 20 and 35S.

Discussion and Conclusion

The best conditions for asexual reproduction in *A. solida* polyps was at intermediate temperatures (20°C) and lower than marine salinities (25 and 30). In contrast, although ephyrae growth was highest at intermediate temperatures as well (20°C), it increased at lower salinities (20). Comparing these results to studies on other *Aurelia* species, we can see differences in optimal rearing conditions regarding temperature and salinity depending on species: *Aurelia aurita* showed highest budding rates at higher temperatures (28°C; Pascual et al., 2015), and *Aurelia labiata* showed higher budding rates at lower temperatures (7 and 10°C; Purcell, 2007). These comparisons highlight the need for laboratory studies for individual species to advance our current understanding on best rearing conditions for the particular species in order to optimize jellyfish culture.

References

Hubot, N., Lucas, C.H., Piraino, S., 2017. Environmental control of asexual reproduction and somatic growth of *Aurelia* spp. (Cnidaria, Scyphozoa) polyps from the Adriatic Sea. PLoS One 12.

Pascual, M., Fuentes, V., Canepa, A., Atienza, D., Gili, J.-M., Purcell, J.E., 2015. Temperature effects on asexual reproduction of the scyphozoan *Aurelia aurita* s.l.: Differences between exotic (Baltic and Red seas) and native (Mediterranean Sea) populations. Mar. Ecol. 36, 994–1002.

Pedersen, M.T., Brewer, J.R., Duelund, L., Hansen, P.L. 2017. On the gastrophysics of jellyfish preparation. Int J Gastron Food Sci. 9: 34–38.

Prieto, L., Enrique-Navarro, A., Volsi, R.L., Ortega, M.J., 2018. The large jellyfish *Rhizostoma luteum* as sustainable resource for antioxidant properties, Nutraceutical Value and Biomedical Applications. Mar. Drugs 16.

Purcell, J.E., 2007. Environmental effects on asexual reproduction rates of the scyphozoan *Aurelia labiata*. Mar. Ecol. Prog. Ser. 348, 183–196.

Purcell, J., Mianzan, H., Frost, J.R., 2015. Jellyfish blooms IV: interactions with humans and fisheries. Vol. 220. Springer

Torri, L., Tuccillo, F., Bonelli, S., Piraino, S., Leone, A., 2020. The attitudes of Italian consumers towards jellyfish as novel food. Food Qual. Prefer. 79.

EX SITU CULTIVATION OF THE MARINE SPONGE *Chondrosia reniformis* IN NOVEL LAND-BASED AQUACULTURE FOR BIOMASS AND COLLAGEN PRODUCTION

K. Schiefenhövel*, M. Schiffer-Harms, W. Schatton, M.J. Slater and J. Henjes

Alfred Wegener Institute, Marine Bioeconomy, Aquaculture Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
*Email: karin.schiefenhoevel@awi.de

Introduction

Marine sponges are one of the most important sources of bioactive compounds used in medicine, industry and as nutraceuticals. As sessile organisms, sponges have developed chemical defense mechanisms to avoid covering by algae, bacteria and infectious microorganisms (Thoms et al., 2006). The Mediterranean sponge species *Chondrosia reniformis* is of special interest due to its high collagen content (Swatschek et al., 2002). However, a large amount sponge biomass is required to obtain a high yield of collagen. To avoid overharvesting of natural sponge resources special culture techniques need to be developed. However, to date no land-based cultivation and high mass production of *C. reniformis* has been possible due to their sensitivity to transportation and high demands on aquaculture conditions (Nickel and Brümmer, 2003).

The present study aims to determine optimal conditions in recirculating aquaculture system (RAS) to allow *C. reniformis* fragments to grow and create a sustainable source of sponge biomass with commensurate maximized collagen production.

Materials and methods

C. reniformis were obtained by SCUBA from the coast of the Conero Promontory (Italy) in the Adriatic Sea and subsequently raised in a recirculation aquaculture system at the Alfred Wegener Institute, Bremerhaven (Germany).

In a first experiment sponge fragments were cut into fragments and glued on an artificial substrate before the effect of nutrient content, light and water drain position on sponge attachment, survival, growth and collagen content were examined at specific intervals. They were fed a mixture of commercial liquid diet and microalgae.

In a second experiment, feeding strategies and feed types were tested. Sponge fragments were fed with either the commercial liquid diet for filter feeders or a novel formulated diet. Sponges were fed once a day for four weeks, then at increased feeding frequency for another four weeks after sacrificing half of the respective fragments. Attachment, fragment growth, survival rate and collagen content were measured at the end of the experiment. Fragment growth was measured photographically. Collagen content was detected by the company KliniPharm GmbH. Collagen was dissolved by alkaline hydrolysis and the solution was analyzed by quantitative color reaction (Roti®-Quant Universal).

Results

In the first experiment, fragments remained attached and after a week began to encrust the substrate. Mean fragment growth curves measured in cm^2 initially increased, but decreased again after one to two months. Mortality was 26.1% in total. Best survival rates were detected under blue light and tank drain at the bottom. Nutrient content (ammonia, nitrite, and nitrate) fluctuated markedly throughout the experiment. Collagen content ranged from 5% to 25% with twenty percent of samples having less than 10% collagen.

In the second experiment, attachment and survival rate was high with 81% and 91%, respectively. Nutrient levels remained low throughout the experiment. A bespoke *C. reniformis* diet was used (diet 1). On average sponge fragments fed with diet 1 had a higher collagen content than those fed with diet 2 (commercial diet) (Fig. 1). After four weeks fed once a day sponge collagen content ranged from 11% to 30% with sixty percent of samples having less than 20% collagen. After another four weeks fed at increased feeding frequency sponge collagen content ranged from 19% to 80% with half of samples exhibiting a collagen content above 30%.

Discussion and conclusion

To avoid high mortality rates of sponge fragments the aquaculture system has to provide stable values of abiotic factors. To increase the possibility of physical adaptation to the new hydrodynamic patterns, moving of fragments during experiments was avoided, hence, measurements of growth rely on two values (at the start and end of experiment).

(Continued on next page)

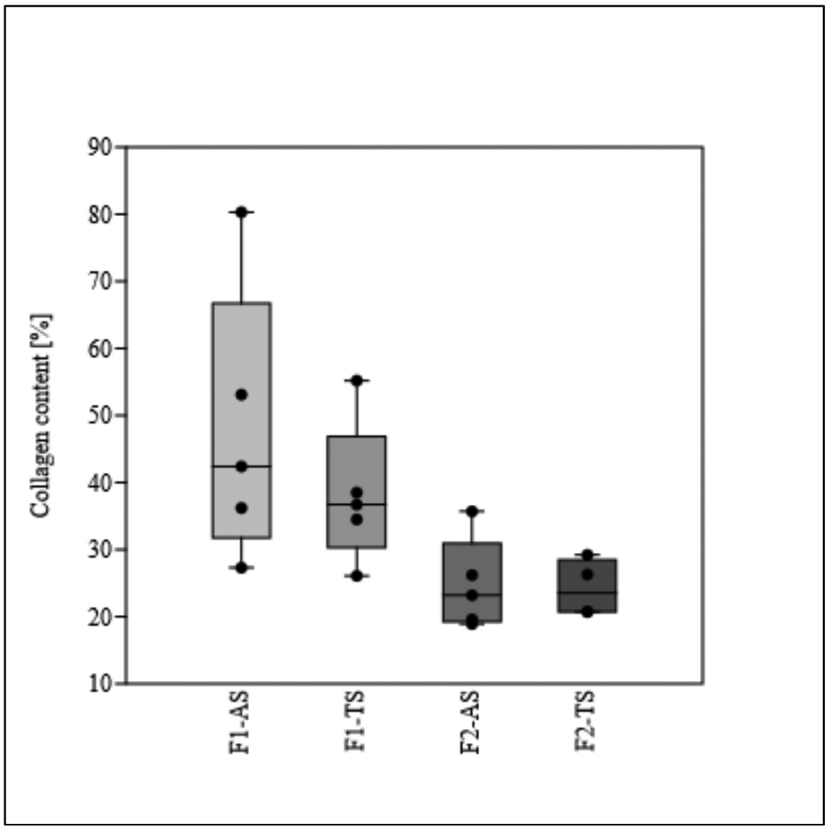


Fig.1 Boxplot of collagen content [%] (F1 = diet 1, F2 = diet 2, AS = artificial substrate, TS = bowl made of clay)

One of the important parameters for successful land-based aquaculture is an optimal feed supply and nutrient content. Additionally, sponge growth is dependent on the amount of feed supplied; high loads of feed can lead to blockage of the ostia and too low feed supply in starving of sponge fragments (Wilkinson, 1983).

In conclusion, an aquaculture system with optimized rearing conditions including stable nutrient water retreatment processes and an optimal feed is essential for survival, attachment, growth, amount of collagen content.

References

Nickel, M., and F. Brümmer. 2003. In vitro sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847). Jorunal of Biotechnology. Vol. 100, Issue 2.

Swatschek, D., W. Schatton, J. Kellermann, W.E.G. Müller and J. Kreuter. 2002. Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. European Journal of Pharmaceutics and Biopharmaceutics 53: 107-113.

Thoms, C., R. Ebel and P. Proksch. 2006. Activated chemical defense in *Aplysina* sponges revisited. Journal of Chemical Ecology. Vol. 32, No.1.

Wilkinson, C.R. 1983. Role of sponges in coral reef structural processes. p.263-274. In: Perspectives on Coral Reefs. D.J. Barnes (Ed.) 277p.

MEDSPON - CHARACTERIZATION OF NEW ANTIBIOTIC PRINCIPLES AGAINST WHO PRIORITY PATHOGENS OF SUSTAINABLE PRODUCED MARINE SPONGES FOR PHARMACEUTICAL APLICATIONS

M. Schiffer-Harms*, K. Schiefenhövel, W. Schatton, M. Slater and J. Henjes

Alfred Wegener Institute, Marine Bioeconomy, Aquaculture Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
Email: Melanie.schiffer@awi.de

Background
Marine sponges are highly valuable sources of bioactive compounds with pharmaceutical potential. Over 55% of almost 300 pharmacological marine products at different stages of clinical exploration are of sponge origin. Among these sponges *Chondrosia reniformis* and *Axinella polypoides*, two Mediterranean sponge species, are of special interest due to the high concentration (> 30%) of type I collagen in the sponge biomass and a broad-spectrum of antibiotic activities, respectively.

One major constraint regarding the industrial application of bioactive substances from marine sponges is a sufficient supply of sponge biomass to process the purified compound. However, removal of large quantities of biomass from the marine habitat might have major ecological consequences. Land-based aquaculture represents an interesting economic alternative to grow marine sponges for production of bioactive compounds. Optimal species-specific conditions for cultivation can be employed in closed recirculating aquaculture systems (RAS) providing sustainable and reliable production and allowing location-independent production of sponge biomass.

Objectives
The aim of the project MedSpon is to develop and establish an aquaculture process for *C. reniformis* and *A. polypoides*, with knowledge from mariculture, by successfully breeding sponge fragments to serve as a source of secondary metabolites. MedSpon will drive the supply of new secondary metabolite sources through convenient recirculating aquaculture system (RAS) conditions for sponge fragments, creating a sustainable, optimised, source of sponge biomass. Strong wild populations of the target species will be studied at Italian field sites, to assess habitat specifications and provide information for mass production in RAS under controlled conditions. Abiotic factors and nutrition will be tested to create optimal rearing conditions for bioactive content. Formation potential of bioactive compounds and associated microbial communities with different sponge species/rearing conditions will be verified to create the maximal bioactive content with highest quality.

Task
For the experiments, the abiotic factors such as temperature, light, flow and nutrient content in RAS will be adapted to the conditions of the native habitat. The sponge individuals of the two species will be cut into similarly sized fragments and are allowed to attach to a clay pot as substrate. Preliminary work has developed a self-designed novel formulated diet for the species *C. reniformis*. Therefore, experiments will concentrate on different feeding strategies. For experiments with fragments of the species *A. polypoides* the parameters light (different spectra), temperature, flow and the filter system will be changed in experimental approaches. Furthermore, three different feeds will be offered to the fragments. Molecular methods (Amplicon sequencing) will be used to determine the symbiont composition in each of the two sponge species in relation to the factors tested in the experiments.

Project consortium
Polytechnic University of Marche, Italy: UNIVPM will co-operate with other partners providing samples for sponge aquaculture ex-situ and produce sponge biomass from sponge aquaculture in-situ to optimize sponge aquaculture in eutrophic and oligotrophic areas. With sound experience in experimental sponge cultivation in-situ and in habitat restoration, UNIVPM activities will be transversal to the other partners.

Alfred Wegener Institute (AWI), Germany: The tasks of AWI will be the detection and adaption of special suitable conditions in a land-based recirculating aquaculture system for cultivation and reproduction of the sponge species with the aim of building up a sustainable source of sponge biomass with a maximized yield of target metabolites.

SpongiPharm EPE, Greece: Possessing the necessary preparatory and analytical laboratory infrastructure, SpoPha will establish primary antibiotic screening of the sponges and their extracts. Sponge candidates will then be identified, and living sponge samples forwarded to the consortium partners for in-vitro sponge culture further developments.

KliniPharm GmbH: KP will discover novel antibiotic principles in sponges. Most interesting is an enzyme based antibiotic activity, e.g. in *Axinella polypoides*, unknown in animals. It blocks the growth of many bacteria of the WHO list of priority pathogens for research and development of new antibiotics.

FROM AQUACULTURE TO AQUACULTURE: PRODUCTION OF THE FISH FEED ADDITIVE ASTAXANTHIN FROM AQUACULTURE SIDESTREAMS IN *Corynebacterium glutamicum*

Ina Schmitt*, Florian Meyer, Nadja A. Henke, Petra Peters-Wendisch and Volker F. Wendisch

Genetics of Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Bielefeld, Germany
E-Mail: ina.schmitt@uni-bielefeld.de

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.

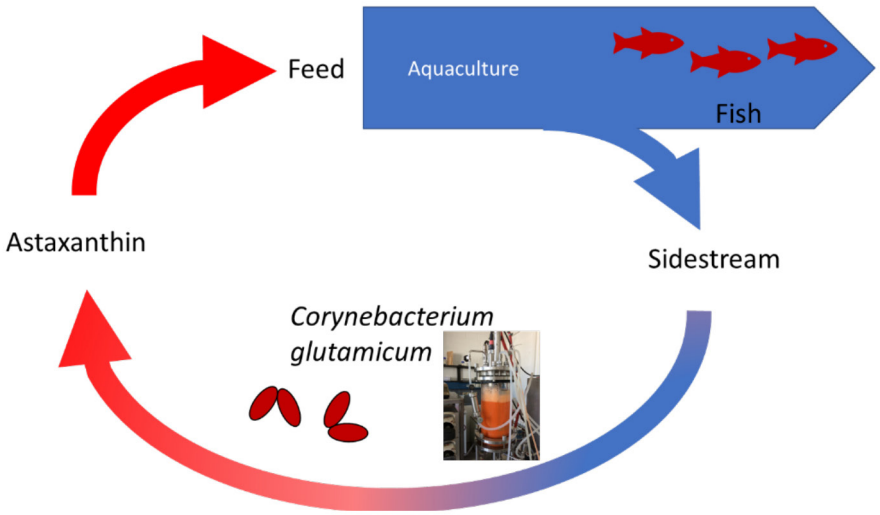
Corynebacterium glutamicum is used in the million-ton-scale fermentative amino acid production (Wendisch, 2020), but has also been engineered for production of functionalized amines, diamines, organic acids, alcohols or isoprenoids (Mindt et al., 2020). *C. glutamicum* naturally synthesizes the yellow C50 carotenoid decaprenoxanthin and was engineered for the production of the red C40 carotenoid astaxanthin (Henke et al., 2016; Henke & Wendisch, 2019). 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.

In this study, we aimed to utilize an aquaculture side stream (AQ) for production of astaxanthin. 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 the native decaprenoxanthin as well as production of astaxanthin. The AQ improved astaxanthin production was scaled-up to 2 L bioreactor fermentations.

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.



(Continued on next page)

Henke, N. A., & Wendisch, V. F. (2019). Improved Astaxanthin Production with Corynebacterium glutamicum by Application of a Membrane Fusion Protein. *Marine Drugs*, *17*(11), 621. https://doi.org/10.3390/md17110621

Henke, N. A., Wiebe, D., Pérez-García, F., Peters-Wendisch, P., & Wendisch, V. F. (2018). Coproduction of cell-bound and secreted value-added compounds: Simultaneous production of carotenoids and amino acids by *Corynebacterium glutamicum*. *Bioresource Technology*, *247*, 744–752. https://doi.org/10.1016/j.biortech.2017.09.167

Henke, N., Heider, S., Peters-Wendisch, P., & Wendisch, V. (2016). Production of the Marine Carotenoid Astaxanthin by Metabolically Engineered *Corynebacterium glutamicum*. *Marine Drugs*, *14*(7), 124. https://doi.org/10.3390/md14070124

Mindt, M., Walter, T., Kugler, P., & Wendisch, V. F. (2020). Microbial Engineering for Production of N-Functionalized Amino Acids and Amines. *Biotechnology Journal*, *15*(7), 1900451. https://doi.org/10.1002/biot.201900451

Wendisch, V. F. (2020). Metabolic engineering advances and prospects for amino acid production. *Metabolic Engineering*, *58*, 17–34. https://doi.org/10.1016/j.ymben.2019.03.008

Wendisch, V. F., Brito, L. F., Gil Lopez, M., Hennig, G., Pfeifenschneider, J., Sgobba, E., & Veldmann, K. H. (2016). The flexible feedstock concept in Industrial Biotechnology: Metabolic engineering of *Escherichia coli*, *Corynebacterium glutamicum*, *Pseudomonas*, *Bacillus* and yeast strains for access to alternative carbon sources. *Journal of Biotechnology*, *234*, 139–157. https://doi.org/10.1016/j.jbiotec.2016.07.022

INNOVATIVE BIODEGRADABLE SEED-MUSSEL COLLECTORS TO OPTIMIZE MUSSEL YIELD

Jildou Schotanus[1*], Jacob J. Capelle[2], Lisanne van den Bogaart[2], Wim Bakker, Tjeerd J. Bouma[1]

[1]NIOZ - Koninklijk Nederlands Instituut voor Zeeonderzoek, Korringaweg 7, 4401 NT Yerseke, The Netherlands
[*]Email: jildou.schotanus@nioz.nl
[2]WMR - Wageningen Marine Research, P.O. Box 77, 4400 AB Yerseke, The Netherlands

To reduce fishing on wild mussel beds Dutch mussel growers increasingly obtain mussel seed (juvenile mussels) through so-called seed mussel collectors (SMCs). A major disadvantage of the current SMC-systems is the increase in costs compared to the traditional fishing of mussel seed from natural reefs. In this study, we tested whether biodegradable (starch-based) socks filled with cockle shells can provide a cost-efficient and sustainable alternative to the regular nylon seed collectors.

While mussel seed is normally harvested from the SMCs and then seeded separately onto culture plots, these biodegradable SMCs can be harvested and seeded in their entirety. We hypothesized that leaving the mussel seed attached to the biodegradable SMC when seeded, will reduce losses caused by predation and hydrodynamic forces. Therefore, mussel seed may be seeded at an earlier and smaller stage and the mussel production will increase.

To examine how the seeding method in combination with seeding time will influence mussel production, a field experiment was carried out on a subtidal culture plot in the Dutch Oosterschelde estuary. Mussels were transplanted in three configurations namely, (1) attached to the intact biodegradable SMC, (2) attached to the biodegradable SMC but with a cut open sock and (3) detached from the SMC and only seeded with loose shells. This experiment was deployed in July when the average mussel seed length was around 1 cm and in August when length was around 2 cm. In addition, a laboratory experiment was conducted to better understand the predation rate by shore crabs and sea stars when mussels are seeded in these three configurations.

After nine months mussel production of the mussels seeded in August was significantly higher when mussels were still attached to the intact biodegradable SMC. Seeding of mussels at an earlier stage, with smaller mussels, was not feasible because of high predation rates by crabs.

Our results show that biodegradable shell-socked SMCs promises to be an effective method to increase mussel production. The next step will be to scale up and examine how different seeding configurations will influence mussel production.

EUROPEAN SEABASS (*Dicentrarchus labrax*) ALLERGENICITY AND FISH QUALITY AFTER EDTA SUPPLEMENTATION

D. Schrama[1,2,*], C. Raposo de Magalhães[1,2], Annette Kuehn[3], S. Engrola[2], P.M. Rodrigues[1,2]

[1]Universidade do Algarve, Campus de Gambelas, Faro, Portugal
[2]CCMAR, Universidade do Algarve, Faro, Portugal
[3]Luxembourg Institute of Health, Department of Infection and Immunity, Esch-sur-Alzette, Luxembourg
E-mail: dschrama@ualg.pt

Introduction

Allergies to fish are a significant public health concern throughout the world and show sensitization prevalence's of up to 2.9% in the general population [1]. The main fish allergen is β-parvalbumin; a small and highly stable muscle protein, and responsible for 95% of the allergic responses. Fish parvalbumins are highly conserved proteins, which bind bivalent ions, like calcium or magnesium. Previous works showed that EDTA is a calcium chelator and might reduce the IgE-binding capacity of the main fish allergen, due to a structural rearrangement resulting in an apo-form. The objective of this study was to diminish the allergenic potential of European seabass through the supplementation of EDTA in fish diets.

Methodology

Twenty-two European seabass juveniles with an initial body weight (IBW) of 174 ± 1.29 g were reared in 500L conical tanks for 98 days. Four different experimental diets were tested (in triplicates) namely, Control (CTRL, commercial diet without EDTA supplementation), 1.5, 3 and 4.5% of EDTA supplementation in the commercial diet, which will be referred as EDTA1.5, EDTA3 and EDTA4.5, respectively. Fish were reared under optimal environmental (dissolved oxygen above 5 mg L^{-1}) and natural photoperiod conditions. Fish were fed twice a day by hand, *ad libitum*. All fish were lethally anaesthetized with tricaine methanesulfonate (MS-222) and sampled for biochemical and quality analysis. Proteins from muscle samples were used for allergenicity quantification by sandwich IgE-ELISA using serum from fish allergic patients.

Results

At the end of the trial, the fish final body weight (FBW) was not significantly affected by any of the diets (Table1). However, feed conversion ratio (FCR) was negatively affected by the EDTA4.5 diet when compared to CTRL (one-way ANOVA, followed by post-hoc Tukey, $p<0.05$).

Fish quality, muscle pH and *rigor mortis* index were measured during the first 72 h *post-mortem*. Muscle pH decreased with time without showing significant differences between the experimental diets. Fish reached almost a full *rigor* index 24 h *post-mortem* with significant differences at T1 (1 hour *post-mortem*) and T48. As mentioned, supplementation with EDTA would induce the apo-form of parvalbumin, a less allergenic structure due to the blocking of the functional epitopes of the protein. The analysis of IgE-reactivity using fish allergic patients' serum (n=6) showed no significant differences in the modulation of parvalbumin and therefore supplementation with EDTA up to 4.5% seems not to reduce the allergenicity of European seabass.

Conclusion

Dietary EDTA supplementation preserved fish quality as edible food product. IgE-ELISA showed that the supplemented chemical into fish feed was unable to modulate fish allergenicity.

As future works a different rearing technique will be tested to reduce the allergenic potential of European seabass. Also, different *post-mortem* treatments will be studied in the two mean Mediterranean species, European seabass and gilthead seabream.

Table1. Growth performance parameters of European seabass fed the experimental diets (CTRL, EDTA1.5, EDTA3, EDTA4.5).

Diet	FBW (g fish $^{-1}$)	FCR
CTRL	367 ± 12	1.50 ± 0.09*
EDTA1.5	363 ± 15	1.64 ± 0.03
EDTA3	345 ± 16	1.64 ± 0.05
EDTA4.5	339 ± 13	1.67 ± 0.05*

FBW – Final body weight, FCR – feed conversion ratio. Data are represented by mean ± standard deviation.
* Significant differences by one-way ANOVA followed by post-hoc Tukey ($p<0.05$)

(Continued on next page)

Acknowledgements

This work received Portuguese national funds from FCT- Foundation for Science and Technology through project UID/04326/2020. This work integrates in project 16-02-01-FMP-0014- Allyfish co-financed by Mar2020 (4107IDNAD50308.18). Denise Schrama and Cláudia Raposo de Magalhães acknowledge FCT for PhD scholarships (SFRH/BD/136319/2018 and SFRH/BD/138884/2018)

Bibliography

1. Klueber, J.; Schrama, D.; Rodrigues, P.; Dickel, H.; Kuehn, A. Fish Allergy Management: From Component-Resolved Diagnosis to Unmet Diagnostic Needs. *Curr. Treat. Options Allergy* **2019**, *6*, 322–337, doi:10.1007/s40521-019-00235-w.

VARIATIONS IN ENERGY UTILISATION EFFICIENCIES OF DIGESTED PROTEIN, FAT AND CARBOHYDRATES IN DIFFERENT FISH SPECIES

L.T.T. Phan[1*], J. Kals[4], K. Masagounder[2], J. Mas-Muñoz[3] & J.W. Schrama[1]

[1] Aquaculture and Fisheries, Wageningen University and Research, Wageningen, the Netherlands.
[2] Evonik Operations GmbH, Hanau-Wolfgang, Germany
[3] De Heus Animal Nutrition B.V., The Netherlands
[4] Livestock research, Wageningen University and Research, Wageningen, the Netherlands
Email: Thuat.Phan@wur.nl

Introduction

Energy evaluation of fish-feeds and ingredients is mostly done on digestible energy (DE) basis. Calculation of the optimal dietary DE content assumes a constant energy utilization efficiency of DE for growth (k_{gDE}). However, k_{gDE} alters with dietary macronutrient composition (Schrama *et al*., 2012; Glencross *et al*., 2017; Rodehutscord and Pfeffer, 1999). With the current and future diversification of ingredients use in fish-feeds, more carbohydrates will be included resulting in more variation in macro-nutrient composition. Energy evaluation on net energy (NE) basis can overcome the shortcomings in DE evaluation. For implementing a NE system for fish, the energy utilization efficiencies of each macro-nutrient (protein, fat and carbohydrate) needs to be estimated. This study assessed the variation in these energy utilisation efficiencies of digested protein, fat and carbohydrates between different fish species: tilapia, trout, African catfish, barramundi, carp, striped catfish and snakehead.

Materials and methods

For this study, the outcome of a meta-analyses on tilapia and trout (Schrama et al., 2018) and four separate experiments on African catfish (71.6 g), carp (28.9 g), snakehead (29.1g), and striped catfish (29.1 g) were used. The experiments had a 4×2 factorial design with three replicates per treatments. In each experiment, four diets were formulated with 2 carbohydrates levels (low vs high) and 2 fat levels (low vs high) and applied at 2 feeding levels. This design aimed to achieve the large contrast in the intake of digested protein, fat and carbohydrates. Data about digestibility and energy balance were collected to conduct the multiple regression between energy retention (*i.e*., growth response) and the intake of digested protein, fat and carbohydrates.

Results

Average over feeding levels and dietary fat levels, increased dietary carbohydrates level decreased the final body weight of African catfish, carp, striped catfish and snakehead by 29, 13, 16 and 10 g, respectively (Table 1). By conducting the multiple regression between retained energy and digested protein, fat and carbohydrates intake, the energy utilisation efficiencies were quantified (Fig 1). Digested fat is used most efficiently compared to digested protein and carbohydrates among studied fish species. The largest variation was found in the utilisation efficiency of digested carbohydrates for growth (Fig 1). The utilisation efficiencies of digested fat were less variable between species than that of digested protein.

Discussion and conclusion

The limited growth by increasing dietary starch on African catfish, carp, striped catfish and snakehead is similar to observations on barramundi (Glencross et al., 2017), Nile tilapia (Schrama et al., 2012) and rainbow trout (Rodehutscord and Pfeffer, 1999). The energy utilisation efficiencies of digestible protein and fat are comparable between studied fish species. This indicates that the ability to use protein and fat for growth is comparable between studied fish species. The energy utilisation efficiencies of digested protein was highest in African catfish, compared to other studied fish species, which needs further studies to explain. The largest variation in the energy utilisation efficiency of digested carbohydrates indicates the large difference in the ability to use carbohydrates for growth in the studied fish species. African catfish, carp and striped catfish can handle the digested carbohydrates efficiently while this ability is limited in barramundi and snakehead.

(Continued on next page)

Table I.
Final body weight of African catfish, carp, striped catfish and snakehead (n=3)

Species	FL	Diet 1 "protein"	Diet 2 "protein" +Carb	Diet 3 "protein" +Fat	Diet 4 "protein" +Carb+Fat	SEM	*P* value D	FL	D x FL
African catfish									
	Low	140.3^{c}	123.0^{d}	141.8^{c}	123.3^{d}	1.25	***	***	***
	High	223.2^{a}	186.3^{b}	226.0^{a}	184.0^{b}				
Carp									
	Low	60.3^{e}	51.9^{f}	59.9^{e}	49.5^{f}	1.08	***	***	***
	High	95.0^{a}	77.0^{c}	87.3^{b}	71.2^{d}				
Striped catfish									
	Low	72^{c}	59^{d}	69^{cd}	63^{cd}	2.43	***	***	***
	High	127^{a}	101^{b}	130^{a}	108^{b}				
Snakehead									
	Low	62.1^{de}	55.5^{f}	65.7^{d}	58.4^{ef}	0.93	***	***	***
	High	108.7^{a}	95.3^{b}	99.3^{b}	87.6^{c}				

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental lipid diet; Diet 4, supplemental starch and lipid diet; carb, carbohydrates; D, diet; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively. ***, P<0.01; *, P<0.05; ns, not significant.
^{abcdef}If interaction effect is significant, means lacking a common superscript differ (*P*<0.05).

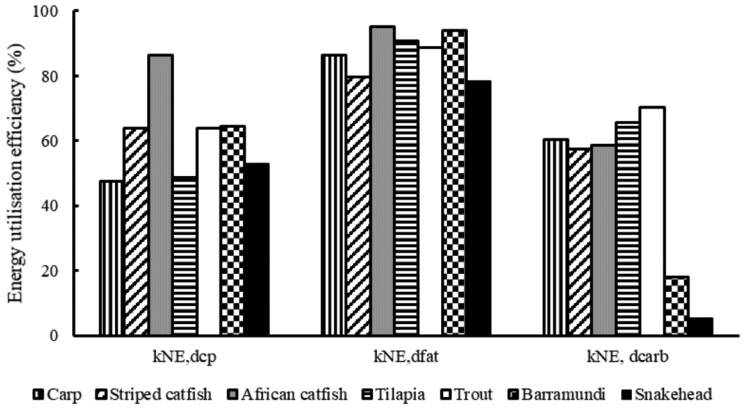


Fig 1. Energy utilisation efficiencies of digestible protein (dcp), fat (dfat) and carbohydrates (dcarb) in tilapia, trout (Schrama *et a*l., 2018), African catfish, carp, barramundi and snakehead (present study).

References

Glencross, B.D., Blyth, D., Bourne, N., Cheers, S., Irvin, S. & Wade, N.M. (2017) An analysis of partial efficiencies of energy utilisation of different macronutrients by barramundi (*Lates calcarifer*) shows that starch restricts protein utilisation in carnivorous fish. British Journal of Nutrition, 117, 500-510.

Rodehutscord, M. & Pfeffer, E. (1999) Maintenance requirement for digestible energy and efficiency of utilisation of digestible energy for retention in rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 179**,** 95-107.

Schrama, J.W., Saravanan, S., Geurden, I., Heinsbroek L.T.N., Kaushik, S.J. & Verreth, J.A.J. (2012) Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus)* and a literature comparision across fish species. British Journal of Nutrition, 108**,** 277-289.

Schrama, J.W., Haidar, M.N., Geurden, I., Heinsbroek, L.T.N. & Kaushik, S.J. (2018) Energy efficiency of digestible protein, fat and carbohydrate utilisation for growth in rainbow trout and Nile tilapia. British Journal of Nutrition, 119, 782-791.

IMPROVING PRODUCTION OF EUROPEAN PIKEPERCH (*Sander lucioperca*) IN AQUAPONICS THROUGH PLANT BIOACTIVE MOLECULES

P. Senff*[,1], O. Polowczyk[2], J. Grosjean[2], S. Milla, C. Robin[2], P. Fontaine[1]

[1]Université de Lorraine, INRAE, UR AFPA, F-54000 Nancy, France
[2]Université de Lorraine, INRAE, LAE, F-54000 Nancy, France
Email : paula.senff@univ-lorraine.fr

Introduction

European pikeperch (*Sander lucioperca*) is a popular food fish and a promising aquaculture species in Europe (Dil 2008, Baekelandt et al. 2018). It is however 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. This limits the intensification of its farming in recirculating aquaculture and aquaponic systems. These are production modes of growing interest for this species and of sustainable intensification of its cultivation (Steinberg et al. 2018). Plant bioactive compounds can be effective feed ingredients that reduce stress and improve fish growth and immunity, but few specific compounds have been identified and tested on pikeperch. Furthermore, plants also release exudates into the medium surrounding their roots, but their influence on an aquaponic system has not been studied to date.

The European INTERREG Research project PERCIPONIE is dedicated to the development of perciculture in aquaponic systems. One of the objectives of the project is to identify bioactive compounds emitted by plants cultivated in aquaponics, or as a food additive with beneficial effects on the growth and immunity of pikeperch. Other objectives of PERCIPONIE are to develop the fish polyculture in aquaponics and to model the dynamics of an aquaponic pilot system. Based on a cross-border consortium of partners, the project brings together stakeholders from research as well as the aquaculture industry for scientific excellence and applicability of results. The collaboration with regional aquaponic companies ensures technical and economic suitability for application.

Materials and Methods

The results of the first project stage will be presented.

A systematic review of the literature from the fields of botany and aquaculture research, is conducted in order to i) identify molecules of interest for the growth and immunity of fish and the families of plants which produce these molecules of interest, either in their tissues or in the form of root exudates, ii) target plants of agronomic interest and / or having a potential positive effect on fish health as a food additive through bioactive molecules, iii) select plant species according to their suitability for hydroponics and iv) assess the compatibility of plants with the range of aquaponic systems involved in the project (from controlled environmental conditions and low species diversity to variable abiotic factors and a wide range of plant species). The results will feed into the other experiments of the PERCIPONIE project and will provide new information on understudied dynamics of aquaponic systems.

References

Baekelandt S, Redivo B, Mandiki SNM, Bournonville T, Houndji A, Bernard B, El Kertaoui N, Schmitz M, Fontaine P, Gardeur JN, Ledoré Y, Kestemont P (2018) Multifactorial analyses revealed optimal aquaculture modalities improving husbandry fitness without clear effect on stress and immune status of pikeperch Sander lucioperca. Gen Comp Endocrinol 258:194–204.

Dalsgaard J, Lund I, Thorarinsdottir R, Drengstig A, Arvonen K, Pedersen PB (2013) Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquac Eng 53:2–13.

Dil H (2008) The European market of the pikeperch for human consumption. In: *Percid fish culture, from research to production*. Fontaine P, Kestemont P, Teletchea F, Wang N (eds) Presses Universitaires de Namur, Namur, Belgium

Steinberg K, Zimmermann J, Meyer S, Schulz C (2018) Start-up of recirculating aquaculture systems: How do water exchange rates influence pikeperch (Sander lucioperca) and water composition? Aquac Eng 83:151–159.

BASQUE MICROALGAE CULTURE COLLECTION (BMCC): HUNDREDS OF MICROALGAE STRAINS FOR BIOTECHNOLOGY PURPOSES

S. Seoane*, J. Bilbao, A. Llorente, E. Blanco-Rayón

Ecology of Marine and Estuarine Plankton Group, Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, 48940, Spain
sergio.seoane@ehu.es

The Basque Microalgae Culture Collection (BMCC) (www.ehu.es/bmcc) is a new microalgae collection recently registered in the World Federation of Culture Collections (WFCC). Sited in the Science and Technology Faculty of the University of the Basque Country (UPV/EHU) (Spain), it possesses more than 700 cultures of microalgae, including species from almost all the taxonomic groups. The major groups in the collection are diatoms, dinoflagellates, haptophytes and chlorophytes, but others such as cyanobacteria and cryptophytes are also well represented. The strains have been isolated from marine, brackish and freshwater ecosystems and it is possible to find pelagic and benthic species. Most of the strains are from the Basque Country (estuaries, coast and reservoirs), but there are maintained strains from all over the world.

The BMCC has recently taken part in different projects related to marine bioprospecting, such as MARBIOM and Algaberri projects. The aim of the MARBIOM project is the discovery of new anti-cancer substances, and it involves the University of Almeria and Pharmamar S.A., among others. Extracts from different strains are being tested in order to find compounds that help to beat this disease. Regarding the Algaberri project, its goal is to obtain compounds that can be used in anti-obesity and anti-neurodegenerative treatments. In this project, the BMCC collaborates with AZTI Company and some research groups of the University of the Basque Country.

The existence of culture collections, with its characterization and maintenance, needs many resources and it is a time-consuming task, but on the other hand, it provides an inestimable source for biotechnology projects to start experiments, saving the time required for the isolation, culturing and characterization of the species.

Bacillus subtilis SPORES ARE EFFICIENT VEHICLES FOR ORAL VACCINATION OF FISH AGAINST VIBRIOSIS

Gabriela Gonçalves[1,2#], Rafaela A. Santos[1,2#], António P. Carvalho[2], Marina Machado[1,3], Lourenço Bonneville[4], Benjamín Costas[1,3], Mónica Serrano[4], Ana Couto[1,2], Carolina Tafalla[5], Patricia Díaz-Rosales[5], Filipe Coutinho[1], Aires Oliva-Teles[1,2] & Cláudia R. Serra[1,2*]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal.
[2] FCUP - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, FC4, 4169-007 Porto, Portugal.
[3] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.
[4] ITQB - Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. República 2780-157 Oeiras, Portugal.
[5] CISA-INIA-CSIC - Animal Health Research Center, Valdeolmos, 28130 Madrid, Spain
[#] equal contribution ; * cserra@ciimar.up.pt

Introduction

Vibriosis, a hemorrhagic septicemia caused by members of the gram-negative genus *Vibrio*, is one of the most prevalent diseases of cultured fish, shrimps and bivalves (1). *V. anguillarum*, the etiological agent of classical vibriosis in warm- and cold-water fish species, is particularly problematic, leading to high mortalities and economic losses in aquaculture (1). Although commercial vaccines have been developed against vibriosis, these are mainly injectable ones, requiring individual handling of fish, which is laborious, time-consuming and may induce stress-related mortalities (2). Present vaccine trend developments focus on alternative methods for mass delivery of antigens, including oral and immersion vaccination (2). Oral vaccine administration incorporated in feed seems to be the preferable method as it reduces fish stress and costs to the minimum and is feasible for larvae and juveniles vaccination (2). Oral vaccines are thus highly demanded by the aquaculture sector, but till today most previous attempts to obtain effective oral vaccines in fish have failed.

One possible strategy is the use of bacterial spores, extremely resistant structures with wide biotechnological applications (3), that survive passage through the gastrointestinal tract. Bacterial spores, in particular those of *Bacillus subtilis,* have been shown to behave as mucosal vaccine adjuvants in mice models (4), but, to date, such technology has not been applied against gram-negative fish bacterial diseases.

To fulfil this gap, we used *B. subtilis* spores as a delivery vehicle for the presentation of the OmpK immunogenic protein, an antigen shared among several *Vibrio* species and evaluated its efficacy in increasing survival of larvae from the model zebrafish (*Danio rerio*) and of juveniles from an economically important aquaculture species, the European seabass (*Dicentrarchus labrax*).

Materials and Methods

The genes encoding for the OmpK protein and the green fluorescence protein (GFP) were PCR amplified containing an N-terminal 6Histines-Tag and cloned into p1CSV-CotY-N and p1CSV-CotY-C plasmid vectors (5), resulting in a translational fusion to the crust protein CotY either C-or-N-terminally. Integration at *B. subtilis* 168 chromosome through a double-crossover recombination event, resulted in the chloramphenicol resistant strains CRS218 (CotY-H6-GFP), CRS219 (H6-GFP-CotY), CRS220 (CotY-H6-OmpK) and CRS221 (H6-OmpK-CotY). Spores of WT *B. subtilis* 168 and its congenic derivatives were obtained after induction of sporulation by nutrient exhaustion in DSM and purified following standard procedures. The display of the target proteins at the surface of the recombinant spores was evaluated by western blot with a commercial anti-His-Tag antibody. Strains carrying the GFP fusions were also observed under a fluorescence microscope.

The vaccination potential of OmpK-carrying spores was first evaluated in zebrafish larvae reared at 28 ºC in 6-wells plates containing egg water. At 6 dpf (days post-fertilization), larvae were treated for 2h with spores suspensions (10^8 CFU mL^{-1}) of each recombinant strain carrying the OmpK-CotY fusions, or from the parental *B. subtilis* 168 strain. At 9 dpf larvae were challenged by immersion with 3×10^8 CFUs mL^{-1} of *V. anguillarum* or 1×10^8 CFUs mL^{-1} of *V. parahaemolyticus*. Cumulative mortalities were registered between 16-24h, and dead larvae removed and safely discarded. The experiment was independently carried out 3 times.

(Continued on next page)

Next, the vaccination potential of OmpK-carrying spores was also evaluated in European seabass. Triplicate groups of 40 European seabass juveniles, were fed diets containing 1×10^9 spores Kg^{-1} feed of either CRS220 (OmpK-diet), CRS218 (GFP-diet) or from the parental *B. subtilis* 168 strain (CTR, control-diet). After 30 days, 10 fish from each tank of the GFP-diet and of the CTR diet were used for blood collection and subsequently used for anti-GFP antibodies detection in fish serum, using an indirect ELISA approach. Simultaneously, 60 fish from tanks of the OmpK-diet and of the CTR diet, were challenged by injection with *V. anguillarum* ($1x10^6$ CFU/fish), while the remaining fish from the same treatments were injected with PBS. Fish survival was followed during 7 days, with dead and moribund fish showing signs of infection daily removed and sacrificed. At the end of the challenge, the remaining fish were euthanized with an overdose of anesthetic. Survival curves were plotted using the Kaplan-Meier method and pairwise comparisons between treatments were performed with nonparametric log-rank test, at 0.05 significance level, in GraphPad Prism version 9.

Results

Both OmpK and GFP proteins were successfully displayed at the surface of the *B. subtilis* spore, although more efficiently in the C-terminal versions. Importantly, zebrafish survival upon challenge with *V. anguillarum* and *V. parahaemolyticus* was increased in magnitudes of 50 to 90% respectively, when previously vaccinated with OmpK-carrying spores. Further, we detected anti-GFP-antibodies in the serum of European seabass fed diets containing GFP-carrying spores and increased European seabass survival by 30% when challenged with *V. anguillarum* if previously fed with a diet containing OmpK-carrying spores.

Conclusions

Overall our results indicate that *B. subtilis* spores can be efficient antigen carriers for oral vaccine delivery in fish.

Funding: SFRH/BD/138187/2018; UIDB/04423/2020 ; UIDP/04423/2020.

References

1. Nurliyana Mohamad, Mohammad Noor Azmai Amal, Ina Salwany Md Yasin, Mohd Zamri Saad, Nurrul Shaqinah Nasruddin, Nurhidayu Al-saari, Sayaka Mino, Tomoo Sawabe. (2019). Vibriosis in cultured marine fishes: a review. Aquaculture 512.
2. Ma J, Bruce TJ, Jones EM, Cain KD. (2019). A review of fish vaccine development strategies: conventional methods and modern biotechnological approaches. Microorganisms 7(11): 569.
3.Zhang X, Al-Dossary A, Hussain M, Setlow P, Li J. (2020). Applications of *Bacillus subtilis* spores in biotechnology and advanced materials. Applied and Environmental Microbiology 86:e01096-20.
4. Copland Alastair, Diogo Gil R., Hart Peter, Harris Shane, Tran Andy C., Paul Mathew J., Singh Mahavir, Cutting Simon M., Reljic Rajko. (2018). Mucosal delivery of fusion proteins with *Bacillus subtilis* spores enhances protection against tuberculosis by Bacillus Calmette-Guérin . Frontiers in Immunology 9:346.
5. Bartels, J., Lopez Castellanos, S., Radeck, J., & Mascher, T. (2018). Sporobeads: The Utilization of the Bacillus subtilis Endospore Crust as a Protein Display Platform. *ACS Synth Biol, 7*(2), 452-461. doi:10.1021/acssynbio.7b00285

EVALUATION OF CYANOBACTERIAL EXTRACELLULAR VESICLES AS ANTIGEN DELIVERY-PLATFORMS FOR FISH

Filipe Coutinho[1], Jorge M. Cardoso[2,3], Steeve Lima[2,3,4], Gabriela Gonçalves[1,5], Lourenço Bonneville[6], Mónica Serrano[6], Aires Oliva-Teles[1,5], Paula Tamagnini[2,3,5], Paulo Oliveira[2,3,5] & Cláudia R. Serra[1,5*]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[2]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, R. Alfredo Allen, 208, 4200-135 Porto, Portugal Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal
[3]IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, R. Alfredo Allen, 208, 4200-135 Porto, Portugal
[4]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[5]FCUP-Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal
[6]ITQB - Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. República 2780-157 Oeiras, Portugal
*cserra@ciimar.up.pt

Introduction

Mycobacterium marinum is the causative agent of Mycobacteriosis, a fish bacterial disease characterized by granulomatous inflammation in multiple organs, with a mortality rate that can range between 30-100% (1). Mycobacteriosis outbreaks have important effects on commercial fish production, in particular that of European seabass (*Dicentrarchus labrax*), a fish species of high economic interest. Mycobacteriosis is also an important infectious disease in zebrafish (*Danio rerio*), associated with severe losses in research facilities. Because no satisfactory vaccine or treatment is available, once a population of fish is infected, the most likely scenario is euthanasia of the entire group. Thus, it is imperative to develop an efficient strategy to prevent mycobacteriosis infections. One possibility is the use of bacterial extracellular vesicles (EVs), which are spherical bilayered structures naturally liberated from the outer membrane of Gram-negative bacteria. Bacterial EVs have been progressively used as carriers of immunogenic antigens (2). An example is the commercially-available EV-based vaccine Bexsero® against *Neisseria meningitidis* serogroup-B15 in humans (3).

Materials and Methods

First, EVs derived from the non-pathogenic and environmentally friendly cyanobacterium *Synechocystis* sp. PCC 6803 wild-type and congenic derivatives Δ*tolC*, Δ*tolC*/Δ*spy*, *ΔfucS* (with distinct vesiculation phenotypes), ranging between 50 and 500 μg LPS mL^{-1}, were tested for their effects on 3 days post fertilization (3 dpf) zebrafish larvae survival. LPS isolated from the same bacterial strains were used for comparison while commercial LPS from *Pseudomonas aeruginosa* (known to induce zebrafish mortality) was used as control. Survival curves were plotted using the Kaplan-Meier method and pairwise comparisons between treatments were performed with nonparametric log-rank test, at 0.05 significance level, in GraphPad Prism version 9. In addition, at days 1 and 5, total RNA was extracted from pools of 5 embryos of each replicate (n=3) from EVs treatments, for analysis of transcript levels of pro-inflammatory cytokines interleukin 1 beta (*il1β*) and tumor necrosis factor (*tnf1α*) and of anti-inflammatory cytokine interleukin 10 (*il10*), by RT-qPCR, using established oligonucleotides. Next, European seabass juveniles were randomly assigned to duplicate groups of 12 fish, and injected with engineered cyanobacterial EVs loaded with the reporter GFP in the following scheme: either one injection with 824 μg EVs containing 16.48 μg GFP; or two injections, separated by 15 days, each with 412 μg EVs containing 8.24 GFP. As controls, one injection of 16.48 μg GFP alone; or PBS were included. At days 15 and 30 after injection, 6 fish from each tank were euthanized for blood collection, plasma extraction and subsequently used for anti-GFP antibodies detection in fish serum, using an indirect ELISA approach.

(Continued on next page)

Results

Neither cyanobacterial EVs from any of the cyanobacterial strains used, nor isolated cyanobacterial LPS, induce significant mortality in zebrafish larvae under the tested conditions. Thus, while 45 μg mL^{-1} *P. aeruginosa* LPS triggered severe mortality (>80%) after 24 h, isolated EVs concentrations of up to 500 μg mL^{-1} from *Synechocystis* may be used without significantly reducing larvae survival even after 5 days of incubation. Accordingly, and in contrast to LPS from *P. aeruginosa* that induced significant upregulation of inflammatory markers at sub-lethal concentration of 35 μg mL^{-1}, EVs isolated from *Synechocystis* strains were shown not to induce significant inflammatory response. Further, we detected anti-GFP-antibodies in the serum of European seabass injected with EVs containing GFP, and no harmful effect could be detected on fish after a 30 days trial.

Conclusions

Our results support that *Synechocystis* EVs structural components are biocompatible with fish even at high concentrations. Cyanobacterial EVs are thus good candidates to be used as carriers of immunogenic antigens in zebrafish and with potential to be used in aquaculture species such as the European seabass. Future work will engineer EVs with heterologous *M. marinum* antigens, and evaluate these as delivery vehicles to immunize zebrafish and European seabass against *M. marinum*.

Acknowledgements

The authors acknowledge the support of the i3S Scientific Platforms "Histology and Electron Microscopy" and "Advanced Ligh Microscopy", members of the national infrastructure Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122). This work was partly funded by the Strategic Funding to UID/Multi/04423/2019 (POCI-01-0145-FEDER-007621), financed by Fundo Europeu de Desenvolvimento Regional (FEDER) funds through the COMPETE 2020 Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project POCI-01-0145-FEDER-029540 (PTDC/BIA-OUT/29540/2017). Fundação para a Ciência e a Tecnologia is also greatly acknowledged for the PhD fellowship SFRH/BD/130478/2017 (SL) and FCT Investigator grant IF/00256/2015 (PO).

References

1. Hashish E, Merwad A, Elgaml S, Amer A, Kamal H, Elsadek A, Marei A, Sitohy M. Mycobacterium marinum infection in fish and man: epidemiology, pathophysiology and management; a review. Vet Q. 2018 Dec;38(1):35-46.
2. Lima S, Matinha-Cardoso J, Tamagnini P, Oliveira P. Extracellular Vesicles: An Overlooked Secretion System in Cyanobacteria. Life (Basel). 2020 Jul 31;10(8):129.
3. Tani C, Stella M, Donnarumma D, Biagini M, Parente P, Vadi A, Magagnoli C, Costantino P, Rigat F, Norais N. Quantification by LC-MS(E) of outer membrane vesicle proteins of the Bexsero® vaccine. Vaccine. 2014 Mar 5;32(11):1273-9.

MODULATION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) GUT MICROBIOTA BY DIETARY INSECT MEAL

F. Rangel[1,2*], L. Gasco[3], F. Gai[4], A. Oliva Teles[1,2], P. Enes[1,2], C. R. Serra[1,2], F. C. Pereira[5]

[1] Department of Biology, Faculty of Sciences, University of Porto, Portugal
[2]CIMAR/CIIMAR Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal
[3]Department of Agricultural, Forest and Food Sciences, University of Turin, Italy
[4]Institute of Science of Food Production, National Research Council, Italy
[5]Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Austria
Corresponding author: claudia.serra@fc.up.pt

Introduction

The aquaculture industry is one of the fastest growing industries of its sector, mostly due to the incremental demand of fish production. However, this generates resource management issues mainly in the use of fish meal (FM), which becomes both ecologically and economically unsustainable. To respond to this issue, novel dietary ingredients are being studied to formulate aquafeeds with suitable dietary profiles for each species. With a high protein and lipid contents, a balanced amino acid profile and a recent authorization for the incorporation in aquafeeds in the European Union, insect meal (IM) positions itself as a strong candidate for FM substitution. Introduction of novel ingredients, however, can impact over the fish immune status and/or growth performance. One of the known effects of introducing novel dietary ingredients is the potential to generate gut microbial communities shifts which are recognized to impact on fish growth, metabolism, and immune status (Ringø et al. 2015; Ringø et al. 2018; Serra et al. 2021). In fact, prebiotic effects have been attributed to IM, with these being commonly associated with the increase of dietary chitin, a structural polysaccharide present in the insects exoskeleton (Perry et al. 2020) However, information about the potential gut microbiota shifts occurring as a consequence of dietary IM inclusion are scarce (Perry et al. 2020). Thus, this study aims to assess the effect of three different IM, namely, *Hermetia illucens* larvae meal (HM), *Tenebrio molitor* larvae meal (TM), and *H. illucens* exuviae meal (HEM) on European seabass gut microbiota modulation.

Materials and methods

To access gut microbiota modulation due to dietary IM, five isoproteic (45%) and isolipidic (18%) diets were used: a control diet (CTR); a diet similar to the CTR with 5% commercial chitin (CHIT5), and three diets with 25% inclusion of HM25, TM25 and HEM2, respectively. The experimental diets were randomly assigned to 5 groups of 15 fish with an initial mean body weight of 53.7 ± 2.67 g. The trial was conducted in a recirculating aquaculture system equipped with 100 L water capacity tanks, thermo-regulated to 22.7 ± 0.3 °C. Fish were fed by hand twice a day, 6 days per week, until apparent visual satiation for 8 weeks. The allochthonous (digesta) and autochthonous (mucosa) communities were aseptically collected, had their DNA extracted and were subjected to a 2-step PCR barcoding of the V4 region of the 16S rRNA followed by Illumina Miseq sequencing (paired-end mode; 2× 300 bp). Amplicon pools were extracted from the raw sequencing data using the FASTQ workflow in BaseSpace (Illumina), demultiplexing was performed with the python package demultiplex (Laros JFJ, github.com/jfjlaros/demultiplex) and barcodes, linkers and primers were trimmed off using BBDuk (BBTools, sourceforge.net/projects/bbmap). DADA2 R package version 1.14.1 (https://www.r-project.org/, R 3.6.2) was used for demultiplexing amplicon sequencing variants (ASVs). Taxonomy assignment was based on SILVA taxonomy database (release 138). Amplicon sequence libraries were analysed using the vegan (v2.4.3) and phyloseq (v1.30.0) packages of the software R. DESeq2 (v1.26.0) implemented in phyloseq was used to determine statistical significant differences in ASV abundances between diets and/or sites. Finally, quantitative PCR of the conserved *chiA* gene was used to assess if the observed microbial shifts were accompanied by a genomic shift towards chitinase-encoding genes.

Results

Overall, the European sea bass gut microbiota was dominated by the phylum Proteobacteria, followed by Firmicutes, Actinobacteria and Bacteroidota. A beta-diversity analysis of the allochthonous and autochthonous communities revealed a stronger impact of the sampled site (digesta versus mucosa) when compared with the impact of the tested diets on community composition, with digesta communities being more diverse than their mucosal counterparts. Nevertheless, an overall impact of diet on the composition of the European sea bass gut microbiota (p=0.004, r^2=0.244, PERMANOVA) could also be observed. While the mucosa-associated microorganisms were more resilient to dietary changes, digesta-associated communities were significantly altered upon HM-based diets leading to an increase in several families belonging

(Continued on next page)

to the Firmicutes and Actinobacteria phyla, with HEM25 presenting a stronger influence. Although genomic potential for chitinolytic activity is recognized for bacteria in both phyla, our results showed that only HEM25 was able to promote a shift towards chitinase-encoding taxa, mainly due to the increase of the *Paenibacillaceae* family, which was not found to significantly increase in HM25. When screening our samples for the presence of chitinase A gene (*chiA*) using primers targeting a conserved region of ChiA, we detected *chiA* in the digesta of fish in all diets, but fish fed the HEM25 diet had significantly higher copy numbers of *chiA* compared to fish fed any of the other diets ($p=0.0077$, ANOVA). These results show an increased *chiA*-driven chitonolytic potential of the European sea bass digesta microbiota fed a HEM25 diet. In conclusion, our results showed a metabolic shift in fish gut microbiota towards chitin-degrading microorganisms due to dietary IM inclusion.

Acknowledgements

This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). P.E. and C.R.S. had scientific employment contracts supported by national funds through FCT. The researcher F.R. was supported by a grant from FCT, Portugal (SFRH/BD/138375/2018)

References

Perry, William Bernard, Elle Lindsay, Christopher James Payne, Christopher Brodie, and Raminta Kazlauskaite. 2020. 'The role of the gut microbiome in sustainable teleost aquaculture', *Proceedings of the Royal Society B: Biological Sciences*, 287: 202001

Ringø, E., Z. Zhou, J. L. G. Vecino, S. Wadsworth, J. Romero, Å Krogdahl, R. E. Olsen, A. Dimitroglou, A. Foey, S. Davies, M. Owen, H. L. Lauzon, L. L. Martinsen, P. De Schryver, P. Bossier, S. Sperstad, and D. L. Merrifield. 2015. 'Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?': n/a-n/a.

Ringø, Einar, Seyed Hossein Hoseinifar, Koushik Ghosh, Hien Van Doan, Bo Ram Beck, and Seong Kyu Song. 2018. 'Lactic Acid Bacteria in Finfish—An Update', *Frontiers in Microbiology*, 9.

Serra, Cláudia R., Aires Oliva-Teles, Paula Enes, and Fernando Tavares. 2021. 'Gut microbiota dynamics in carnivorous European seabass (Dicentrarchus labrax) fed plant-based diets', *Scientific reports*, 11.

ISOLATION OF PUTATIVE PROBIOTICS WITH CHITIN-METABOLIZING ABILITIES FROM EUROPEAN SEA BASS (*Dicentrarchus labrax*) GUT MICROBIOTA

F. Rangel[1,2*], R. A. Santos[1,2], M. Monteiro[1,2], L. Gasco[3], F. Gai[4], A. Oliva Teles[1,2], P. Enes[1,2], C. R. Serra[1,2]

[1] Department of Biology, Faculty of Sciences, University of Porto, Portugal
[2]CIMAR/CIIMAR Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal
[3]Department of Agricultural, Forest and Food Sciences, University of Turin, Italy
[4]Institute of Science of Food Production, National Research Council, Italy
Corresponding author: fjorangel@gmail.com

Introduction

In aquaculture, finding alternative ingredients to fish meal (FM) is urgently needed, since the depletion of fish wild stocks has led to a decrease in FM availability, thus increasing FM price. Plant feedstuffs (PF) may partially replace FM in aquafeeds (Tacon et al. 2011). However, PF use has its own drawbacks. In terms of resources management, PF are in direct competition with human food and have a high demand for land and water for production. Moreover, the presence of anti-nutritional factors, and, imbalanced amino acid profile, diminishes its effectiveness as an FM substitute in aquafeeds (Gatlin et al. 2007; Henry et al. 2015). Insect meal (IM), recently authorized for use in aquafeeds, positions itself as a promising aquafeed commodity due to its high protein content, balanced amino acid profile and high lipid contents. However, insects are also rich in chitin, a structural polysaccharide present in the insect's exoskeleton, whose non-digestibility has been linked with lower fish performance and nutrient digestibility (Henry et al. 2015; Gasco et al. 2019). To overcome the chitin impairments of IM inclusion in aquafeeds, IM can be submitted to chemical or enzymatic treatments to promote chitin breakdown before being added to diets, but these processes have high costs, low yield, residual acidity, and may result in serious environmental pollution (Harish Prashanth and Tharanathan 2007). Alternatively, probiotic (PRO) bacteria, knowing to possess chitinase activity (e.g. *Bacillus subtilis*, (Askarian et al. 2013)), could be a solution. As such, this proposal aims to isolate, from European sea bass gastrointestinal tract, PRO bacteria capable of producing chitinases to improve the use of high IM-containing diets, supporting cost-effective fish growth without compromising welfare and health status.

Materials and methods

Based on the adaptability of gut microbial communities, chitin-containing diets were fed to promote microbial shifts towards chitin-metabolizing bacteria. Five isoproteic (45%) and isolipidic (18%) diets were formulated: a control diet (CTR); a diet similar to the CTR with 5% chitin supplementation (CHIT5); and 3 other diets with 25% inclusion of either *Hermetia illucens* (HI) larvae meal (HM25), *Tenebrio molitor* larvae meal (TM25) or HI exuviae meal (HEM25). After 8 weeks feeding trial, whole guts were aseptically excised and squeezed to collect the digesta contents. To select for aerobic bacterial sporeforming isolates, serial dilutions were prepared and heat-treated before inoculation on Luria Bertani (LB) medium (Nicholson 1990). The potential PRO were comprehensively screened *in vitro*, concerning their putative PRO traits, namely: sporeforming ability; chitinolytic activity; antibiotic resistance; haemolytic activity; and gut-survival aptitude.

Results

From the obtained microorganisms, from each diet and dilution, colonies presenting different morphologies were randomly selected for further analysis, resulting in a total of 363 isolates. Sporulation ability was confirmed by phase-contrast microscopy of cultures submitted to nutrient exhaustion by cultivation on DSM. These isolates were sequentially gauged for their chitin metabolization ability, resulting in a group of 40 isolates with promising chitinolytic activities. The remaining 40 isolates were subjected to full taxonomic identification followed by haemolytic activity characterization narrowing the isolate number to 20. Antibiotic resistance screening of the 20 candidates revealed that all isolates, but one, were resistant to at least one of the tested antibiotics and, as such, total chitinolytic activity was determined for all 20 isolates. Within these, the 4 isolates presenting the highest chitinolytic activity were selected and assessed regarding their gut-survival aptitude and total chitinolytic activity in gut-like conditions. All the selected isolates performed well in the tested conditions with 2 isolates performing better than the rest, namely FI645 and FI658. The best PRO candidates will be subsequently evaluated *in vivo*, considering their potential to modulate fish gut microbiota, aiming to improve chitin utilization in IM-based diets.

(Continued on next page)

Acknowledgements

This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. P.E. and C.R.S. had scientific employment contracts supported by national funds through FCT. F.R., R.A.S, and M.M. were supported by a PhD grant from FCT, Portugal, respectively SFRH/BD/138375/2018, SFRH/BD/131069/2017, and SFRH/BD/114995/2016.

References

Askarian, Fatemeh, Sigmund Sperstad, Daniel L. Merrifield, Arun Kumar Ray, and Einar Ringø. 2013. 'The effect of different feeding regimes on enzyme activities of gut microbiota in Atlantic cod (Gadus morhuaL.)', *Aquaculture Research*, 44: 841-46.

Gasco, Laura, Ilaria Biasato, Sihem Dabbou, Achille Schiavone, and Francesco Gai. 2019. 'Animals Fed Insect-Based Diets: State-of-the-Art on Digestibility, Performance and Product Quality', *Animals*, 9: 170.

Gatlin, Delbert M., Frederic T. Barrows, Paul Brown, Konrad Dabrowski, T. Gibson Gaylord, Ronald W. Hardy, Eliot Herman, Gongshe Hu, Åshild Krogdahl, Richard Nelson, Kenneth Overturf, Michael Rust, Wendy Sealey, Denise Skonberg, Edward J Souza, David Stone, Rich Wilson, and Eve Wurtele. 2007. 'Expanding the utilization of sustainable plant products in aquafeeds: a review', *Aquaculture Research*, 38: 551-79.

Harish Prashanth, K. V., and R. N. Tharanathan. 2007. 'Chitin/chitosan: modifications and their unlimited application potential—an overview', 18: 117-31.

Henry, M., L. Gasco, G. Piccolo, and E. Fountoulaki. 2015. 'Review on the use of insects in the diet of farmed fish: Past and future', *Animal Feed Science and Technology*, 203: 1-22.

Nicholson, WL. 1990. 'Sporulation, germination, and outgrowth', *Molecular biological methods for Bacillus*.

Tacon, Albert GJ, Mohammad R Hasan, and Marc Metian. 2011. 'Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects', *FAO Fisheries and Aquaculture technical paper*: I.

GUT MICROBIOTA DYNAMICS IN *Solea senegalensis* JUVENILES FED FORTIFIED DIETS

Fábio Rangel[1], S. Pereira[1,2], P. Santos[1], W. Pinto[3], I. Blanquet[4], L. Conceição[3], B. Costas[1,2], C. R. Serra[1,5 *]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[2] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[4] Safiestela SA (SEA8), Lugar Do Rio Alto, Estela, Póvoa de Varzim, Portugal
[5] FCUP - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, FC4, 4169-007 Porto, Portugal
* cserra@ciimar.up.pt

Introduction
The gut microbiota plays a central role in fish health and performance. Gut microorganisms perform or facilitate a series of digestive, metabolic, and immune-stimulating processes vital for host fitness (1). Imbalances in the gut microbiota composition, a process called dysbiosis, are linked to the development of multifactorial diseases (1,2). Aquaculture practices, such as fish transportation, are often associated to an increase of fish stress, morbidity and mortality, and have the potential to generate gut microbial community shifts, with an impact on fish growth, metabolism, and immune status (2,3). Here, we examined whether the gut microbial population of Senegalese sole juveniles was changed by an acute stress (standardized 30 min transportation), and if such changes could recover, when fish were fed diets fortified with antioxidants and β-glucans, known as effective immunostimulants in fish production (4).

Materials and methods
Triplicate groups (80fish/tank) of Senegalese sole juveniles (5g) were fed three diets, formulated and manufactured by SPAROS, with high-quality ingredients: CTRL, rich in high quality fish, squid and krill meals; CTRLox, CTRL supplemented with natural antioxidants (i.e. vitamins and curcumin); and CTRLas, CTRL supplemented with antioxidants and β-glucans. A fourth commercial diet was used as positive control. Each diet was administered in 8 daily meals and fed to apparent satiety. Animals were fed the experimental diets for 7 days and submitted to an acute stress by a standardized 30 min transport. Fish were then returned to the experimental tanks and fed the same dietary treatments for additional 7 days. After this period, all fish were fed the commercial diet for the last 7 days of the experiment. Fish were sampled at the beginning (time 0), prior transport (time 7), 7 days following transport (time 14) and at the end of the feeding trial (time 21). At each sampling point, intestines of 5 fish per tank were aseptically excised, and pooled into one sample to overcome inter-fish variation and assess differences between dietary groups. DNA extraction and polymorphism analysis of 16S rRNA genes by Denaturing Gradient Gel Electrophoresis (DGGE) and by Next-Generation Sequencing (NGS) using the Illumina´s MiSeq platform (paired-end mode; 2× 300 bp), was essentially performed as previously described (5).

Results
The Senegalese sole gut microbiota was dominated by the phylum Tenericutes, followed by Proteobacteria, Firmicutes, and Actinobacteria. Although the tested experimental diets did not promote significant changes in the gut microbiota diversity indices, a reduction on microbial richness and microbial diversity was seen after the acute stress induction. Regarding differently abundant genera, the experimental diets CTRLas and CTRLox significantly reduced the % of *Cellulosilyticum* spp. *(p<0.001)*, a poorly characterized genus of cellulolytic, acetic acid producing anaerobic sporeforming bacteria; and of *Vibrio spp*. *(p=0.011)*, a more diverse genus, that includes species widely distributed in aquatic and marine habitats, many of which are considered opportunistic bacterial pathogens for fish (e.g. *V. scophthalmi , V. anguillarum, V. vulnificus*) causing Vibriosis.

Acknowledgments
This work was supported by Project FEEDMI (39948), financed by Portugal and the European Union through FEDER, COMPETE 2020, and CRESC Algarve 2020, in the framework of Portugal 2020. F.R. holds a PhD grant (SFRH/BD/138375/2018) and C.R.S. has a scientific employment contract, both supported by national funds through FCT.

References
1. Rinninella, E. *et al*. 2019. Microorganisms 7, 14.
2. Brugman, S. *et al*. 2018. Front. Nutr. 5, 80.
3. Kraimi, N. *et al*. 2019. Physiol. Behav. 210, 112658.
4. Vallejos-Vidal, *et al*. 2016. Fish & Shellfish Immunology 56, 34-69.
5. Serra, C., *et al*. 2021. Scientific reports, 11.

FUNCTIONAL ADDITIVES IN SELECTED EUROPEAN SEA BASS (*Dicentrarchus labrax*) GENOTYPES: EFFECTS ON STRESS RESPONSE ANDGILL ANTIOXIDANT RESPONSE

A Serradell[a*], S. Torrecillas[a], A. Makol[b], F. Acosta[a], M.S. Izquierdoa, F. Allal[c], P. Haffray[d], A. Bajek[e] and D. Montero[a]

[a]Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de LasPalmas de Gran Canaria, Crta. Taliarte s/n, 35214, Las Palmas, Canary Islands, Telde, Spain
[b]Delacon Biotechnik GmbH, Weissenwolffstrasse 14, 4221, Steyregg, Austria
[c]MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34250 Palavas-les-Flots, France
[d]SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[e]Ecloserie Marine de Graveline (EMG) Ichtus, Route des Enrochements, 59820Gravelines, France

* Corresponding author E-mail: antonio.serradell101@alu.ulpgc.es

Introduction

An adequate nutritional strategy acquires a great importance enhancing the potential of selective breeding programs on aquaculture production (Callet *et al.,* 2017). The substitution of FM and FO by alternative and more sustainable ingredients may affectfish growth, welfare and health (Montero and Izquierdo, 2010), impairing the geneticallyselected traits. Functional additives have shown the ability to reduce this effect in commercial based diet formulas (Giannenas *et al.,* 2012) by enhancing growth performance (Hoseinifar *et al.,* 2015), welfare (Serradell *et al.*, 2020), and health (Torrecillas *et al.,* 2019). The objective of the present study was to evaluate the potential of different functional additives to attenuate stress response and antioxidant status on a selected genotype comparatively to non-selected European sea bass (*Dicentrarchus labrax*) juveniles fed a low FM and FO diet.

Material and methods

Four isoenergetic and isonitrogenous diets with a low FM and FO content (10% and 6% respectively) supplemented with a 0.05% galactomannan oligosaccharides (GMOS), a 0.02% of two different mixtures of essential oils (PHYTO 1 and PHYTO 2) and acontrol diet (with no supplementation) were fed to two European sea bass genotypes,high grow genetically selected (HG) (by EMG breeding company since several generation with SYSAAF advice), and non-genetically selected (UN). The experimental diets were fed for 72 days (6 days/week, 3 times/day). Afterwards, fish were subjected toan oxidative stress challenge by bath with hydrogen peroxide (H_2O_2) at a final concentration of 50 ppm for 1 hour. At the end of the feeding trial (t=0h), and along the*in vivo* challenge test (2h, 4h, 24h after the exposure) plasma cortisol concentrations (ng/mL) as well as the relative gene expression of different genes in gill, including genesrelated with the antioxidant fish capacity as *sod, cat, gpx*, mitochondrial function genesas *Na-K-ATPase, ucp1*, *ucp* 3, *nd5* and proinflammatory genes as *interleukin-1β*, *nf- Kβ*, *tnf- α*, *cyb*, *coxi* and the proapoptotic *caspase-9* were evaluated.

Results

HG fish presented a significant ($p < 0.05$) lower basal circulating plasma cortisol level than UN fish at t= 0h before the *in vivo* challenge test, with concentrations ranging between 280.1 ± 80.9 ng/ml and 387.7 ± 84.1 ng/ml for HG and UN fish groups, respectively. Unless no significant, HG fish presented an improved acute stress response in relation to higher plasmatic cortisol concentration than UN fish (2h post exposure). HG fish fed Control and GMOS presented an increase in cortisol concentrations of 3.2 and 2.7 fold times in relation to their basal levels, while those HG fish fed PHYTO 1 and PHYTO 2 diets presented an increase of 2.5 and 2.2 fold times respectively in relation to their basal level. Similarly, UN fish fed Control and GMOS diets, despite presented a higher basal circulating cortisol levels than HG fish groups, the exposure to the stressor induced an increase of cortisol level of 1.8 and 1.7-fold (2h post exposure) in relation to basal levels, while UN fish fed PHYTO 1 and PHYTO 2 diets presented an increase on plasmatic cortisol concentrations (2h post exposure) of 1.6 and 1.4-fold in relation totheir basal level. Twenty-four hours after the exposure to the stressor (t=24h) fishbelonging to both genotypes (HG and UN) presented a reduction on circulating plasma cortisol values regardless the dietary treatment fed. At t=2h after oxidative stress, UNfish showed higher ($p < 0.05$) gill relative gene expression of *sod, cat* and *gpx* than HG fish. When fish were fed the functional diets (PHYTO 1, PHYTO 2 or GMOS), both HG and UN fish presented an up-regulation of *cat* relative gene expression 2h after H_2O_2 exposure. In contrast, those fish fed the

(Continued on next page)

Control diet showed higher *cat* relative gene expression values at t= 4h after oxidative stress. *Interleukin-1β* relative gene expression values were up-regulated ($p < 0.05$) in UN fish after oxidative stress in relation to *interleukin-1β* basal relative gene expression values (t=0h). At t= 2h after exposure, UN fish fed the functional diets presented an up-regulation ($p < 0.05$) of the gene expression levels compared to fish fed the Control diet. At t=4h fish fed the Control diet presentedan up-regulation on *interleukin-1β* gene expression values similar to those presented by fish fed the supplemented diets.

In summary, multi-traits EMG selected European sea bass genotype has a higher capacity of response against oxidative stress than UN fish, in relation to a more efficient acute response in terms of cortisol production two hours after H_2O_2 exposure. The recovery of basal levels for HG groups seems also to be more efficient in selected groupsthan in unselected populations, regardless of the diet fed. The use of functional diets up- regulated UN fish antioxidant gene expression, especially *cat,* after the oxidative stress challenge.

Bibliography

Callet, T., *et al*. (2017). *PLoS One*, *12*(10), e0186705.
Hoseinifar, S.H., *et al*. Fish Shellfish Immunol. 45 (2015) 27–32.
Montero, D., & Izquierdo, M. (2010). Fish oil replacement and alternative lipid sourcesin aquaculture feeds, 439-485.
Serradell, A., *et al*. (2020). *Fish & shellfish immunology*, *100*, 219-229. Torrecillas, S., *et al*. (2019). *Plos one*, *14*(9), e0222063.

Acknowledgements

This research was funded by the EU Horizon 2020 project AquaIMPACT (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture); number: 818367.

AQUACULTURE IN THE DUTCH NORTH SEA: TECHNICAL PRESENTATION OF TWO CONCEPTS

Jorrit-Jan Serraris[1], Kika Jonker[2], Linda Kemp[1]

(1) Maritime Research Institute Netherlands (MARIN). P.O. Box 28, 6700 AA, Wageningen (Netherlands)
Email: j.w.serraris@marin.nl, (2) kmmjonker@gmail.com

Introduction

More and more offshore wind parks are being developed in the Dutch North Sea. Multi-use of the space between the wind turbines is foreseen for nature reinforcement and development, production of renewable energies, mariculture and passive fishing activities.

Inspired by the international aquaculture industry the present paper presents an investigation into the application of aquaculture within Dutch offshore wind parks. The paper touches upon relevant aspects of aquaculture and focusses on the technical aspects.

Background

The feasibility of aquaculture within Dutch offshore wind parks depends on many aspects, including: government and legislation: which activities are allowed within offshore wind farms and which fishing techniques can be applied; biological: which fish species are suitable for cultivation in the Dutch North Sea; ecological: what is the impact on the ecosystem; economical: what will be a viable business case; communal: who will work in the aquaculture industry and what is the communal impact; fish welfare: how to guarantee the welfare of the fish; technical: what are the design conditions for aquaculture systems in the Dutch North Sea and what are robust designs?

Ref [1] gives insight in governmental, ecological and economical aspects. Ecological, biological and economic viability of potential species for aquaculture are described in detail in Ref [2].

The present paper adds to the exploratory research from the technical viewpoint by means of presentation of two potential aquaculture concepts. The suggested systems are patent-free; we encourage further development of these concepts by others.

Technical design

Selected site: Borssele II
Environmental conditions and design loads

Suggested concept

Concept I: closed net pen: philosophy, design, loads, mooring
Concept II: Seabed based large fish cage: philosophy, design, loads, mooring

Figure 1: Concept I: Closed net pen within offshore wind park

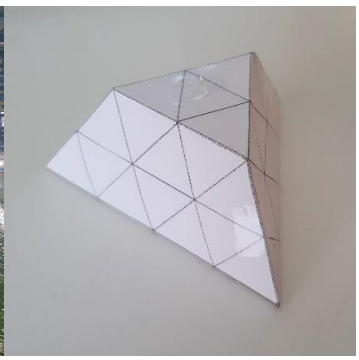


Figure 2: Concept II: seabed based large fish cage

(Continued on next page)

Conclusions and recommendations

The present research indicates the technical feasibility of the two suggested systems. Further technical developments are recommended on: determination of the environmental loads; improvement of the design; logistic operations including installation, operation and maintenance. Furthermore multidisciplinary research is required to develop the concepts further including governmental, ecological and economical research aspects.

References

[1] Lisa Valerie Putter (june 2020), The Identification of businesspartners related to multi-use within wind farm Borssele, https://kennisdelen.rvo.nl/file/download/55080423/scriptie-the-identification-of-business-partners-related-to-multi-use-within-windfarm-borssele-l-putter.pdf

[2] Jansen, H.M., Van Den Burg, S., Bolman, B. et al. The feasibility of offshore aquaculture and its potential for multi-use in the North Sea. Aquacult Int 24, 735–756 (2016). https://doi.org/10.1007/s10499-016-9987-y

THE POTENTIAL FOR COMBINED PRODUCTION OF SALINITY TOLERANT TILAPIA AND SHRIMP IN BRACKISH WATER PONDS IN INDONESIA

Priadi Setyawan[1,2*], Mark Camara[1], John Bastiaansen[1], and Hans Komen[1]

[1]Animal Breeding and Genomics, Wageningen University and Research
[2]Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries Indonesia.
*Corresponding author. E-mail: priadi.setyawan@wur.nl

Introduction

The potential area for brackish water aquaculture in Indonesia is about 2.25 million ha of undeveloped and marginal land concentrated along the coast of Java Island, which contributed 614,518 ton in 2017 with the total value of around 1,59 billion USD. Total production is spread between traditional (216,791 ton), semi-intensive (360,617 ton) and intensive (37,109 ton) farming systems, and consists mostly of smallholder shrimp farmers operating low input traditional and semi-intensive systems.

Shrimp farming occupies around 53% of Indonesian aquaculture production in brackish water (1.15 million ton in 2017) second only to tilapia (1.27 million ton) (MMAF 2018). However, in recent years mass mortality and farm failures have made shrimp farming a high-risk industry, and resulted in the abandonment of land and ponds in many traditional shrimp farm areas. Consequently, many small-scale farmers are looking for alternative crops to secure their livelihoods. Promising alternatives are rotational cropping or co-culture of tilapia and shrimp.

Tilapia farming is predicted to become a key industry in Indonesia (Phillips et al. 2015). Fisheries extension officers in many areas in North Coast Java report that the number of tilapia farmers in brackish water ponds is rapidly increasing, and milkfish production declined from 747,445 tons in 2016 to 701,319 ton in 2017 (MMAF 2018). However, tilapia in high salinity ponds have lower growth rates, survival, and reproduction (Popma and Masser 1999, Cruz-Suarez et al. 2006, Bœuf and Payan 2001, Cnaani and Hulata 2011). The prevalence and economics of these systems have not been studied sufficiently to determine whether they are economically viable and sustainable. economically viable and sustainable.

The objective of this study was to review the current status of combined tilapia and shrimp farming in Indonesia as a foundation for more in-depth economic analyses and to identify challenges, barriers, and opportunities that must be addressed in the near future.

Materials and methods

We conducted a survey of farmers in four provinces on Java Island (Banten, Central, East, and West) and to determine the current practices related to shrimp and tilapia farming in brackish water. 224 farmers were asked a series of questions about which species they farm, the systems they use, and the income they generate. To describe the patterns and trends, we summarized the number of farmers who earn low, medium and high incomes as percentages for each of provinces. We categorized the farming systems implemented in each province as monoculture, polyculture, mono-rotation, and poly-rotation and present these data as percentages. We also summarized the types of water aeration, pond lining systems, the use of commercial feed, the feeding rate and type of tilapia farmed as percentages.

Results

Our survey results show those farming practices vary between regions, and that polyculture and rotational farming systems are much less common in Banten than other regions of Java. We also found that the use of commercial feed and feeding rates varies between regions, with Banten farmers completely reliant on commercial feed, East Java farmers least reliant on commercial feed, and Central and West Java intermediate. More than half of farmers in Central Java, West Java and East Java applied commercial feed at less than 3% of fish biomass per day, while 56.3% of farmers in Banten applied a higher rate of 3-5% biomass per day.

Furthermore, the income levels of farmers vary between regions. Most farmers in Java Island (46.4-61.1%, depending on the province) are low-income earners. Medium income earners account for around a third, and less than a quarter of the farmers surveyed are high income earners. This regional variation could be affected by many factors that need to be analyzed in further study.

(Continued on next page)

Discussion and conclusions

Recurrent shrimp farming failures caused by disease outbreaks have resulted in an important shift in farming systems in brackish water ponds in Indonesia. Shrimp monoculture is now only practiced in the most suitable areas, and a majority of shrimp farmers have shifted to tilapia or low-density shrimp production in polyculture systems. Farmers are implementing a variety of production rotational and polyculture systems in Central, East and West Java, but much less so in Banten. It seems that the lowest percentage of ponds in production driven their farming system.

However, despite the advantages, farming tilapia in abandoned brackish water shrimp ponds faces some challenges and obstacles. Currently available salinity tolerant tilapia strains show large variation in growth and survival, and further work is required to 1) develop an optimised breeding strategy to improve growth rate and survival of tilapia grown in shrimp ponds under fluctuating and unpredictable salinities, and 2) assess and compare the economic viability of various shrimp-tilapia farming systems.

Small scale shrimp tilapia farmers in all four provinces are dominated by low-income earners who practised low input production system which presumably ended up in a low productivity trap. Government support is needed to promote them to be more productive through providing a better financial access and supporting irrigation system, road, electricity, and other infrastructures in brackish water area.

References

MMAF (2018) Marine and Fisheries in Figures 2018. Ministry of Marine Affairs and Fisheries, Jakarta

Rimmer MA, Sugama K, Rakhmawati D, Rofiq R, Habgood RH (2013) A review and SWOT analysis of aquaculture development in Indonesia. Reviews in Aquaculture 5 (4):255-279. doi:10.1111/raq.12017.

CO_2/PH IMPACTS THE SURVIVAL OF EUROPEAN EEL (*Anguilla anguilla*) EMBRYOS WHEN INCUBATED IN RECIRCULATION AQUACULTURE SYSTEMS

Daniela E. Sganga[1*], Sebastian N. Politis[1], Flemming T. Dahlke[2], David Mazurais[3], Ana Servili[3], Sune R. Sørensen[1,4], Ian A. Butts[5], Francesca Bertolini[1] and Jonna Tomkiewicz[1]

1 Technical University of Denmark, National Institute of Aquatic Resources, Lyngby, Denmark
2 Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
3 Ifremer, Marine Environmental Science Laboratory UMR 6539, Plouzané, France
4 Billund Aquaculture, Billund, Denmark
5 Auburn University, School of Fisheries, Auburn, Alabama, United States of America
E-mail: delsg@aqua.dtu.dk

Introduction

Considerable efforts have been made in the past years towards closing the European eel life cycle in captivity (Tomkiewicz et al., 2019), including enhanced environmental conditions for embryonic and larval culture (Politis et al., 2017, 2018). Still, effects of pH fluctuations on eel early life stages remain unknown. Fish embryos and larvae are expected to be particularly sensitive to pH changes, as organs for pH regulation are not fully developed (Ishimatsu et al., 2008). The aim of this study was to evaluate the sensitivity of eel embryos to fluctuations in pH/CO_2 and identify the developmental timing and functionality of related regulatory pathways.

Materials and methods

Following a standardized gamete production and fertilization protocol (Tomkiewicz et al., 2019), European eel embryos were moved to three custom designed RAS units supplying upwelling incubators. Embryos were incubated at optimal temperature and salinity and at three different PCO_2/pH conditions: (1) control-PCO_2 (400 µatm CO2, pH 8.1), (2) high-PCO_2 (1000 µatm, pH 7.7) and (3) extreme-PCO_2 (3000 µatm, pH 7.0). Embryonic survival was assessed at 4, 24, and 48 hours post-fertilization (hpf). Samples of embryos were taken at 24 hpf for morphological measurements, and at 24 and 48 hpf to determine the expression levels of 11 target genes: hsp70 and hsp90, involved in physiological stress/repair response; aqp1 and aqp3, which are water and solute transporters; nkcc1a, ncc and car15, involved in acid-base regulation; crfr1 and crfr2, that mediate in responses to stress; and GABAAα6b and GABAAα12, related to inhibitory neurotransmission.

Results

Survival was similar for all pH/CO_2 conditions at 4 hpf. At 24 hpf and onwards, survival significantly declined at extreme PCO_2 compared to the control and high PCO_2 treatment (Fig. 1A). The egg diameter at 24 hpf was smaller at high and extreme PCO_2 with a related reduction of the perivitelline space (Fig. 1B). The expression of aqp3, hsp70, crfr2 and ncc significantly increased from 24 to 48 hpf, although they were not affected by the pH/PCO_2 treatments. Conversely, crfr1, GABAAα6b and GABAAα12 expression, decreased over time for all treatments. Finally, the expression levels of aqp1, hsp90, car15, and nkcc1a genes were similar across treatments and developmental times.

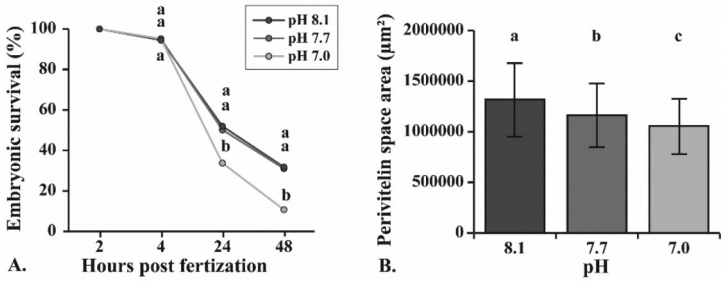


Figure1. Survival (A) and perivitelin space area (B) of European eel embryos incubated at control, high and extreme-PCO_2 conditions.

(Continued on next page)

Discussion

At increased pCO2 levels, egg swelling was impaired resulting in a reduction in egg size. Here, high concentrations of H^+ may have an inhibitory effect on colloidal processes leading to a reduction in water uptake during perivitelline space formation (Eddy & Talbot, 1983). This could negatively affect the embryos ability to osmoregulate and therefore lead to increasing mortality as was observed for the high and extreme PCO_2 conditions. Such stressful conditions, are expected to lead to upregulation of heat shock proteins (Roberts et al., 2010). In European eel embryos, hsp70 and hsp90 expression was unaffected by changes in PCO_2, even though these genes are upregulated in response to salinity and thermal stress in early larval stages (Politis et al., 2017, 2018). Similarly, crfr1 and crfr2 transcription levels, genes also involved in stress response, were not affected by acidification. Expression of crfr2 increased in all treatments from 24 to 48 hpf, whereas crfr1 showed an opposite trend. This is similar to what was observed for zebrafish embryos, which relates to their inability to synthesise cortisol until after hatching (Alderman & Bernier, 2009). On the other hand, the expression of acid-base regulatory genes was similar for all treatments. Here, nkcc1 and CA15 expression remained constant, but ncc increased towards 48 hpf suggesting an important role in osmoregulation in late developmental and early larval stages. In conclusion, the underlying mechanisms investigated in this study, seem to be under development during early embryonic ontogeny and thus not yet matured to regulate a molecular response to acidification. Nevertheless, these findings show that exposure to high PCO_2 conditions can impair normal development in this species and should be controlled in order to optimize European eel culture.

References

Alderman SL & Bernier NJ (2009). Ontogeny of the corticotropin-releasing factor system in zebrafish. Gen Comp Endocr, 164:61-69

Eddy FB & Talbot C (1983). Formation of the perivitelline fluid in Atlantic salmon eggs (*Salmo salar*) in fresh water and in solutions of metal ions. Comp Bioch Phys C, 75:1-4

Ishimatsu A, Hayashi M & Kikkawa T (2008). Fishes in high-CO2, acidified oceans. MAR Ecol Prog Ser, 373:295-302

Roberts RJ, Agius C, Saliba C, Bossier P & Sung YY (2010). Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. J Fish Dis, 33:789-801

Politis SN, Mazurais D, Servili A, Zambonino-Infante JL, Miest JJ, Sørensen SR, ... & Butts IA (2017). Temperature effects on gene expression and morphological development of European eel, *Anguilla anguilla* larvae. PLoS One, 12:e0182726

Politis SN., Mazurais D, Servili A, Zambonino-Infante JL, Miest JJ, Tomkiewicz J & Butts IA (2018). Salinity reduction benefits European eel larvae: Insights at the morphological and molecular level. PloS one, 13:e0198294

Tomkiewicz J, Politis SN, Sørensen SR, Butts IAE, Kottmann JS (2019). European eel – an integrated approach to establish eel hatchery technology in Denmark. In Eels Biol Monit Manag Cult Exploit Proc First Int Eel Sci Symp. 340-374

EFFECTS OF DIETARY LIPID LEVEL AND ENVIRONMENTAL TEMPERATURE ON THE SEVERITY OF STEATOSIS IN ATLANTIC SALMON FED SUBOPTIMAL LEVEL OF CHOLINE

Daphne Siciliani[1], Gerd M. Berge[2], Trond M. Kortner[1], Elin C. Valen[1], Elvis M. Chikwati[1], Åshild Krogdahl[1]

[1]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
[2]NOFIMA, Norwegian Institute of Food, Fisheries and Aquaculture Research, Sunndalsøra, Norway
E-mail: daphne.siciliani@nmbu.no

Introduction
Hypervacuolization and a whitish appearance of the pyloric caeca due to excessive lipid accumulation in the tissue are the main symptoms of the lipid malabsorption syndrome (LMS)[1]. LMS frequently affects Atlantic salmon *(Salmo salar L.)* fed with plant-based feeds containing high lipid level concomitant with low choline. Choline, free or as part of phosphatidylcholine, is required for lipoprotein assembly and secretion, and therefore essential for lipid transport[1]. The requirement for post-smolt Atlantic salmon fed with a plant-based diet containing 29% lipid has been estimated by Hansen et. al[2] to be around 3.4 g/kg. However, choline requirement most likely vary with production conditions, environmental and dietary factors. Fish at later stages of development need increasing level of lipids in the diet, and therefore, higher level of choline may be necessary to secure an adequate lipid transport. The results to be presented are part of an experiment conducted to evaluate how choline requirement in Atlantic salmon is affected by dietary lipid level and possible interaction with environmental temperature.

Materials and methods
Four choline deficient plant-based diets were formulated differing in lipid levels: 16%, 21%, 26% and 31%, respectively and fed to Atlantic salmon raised in fresh water at two different environmental temperatures: 8 and 15 degrees. Duplicate tanks of fish were used for each treatment.

Following an 8-week feeding trial, six fish from each tank were sacrificed and body measures taken. Thereafter, the fish were opened, and the organ package removed. Liver and intestines were separated from the other organs and cleaned of external lipid. Weights of liver, and the intestine separated into the pyloric region, mid and distal intestine sections were weighed and sampled. Tissue samples from all the fish were processed to perform gene expression analyses. Additional samples were processed to evaluate histological changes and steatosis level. Enterocyte vacuolization was scored according to their morphological appearance and graded as normal, mild, moderate, marked and severe (Fig.1). To conduct further chemical and digestibility evaluations, feed and feces were collected to be analyzed for dry matter, ash, crude protein, lipid and starch. The digestibility of the macronutrients was assessed by using Yttrium oxide as internal marker. Plasma was collected and stored for evaluation of plasma biomarkers of nutritional status: glucose, free fatty acids, cholesterol and total triacylglycerides.

Results
Growth performance was, as expected substantially, higher in fish raised at 15 degrees compared to those raised at 8 degrees, whereas no significant effect of dietary lipid level was observed.

The histological examination showed a significantly higher degree of vacuolization in the pyloric caeca of fish fed higher lipid levels compared to those fed lower lipid levels. Suggesting a clear dose response effect (Fig.2).

In parallel, the profiling of targeted genes involved in lipid uptake and transport, cholesterol metabolism, phosphatidylcholine and fatty acid synthesis, revealed a similar pattern.

However, preliminary results show that gene expression is highly influenced by the lipid level, rather than by the environmental temperature.

Other results of the experiment are under evaluation and will be presented at the conference.

Conclusions
The results obtained so far indicate that choline requirement is mainly affected by the lipid level, whereas the temperature effects show a less clear picture. Further data analyses will be conducted to achieve a clearer overall conclusion.

(Continued on next page)

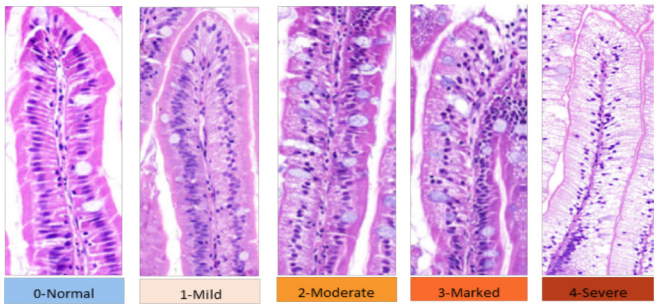


Figure 1: *Illustration of score values indicating severity of vacuolation of the pyloric caeca tissue. Presence of supranuclear vacuoles was the main criteria for the scores, i.e. normal, mild, moderate, marked and severe. Photo by Elvis M. Chikwati*

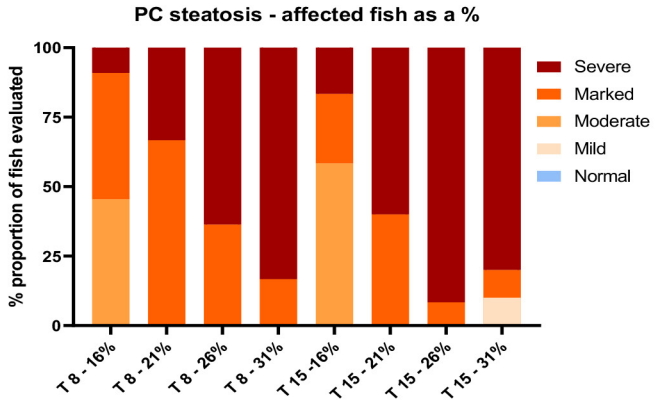


Figure 2:*Scoring of steatosis level in the pyloric caeca tissue*

References

1. Hansen, A. K. G. *et al*. Choline supplementation prevents diet induced gut mucosa lipid accumulation in post-smolt Atlantic salmon (Salmo salar L.). *BMC Vet. Res.* **16**, 1–15 (2020).
2. Hansen, A., Kortner, T., Denstadli, V., Måsøval, K., Björkhem, I., Grav, H., & Krogdahl, Å. Dose–response relationship between dietary choline and lipid accumulation in pyloric enterocytes of Atlantic salmon (Salmo salar L.) in seawater. British Journal of Nutrition, 123(10), 1081-1093

Funding

The experiment is one of a series conducted under the GutMatters project funded by The Norwegian Seafood Fund (FHF, project 901435).

DEFINING NUTRITIONAL REQUIREMENTS OF RAINBOW TROUT FOR THE FUTURE BY REVISITING THE PAST

T. S. Silva*[1], A. Kause[2], F. Soares[1], A. Nobre[1], J. Dias[1], L. E. C. Conceição[1]

[1] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal.
[2] Natural Resources Institute Finland (Luke), Genomics and Breeding, Myllytie 1, FI-31600 Jokioinen, Finland
E-mail: tomesilva@sparos.pt

Introduction

Nutritional requirements of fish are classically determined through dose-response trials under controlled settings and generally defined as the nutrient amounts required to cover basal/maintenance costs plus whatever required to achieve a certain growth (or nutrient retention). Despite the cumulative knowledge acquired on the nutritional requirements of fish (see, e.g., NRC 2011), there is the possibility that, as fish traits change due to (e.g.) genetic improvement programs, fish nutritional requirements may be affected, eliciting the need to revise nutritional guidelines accordingly.

It is challenging to quantify the genetic improvement in feed utilisation and growth efficiency across years. As a complementary approach, it is possible to analyse annual trends of data on fish traits, with a "year effect" being used as a proxy of the "genetic effect" on fish traits. In particular, we assume that, if genetic improvement has affected fish traits in a meaningful way, one should be able to observe these changes along time, as genetic improvements accumulate.

We performed a meta-analysis of rainbow trout growth trials (from both published peer-reviewed sources and in-house trials) covering the 1979-2019 time period to determine: a) whether there is evidence of changes in important fish traits (e.g. feed intake rates, maximum growth capacity, growth efficiency) along time; b) what possible changes in nutritional requirements should we expect as a consequence of these changes in fish traits.

Materials and methods

For the analysis, information on a total of 24 rainbow trout growth trials (representing 172 tanks/cages/units) was collected from either scientific literature or in-house trials of SPAROS Lda, covering the time span between 1979 and 2019. This effort focused on sources which provided explicit information on growth, water temperature, feed intake and diet composition. When available, information on whole body composition was also collected from each source. For each period between measurements, aggregate estimates of relevant factors and responses (year, average temperature, average body weight, average growth, average feed intake, average digestible energy intake, average digestible protein intake, average energy gain, average protein gain) were calculated, resulting in a dataset with 534 points used for downstream analysis. Meta-analysis was performed by modelling relevant fish traits as a function of the factors (or transformed factors) using least-squares and quantile linear regression. Additional information about time-dependent trends of important traits (growth, FCR, fillet yield, viscerosomatic index) in rainbow trout, provided by Luke and obtained using an independent genetic trend analysis of the Finnish national breeding program, was also used to support this analysis (Kause et al. 2021).

Results

The results do not show strong evidence of year effects (and, thus, potential genetic improvement effects) on feed intake levels or maximum growth rates. Instead, there is a progressive improvement over time in traits related to growth efficiency (i.e. minimum FCR, maximum protein and energy retention efficiencies).

This improvement in growth efficiency is consistent with the independent estimates of the genetic improvement of rainbow trout traits in Luke's breeding program (red points/lines in Figure 1, Kause et al. 2021), which have been estimated avoiding the confounding effects of nutritional factors.

Discussion and Conclusion

Overall, the results support the notion that, at least in rainbow trout, genetic improvements have led to cumulative changes in traits that can affect fish nutritional requirements. We discuss the possible impacts of these trait changes on nutritional requirements and optimal feed formulations.

(Continued on next page)

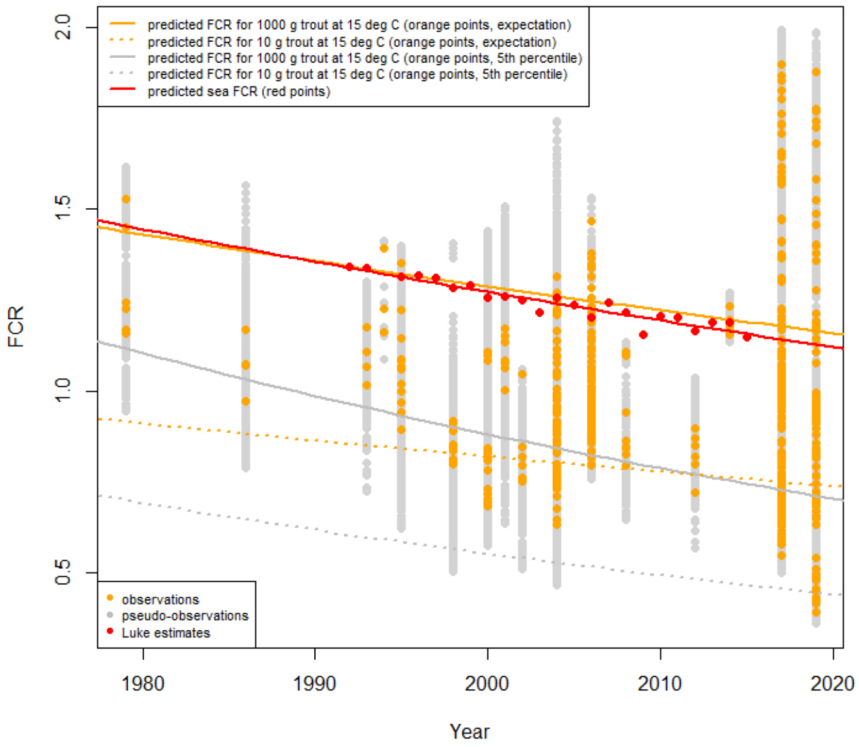


Figure 1 – Scatter plot showing measured FCR values as a function of year. Orange points represent measurements used for the analysis, grey points represent pseudo-measurements obtained by interpolation (not used for analysis), and red points represent Luke's estimate of genetic improvement. The effect of year on rainbow trout FCR estimated by Luke (red line) is consistent with the effects estimated in the current analysis (other lines).

Acknowledgements

This work was funded by the EU Horizon 2020 AquaIMPACT project (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture; 818367).

References

National Research Council. (2011). Nutrient requirements of fish and shrimp. National academies press.

Kause A., Koskinen H., Nousiainen A. (2021). Role of selective breeding in the improvement of feed efficiency at rainbow trout farms. Aquaculture Europe 2020. Book of Abstracts.

CHARACTERIZATION OF ANTIOXIDANT AND ANTI-ENZYMATIC ACTIVITIES OF ALGAL EXTRACTS FROM THE PORTUGUESE COAST – POSSIBLE VALUABLE INGREDIENTS FOR FISH DIET SUPPLEMENTATION

Nádia Silva[1,4*], Vânia P. Roberto[1,4], Leonardo Mata[1], Katia Pes[1,4], Maria Graça Miguel[3], Paulo J. Gavaia[1,4]

[1] Center of Marine Sciences, University of Algarve, Faro, Portugal
[2] Sparos Lda, Olhão, Portugal
[3] Faculty of Sciences and Technology (FCT), University of Algarve, Faro, Portugal
[4] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
nsilva@ualg.pt

Introduction
Macroalgae are natural sources of functional proteins and carbohydrates, carotenoids, phenolic compounds and vitamins. The use of macroalgae in fish diets has improved trends in fish growth, physiology, stress resistance, immune system, and fillet muscle quality. Nevertheless, very few macroalgae species have been investigated as potential components in finfish diets, reviewed in (Kamunde et al., 2019; Wan et al., 2019). Due to the variable nutritional content of macroalgae, a previous characterization of their properties is essential.

Methods
Here, we performed a characterization of biological activities in seven macroalgae species naturally occurring in the southwest region of the Portuguese coast: the brown algae: *Cystoseira sp*., *Fucus vesiculosus, Sargassum vulgarae*; the red algae: *Asparagopsis armata*, *Sphaerococcus coronopifolius*, *Plocamium cartilagineum* and the green algae: *Codium vermilara*. The algae were collected by diving and were dried in the shadow at room temperature. Ethanolic extracts were prepared (100mg/ml) and further characterized by evaluation of total phenolic content and *in-vitro* antioxidant activity, by determining superoxide, DPPH and NO scavenging activity. *In-vitro* evaluation of anti-diabetic, anti-inflammatory and antioxidant capacity was done by measuring inhibitory activities of the extracts on the enzymes: lipoxygenase, acetylcholinesterase, tyrosinase, xanthine oxidase, α-glucosidase and α-amylase.

Results
Brown algae, namely *Fucus vesiculosus,* and *Sargassum vulgarae*, demonstrated to have the highest phenolic content (Fig. 1A) and antioxidant capacity (Fig.1B).

Brown algae also revealed an enhanced enzyme inhibitory activity when compared with green and red algae, especially anti-diabetic effects, by inhibiting α-amylase and glucosidase enzymes as shown by the very low IC50 values (Fig 2). Anti-inflammatory activity by inhibition of lipoxygenase and acetylcholinesterase was prevalent in all extracts although at higher concentrations.

Conclusions
Extracts of seven species of naturally occurring marine macroalgae of in the southwest coast of Portugal were characterized for the first time regarding their antioxidant capacity and anti-enzymatic activity. There was individual variability regarding antioxidant levels but the brown algae *Fucus vesiculosus, Sargassum vulgarae* and *Cystoseira sp*., presented the highest levels of phenolic content and antioxidant capacity. *Fucus vesiculosus* and *Sargassum vulgarae* showed also good levels of anti-enzymatic activity in all analysis performed especially anti-diabetic activity by inhibiting α-amylase and α-glucosidase. Supplementation of finfish diet with these algae or algae extracts may bring benefits for the aquaculture industry and constitute a valuable functional ingredient in fish feeds.

(Continued on next page)

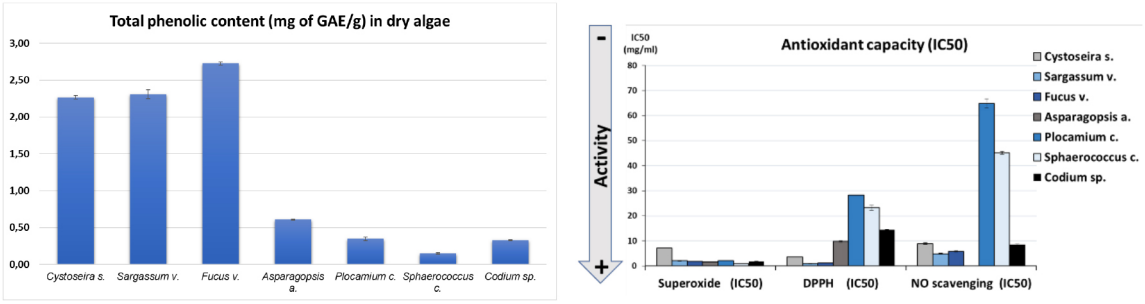


Figure 1 – (A) Total phenolic content (mg/g gallic acid equivalent GAE) and (B) antioxidant capacity (IC50 = mg/mL) of ethanolic extracts of *Cystoseira sp.*, *Fucus vesiculosus, Sargassum vulgarae*, *Asparagopsis armata*, *Sphaerococcus coronopifolius*, *Plocamium cartilagineum* of the Portuguese coast. Extracts with no inhibitory enzymatic activity are absent from the graph.

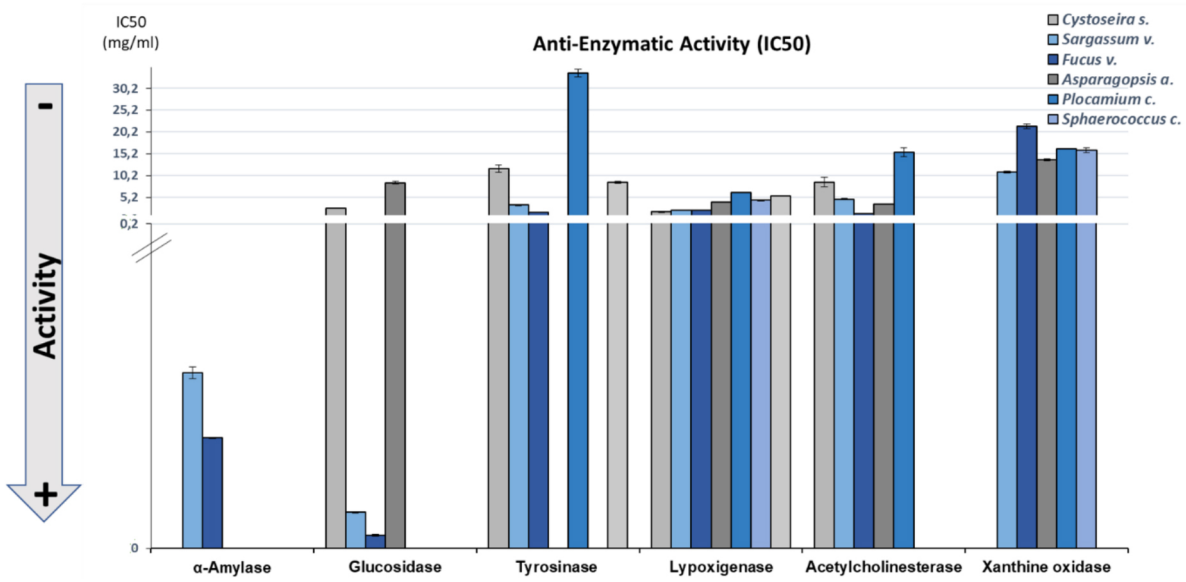


Figure 2 – Anti-enzymatic activity of enzyme inhibitory activities (IC50 = mg/mL) of ethanolic extracts of *Cystoseira sp.*, *Fucus vesiculosus, Sargassum vulgarae*, *Asparagopsis armata*, *Sphaerococcus coronopifolius*, *Plocamium cartilagineum* of the Portuguese coast. Extracts with no inhibitory enzymatic activity are absent from the graph.

Acknowledgments

Financial support from the FCT (grant UID/Multi/04326/2020) and European Maritime and Fisheries Fund (EMFF/ FEAMP) through the National Operational Program MAR2020 (grant ALGASOLE-16-02-01-FMP-0058).

References

Kamunde, C., Sappal, R., Melegy, T.M., 2019. Brown seaweed (AquaArom) supplementation increases food intake and improves growth, antioxidant status and resistance to temperature stress in Atlantic salmon, Salmo salar. PLoS One 14, e0219792.

Wan, A.H.L., Davies, S.J., Soler-Vila, A., Fitzgerald, R., Johnson, M.P., 2019. Macroalgae as a sustainable aquafeed ingredient. Rev. Aquac. 11, 458–492.

DID YOU KNOW THAT HETEROTROPHIC FEED CAN IMPROVE CORAL THERMAL STRESS RESPONSE?

Andreia F. Silva[1*], Ana P.L. Costa[2], Davide A.M. Silva[2], Sílvia S.F. Pires[2], Andreia C.M. Rodrigues[2], Amadeu M.V.M. Soares[2], Jorge Dias[3], Teresa Baptista[1] and Rui J.M.Rocha[2,4]

School of Tourism and Marine Technology, Institute Polytechnic of Leiria (Peniche,Portugal)
CESAM & DeBio, Aveiro University (Portugal)
SPAROS Lda, Olhão (Portugal)
RIASEARCH Unipessoal, Lda, Murtosa (Portugal)
* andreia.filipa.cs@gmail.com

Introduction

The effects of climate change have been observed all over the world, specifically on coralreefs, which may have an irreparable impact on biodiversity and ecosystem services in theoceans. When they are under stress, the photosynthetic corals can lose theirendosymbionts, (dinoflagellates from the genus *Symbiodinium,* usually known as zooxanthellae) being this process described as mass coral bleaching. Climate change havebeen associated with the rise of sea surface temperature and the occurrence of marine heatwaves. The frequency at which marine heatwaves occur has increased over the last few decades, resulting in a higher frequency at which coral bleaching events occurs. As aconsequence, coral species most susceptible to these events suffer irreversible damage, aszooxanthellae represents a substantial part of their nutrition, which can lead to coral degradation and, in more extreme cases, to their death, shifting the structure of coral reefs.However, in addition to autotrophic nutrition, provided by the products of photosynthesis,photosynthetic corals can also feed by heterotrophy, and are consequently classified as mixotrophic animals. According with several studies, this feeding strategy can contributeto corals resilience to stress.

Methodology

In this context, the aim of this study was to evaluate the response to heat stress of mini colonies of *Palythoa sp*. (subclass Hexacorallia; order Zoantharia) after four months of culture without (NF) and with (F) feed supply. Immediately after this period the thermal stress test was performed: two groups (25NF and 25F) remained with the culture temperature (25±1°C) while other two groups (30NF and 30F) were exposed to a gradualwater temperature increase during 24 hours until reaching 30±1°C. This temperature wasmaintained during eight days. Fed corals were compared with corals without feed supply,in order to evaluate the diet effect in a scenario of thermal stress. To support this experimental assay, an evaluation of photobiology parameters, oxidative and thermal stress biomarkers, oxidative damage, and energy reserves was carried out. No mortality was recorded during the experimental assay.

IMPLEMENTATION OF A MACROALGAE SUPLEMENTED FEED FOR SENEGALESE SOLE (*Solea senegalensis*) REARING

Nádia Silva[1,2], Vânia P. Roberto[1,2], Catarina Oliveira[1], Leonardo Mata[1], Michael Viegas[3], Elsa Cabrita[1], Paulo J. Gavaia[1,2*]

[1] Center of Marine Sciences, University of Algarve, Faro, Portugal
[2] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
[3] Sparos Lda, Olhão, Portugal
*pgavaia@ualg.pt

Introduction
Macroalgae offer nutritional added value in fish diet, since they were shown to have beneficial effects in fish growth, physiology, stress resistance, immune system, and fillet muscle quality and have been successfully introduced in feed for farm animals (Kamunde et al 2019; Wan et al, 2019; Nazarudin et al, 2020). Nevertheless, the use of macroalgae in the production of Senegalese sole have never been tested and the potential effects on growth, reproduction and skeletal development are still to be determined. The aim of this study was to assess the benefits to Senegalese sole performance by incorporating dry algae, rich in phylloquinone and antioxidants, into a semi-humid diet specifically formulated and optimized for *S. senegalensis* rearing.

Methods
After establishing a macroalgae panel, previously characterized for their phylloquinone and antioxidant content, we selected *Plocamium cartilagineum*, *Sargassum vulgare* and *Cystoseira sp*. for incorporation (5% dw) into *S. senegalensis* feed. The dietary experiment was carried out during alevin 3 stage (mean weight 16g) and was carried for 4 months. The main parameters determined included growth, bone quality and acute stress resistance.

Results
Fish showed a good acceptance of the new diets and during the experimental period of 117 days increased over 3-fold in wet weight. Survival was not affected by dietary supplementation with the selected seaweeds. Algae supplementation had a small effect on growth parameters: final weight amongst groups was similar and feed efficiency and survival rate were not affected (Table 1). The condition factor (K%) was lower in *Sargassum vulgare* fed groups ($p<0.05$) and daily weight gain was smaller for *Cystoseira sp.* and *Plocamium cartilagineum* groups ($p<0.01$) but overall, the specific growth rate (SGR%) was not influenced by macroalgae supplementation in the diet.

Analysis of bone quality was determined by vertebral mineral content and revealed a decrease in the levels of calcium and especially phosphorus in the fish fed with *P. cartilagineum* supplemented diet (Table 2). Fish fed with *S. vulgare* supplemented diet also showed a slight decrease in phosphorous levels, but not in calcium. Nevertheless, these differences were not reflected in less radio-dense bone as diagnosed by X-Ray analysis (data not shown).

Stress resistance was performed by air exposure challenge, which led to an acute stress response characterized by an increase in the cortisol levels in all groups (Figure 1). Nevertheless, the timing and dimension of the response was different for each diet, while the *P. cartilagineum* fed group followed a similar response to the control diet group, the amplitude of the cortisol peak at 1.5h was significantly smaller ($p<0.001$). *Cystoseira sp.* fed group reached the cortisol peak at 1h while recovery to pre-stress levels occurred at 1.5h, sooner than control group. *S. vulgare* fed group displayed the highest stress response with cortisol peak occurring at 1h and continuing to rise at 1.5h. By 3h post-stress all groups had reached basal levels of cortisol except *S. vulgare* ($p<0.0001$). The secondary response to stress was evaluated by plasmatic glucose and lactate levels. *Cystoseira sp.* group displayed an earlier increase in glucose levels (at 0.25h, $p<0.05$), while *S. vulgare* fed diet displayed an increase at 3h in both glucose ($p<0.001$) and lactate levels ($p<0.0001$). *P. cartilagineum* fed group had levels of glucose and lactate similar to control group at all timepoints.

Conclusions
Supplementation of macroalgae rich in phylloquinone and antioxidants showed no deleterious effects on growth, feed efficiency parameters and survival. On the other hand, bone quality of *Solea senegalensis* fed with *P. cartilagineum* and *S. vulgare* enriched diet may be affected since phosphate content was reduced. Regarding acute stress response, *Cystoseira sp,* enriched diet allowed for a faster recovery of the stress condition, while *P. cartilagineum* supplemented diet led to a decrease of cortisol levels. Further studies at molecular and histological levels will provide further insights on the mechanisms giving rise to these distinct physiological stress responses. As it stands the benefits/drawbacks of using these macroalgae in *S. senegalensis* diet need further experiments and will be reinforced by analysis of immune response and nutrient content in the fish.

(Continued on next page)

Table 1: Fish performance and feed efficiency in *Solea senegalensis* fed algae supplemented diets.

	Control	*Cystoseira sp.*	*S. vulgarae*	*P. cartilagineum*
Initial weight (g/fish)	16.61±6.35	16.9±6.98	14.95±6.68	16.69±6.56
Final weight (g/fish)	62.71±21.3	58.73±24.14	61.70±26.22	58.76±26.10
SGR% weight	1.15±0.02	1.08±0.01	1.23±0.02	1.09±0.06
K%	1.57±0.178	1.48±0.167	1.47±0.147*	1.53±0.191
Daily weight gain	0.40±0.002	0.36±0.01**	0.40±0.035	0.37±0.04**
Conversion ratio	1.03±0.35	1.10±0.62	1.17±0.29	1.05±0.16
Survival (%)	89.6±6.87	89.6±10.80	89.58±8.83	93.06±1.96

Data are expressed as mean ± SD, N=50, One-way Anova (*p<0.05; **p<0.01); K%=(Px100)/L^3, condition factor; SGR%=(e^g-1)*100, g=(ln Wf-ln Wi)/(Tf-Ti)

Table 2: Mineral contents of vertebral column from *Solea senegalensis* fed with macroalgae supplemented diet. Values are presented as % dry weight (n=3, total per treatment =9; One-way Anova:*p<0.05; **p<0.01).

(%)	Control	*Cystoseira sp.*	*S. vulgarae*	*P. cartilagineum*
Vertebrae dry weight	43.01±1.89	39.68±2.59	43.25±9.45	41.87±14.16
Calcium	24.64±2.08	22.06±2.20	23.10±1.27	19.36±0.70 *
Phosphorus	9.62±0.60	8.20±0.66	7.69±1.06 *	4.83±0.50**
Ca/P	2.56±0.05	2.69±0.06	3.02±0.238	4.02±0.03 **
K	0.36±0.04	0.38±0.09	0.35±0.02	0.48±0.05
Mg	0.42±0.01	0.41±0.05	0.42±0.02	0.37±0.01
Na	0.94±0.01	1.04±0.16	0.92±0.01	0.77±0.09

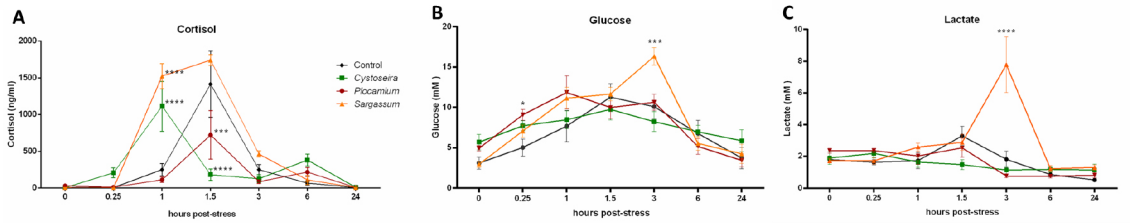


Figure 1 – Characterization of acute stress response of *S. senegalensis* fed experimental diets through time, by analysis of plasma cortisol (A), glucose (B) and lactate (C) levels. Data are expressed as mean±SEM. Two-way Anova analysis was performed using time and diet as variables, followed by Dunnet's multiple comparisons against the control group for each timepoint; *p<0.05; ***p<0.001;****p<0.0001.

Acknowledgments

Financial support from the FCT (grant UID/Multi/04326/2020) and European Maritime and Fisheries Fund (EMFF/ FEAMP) through the National Operational Program MAR2020 (grant ALGASOLE-16-02-01-FMP-0058). The authors would like to acknowledge the support and technical assistance of João Reis and Miguel Patusco at Ramalhete Marine Station.

References

Kamunde, C., Sappal, R., Melegy, T.M., 2019. Brown seaweed (AquaArom) supplementation increasesfood intakeand improves growth, antioxidant status and resistance to temperature stress in Atlantic salmon, Salmo salar. PLoS One 14, e0219792.

Wan, A.H.L., Davies, S.J., Soler-Vila, A., Fitzgerald, R., Johnson, M.P., 2019. Rev. Aquac. 11, 458–492.

Nazarudin M.F., Yusoff F., Idrus E.S., Aliyu-Paiko M. 2020. Aquac. Rep. 18, 100488.

EFFECT OF MACROALGAE DIETARY SUPPLEMENTATION ON THE RESISTANCE TO PATHOGENS IN *Solea senegalensis*

Nádia Silva[1,2], Rui Sousa[3], Vânia P. Roberto[1,2], Michael Viegas[4], Florbela Soares[3], Catarina Marques[3], Pedro Pousão-Ferreira[3], Paulo J. Gavaia[1,2]

[1] Centre of Marine Sciences, University of Algarve, Faro, Portugal
[2] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal
[3] Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO), Olhão, Portugal
[4] Sparos Lda, Olhão, Portugal
pgavaia@ualg.pt

Introduction

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species in the aquaculture of SouthernEuropean countries. Aquaculture produced fish are potentially subjected to stress and pathogens due to several environmental factors. *S. senegalensis* is especially vulnerable to diseases such as Pasteurellosis or Flexibacteriosis (Toranzo et al., 2005). Previous studies reported that macroalgae can be used as an antioxidant additive, and are natural sources of liposoluble vitamins, such as vitamins K1, E and A, which are known to improve immune competence (Kraan, 2013). With this work we propose to increase health and well-being of *S. senegalensis* under aquaculture conditions, by using diets supplemented with macroalgae as natural sources of liposoluble vitamins and antioxidants, in this way promoting higher resistance to disease outbreaks and avoiding the use of antibiotics and other treatments.

Methods

The experiment was carried out at Ramalhete Marine Station Facilities at the University of Algarve (Faro, Portugal) in a RAS system (temperature: 17-19ºC; salinity 36ppt; photoperiod 14h light:10h dark; dissolved oxygen above 85% saturation level). Fish (weighing on average 16.22±0.9g wet weight) were maintained for 9 months and feed with a commercial diet supplemented with 5% dry algae: *Plocamium cartilageneum*, *Cystoseira sp*., *Sargassum sp*. and a control diet supplemented with 5% wheat (Sparos, Lda, Olhão, Portugal). At the end of the trial a challenge with *Photobacterium damselae* sub. *piscicida* was performed in IPMA/EPPO (Olhão, Portugal). For that, 19 fish from each experimental diet (weighing on average 66.2±26.9g wet weight) were subdivided into 3 groups and received $5x10^8$ CFU/ml of *Photobacterium damselae* sub. *piscicida* through intraperitoneal inoculation (0,1ml). Another 18 (3x6) fish from the control diet were divided into 3 subgroups and received a saline control inoculation. Mortality was recorded daily.

Results

This study intended to evaluate the effects of dietary macroalgae inclusion in the resistance to pathogens, namely mortality, in sole.

Statistical analysis showed no significant differences between group diets in each sampling time. Nevertheless, *P. cartilageneum* and *Cystoseira sp*. enriched feed had slightly better results in the mortality assay at 120h (95,24% vs 100%).

Conclusions

Algae supplementation in fish diet has the potential to improve resistance to disease outbreaks. Here supplementation with 5% *Plocamium cartilageneum*, *Cystoseira sp*. and *Sargassum sp*. showed no significant effect in resistance of *S. senegalensis* juveniles to *Photobacterium damselae* sub. *Piscicida*. However other algae or higher algae incorporation rates should be tested to determine the potential benefits of macroalgae as natural sources of bioactives for dietary enrichment.

(Continued on next page)

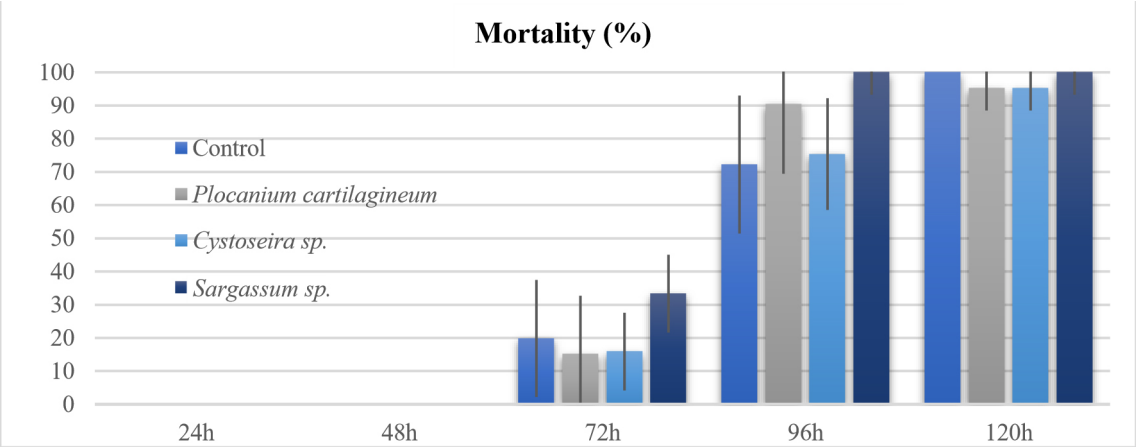


Figure 1 – Characterization *Solea senegalensis* mortality fed with 3 diets, after infection with *Photobacterium damselae* sub. *piscicida*. Data are expressed as % mortality ±SD. Statistics was performed using Kruskal Wallis one-way anova .

Acknowledgments

Financial support from the FCT (grant UID/Multi/04326/2020) and European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Program MAR2020 (grant ALGASOLE-16-02-01FMP-0058) and SAUDE&AQUA (MAR-02.05.01-FEAMP-0009).

The authors would like to acknowledge the support and technical assistance of João Reis and Miguel Patusco at Ramalhete Marine Station.

References

Kraan, S., 2013. Pigments and minor components in algae. In: Dominguez, H. (Ed.), Functional Ingredients from Algae for Foods and Nutraceuticals. Woodhead Publishing, Cambridge, p. 205.

Toranzo, A.E., Magariños, B., Romalde, J.L., 2005. A review of the main bacterial fish diseases in mariculture systems. Aquaculture 246, 37–61.

MEASURING STRESS IN CULTURED CORALS*:* SHOULD WE USE THE SAME YARDSTICK?

Davide A. M. Silva*[1]; Ana P. L. Costa[1]; Andreia C. M. Rodrigues[1]; Mario G. G. Pacheco[1], Amadeu M.V.M. Soares[1] and Rui J.M. Rocha[1,2]

[1] CESAM – Centro de Estudos de Ambiente e do Mar, Departamento de Biologia, Universidade de Aveiro Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[2] RIASEARCH Unipessoal Lda, Murtosa, Portugal
Email: dams@ua.pt

Introduction

Scientific interest for photosynthetic corals has been steadily rising due to climate change, but also because these organisms are increasingly cultured for marine conservation purposes, bioprospection and the aquarium trade (Leal et al., 2013). Nevertheless, the intricate physiology of these organisms is still not well known. Furthermore, photosynthetic corals are unique in the animal kingdom, on the one hand, due to the sheer simplicity of their tissues, and on the other hand due to the complexity of several different symbionts sharing the same organism. The increasing awareness in these organisms has been fostering the scientific community interest, and numerous studies have been developed, namely toxicological trials in which the use of organic solvents is customary. The work described here sheds light on the physiologic response to one of the more commonly used solvents, methanol, of three distinct coral species (*Montipora digitata*, *Sarcophyton glaucum* and *Zoanthus* sp.). These species represent the major groups of cultured photosynthetic corals (*Scleractinia, Alcyonacea, Zoantharia*), providing crucial insights regarding the physiology of cultured corals.

Materials and methods

Coral specimens were collected in the Indo-Pacific and acclimated for one month in standardized modular systems (Rocha et al*.,* 2015). After acclimation, colonies were fragmented and randomly assigned to different methanol concentrations (from 0.01 mL L-1 to 2.9 mL L-1) for 96 h. After the trial, we assessed the antioxidant response (catalase activity – CAT, glutathione s-transferase activity – GST and total glutathione – tGSH), the lipidic peroxidation – LPO and the photosynthetic activity using rapid light curves (maximum electron transport rate – ETRmax and maximum quantum yield – F_v/F_m). Data were analysed in R (2021) with a principal component analysis – PCA.

Results

The principal component analysis clearly shows separate groups representing the different species. The PC1 is sufficient to separate the species and is responsible for the variation within the *Montipora digitata* group. The antioxidant response and lipid peroxidation are the variables that contribute more to the PC1 explaining 40.58% of the variation. The PC2 is responsible for the variation within the groups, more notably within the zoanthid but also within the *Sarcophyton glaucum*. The photosynthetic indicators, F_v/F_m and ETRmax, are the variables that contribute the most to the PC2, which explains 20.03% of the total variation.

Discussion

The response of the zoanthid is stronger in photosynthetic indicators. This behaviour is intriguing given the fact that zoanthids usually prioritize autotrophy over heterotrophy (Leal et al., 2017), and consequently, conserving the photosynthetic apparatus was expected, although not observed. The response of *Montipora digitata* is reflected mainly throughout the antioxidant response. This reaction is typical to several other organisms when faced with chemical stressors (*e.g.* Rodrigues et al. 2015). Finally, the *Sarcophyton glaucum* response is somewhat less expressive than the other species. Nonetheless, the response is more variable throughout the photosynthetic apparatus. Given the results, our study highlights the need to consider the physiological intricacies of apparently similar organisms that are often entitled to the same living being. The oversimplification of the *Anthozoa* class hinders the unfolding of the physiology of these organisms, which is the cornerstone to better understand these organisms, and ultimately better culture techniques.

(Continued on next page)

Figures

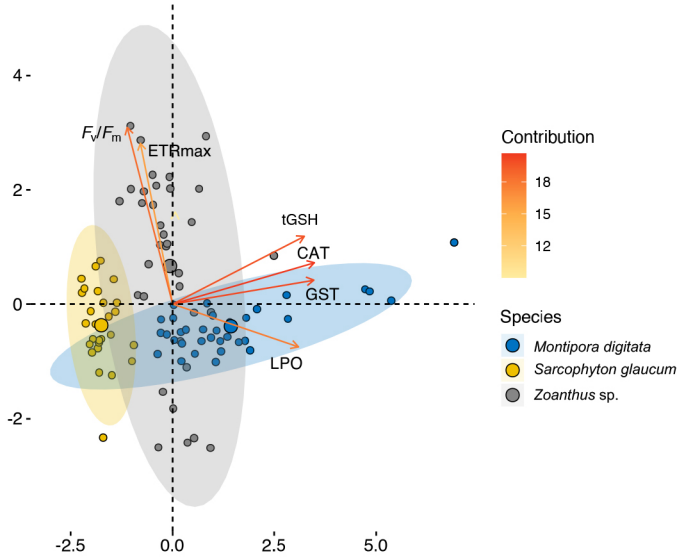


Figure 1 – Principal Component Analysis (PCA) showing variation between species in terms of antioxidant response (CAT, GST, tGSH), lipid peroxidation (LPO) and photosynthetic activity (F_v/F_m and ETRmax).

References

Leal, M.C., Calado, R., Sheridan, C., Alimonti, A., Osinga, R., 2013. Coral aquaculture to support drug discovery. Trends Biotechnol. 31, 555–561.

Rocha, R.J.M., Bontas, B., Cartaxana, P., Leal, M.C., Ferreira, J.M., Rosa, R., Serôdio, J., Calado, R., 2015. Development of a Standardized Modular System for Experimental Coral Culture. J. World Aquac. Soc. 46, 235–251.

Leal, M.C., Rocha, R.J.M., Anaya-Rojas, J.M., Cruz, I.C.S., Ferrier-Pagès, C., 2017. Trophic and stoichiometric consequences of nutrification for the intertidal tropical zoanthid *Zoanthus sociatus*. Mar. Pollut. Bull. 119, 169–175.

Rodrigues, A.C.M., Gravato, C., Quintaneiro, C., Golovko, O., Žlábek, V., Barata, C., Soares, A.M.V.M., Pestana, J.L.T., 2015. Life history and biochemical effects of chlorantraniliprole on *Chironomus riparius*. Sci. Total Environ. 508, 506–513.

SEXUAL REPRODUCTION IN THE GREEN MACROALGA *Codium tomentosum* – A "NEW SPECIES" FOR AQUACULTURE

Gonçalo Silva Marinho*[1,2], Maria Francisca Sá[1,2], Isabel Sousa-Pinto[1,2]

*corresponding author: goncalomarinho@hotmail.com
[1] Department of Biology, Faculty of Sciences of the University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros de Leixões, 4450-208 Matosinhos, Portugal

Introduction

AquaVitae (AV) is a research and innovation project funded by the European Union's Horizon 2020 program. AV's overall objective is to introduce new, and expand existing, low trophic species products and processes to marine aquaculture value chains across the Atlantic. Diversification of macroalgae production is paramount if macroalgae are to reach their full potential for the provision of food, feed, and biomass for many other applications. Therefore, species of macroalgae not yet fully exploited commercially but with recognised potential were investigated to optimise production conditions.

Codium tomentosum is a valuable source of food and biomass, which is currently cultivated at a pilot scale in land-based aquaculture. Cultivation is carried out through biomass fragmentation followed by vegetative propagation in tumble culture, which may result in reduced genetic diversity and reduce technology options for its cultivation.

To develop a new hatchery protocol based on sexual reproduction to produce seedlings of *C. tomentosum* it is essential to understand the basic reproduction biology of the species. Sexual reproduction in the *Codium* genus is performed through zygotes resulting from the gamete fusion. The zygote germinates into a diploid germling, a siphonous filament, which eventually develops into the adult thalli. The peak of sexual maturation for *C. tomentosum* has been reported to be reached in winter, but no comprehensive analysis on the seasonal reproductive status of the species has been done until now. Moreover, the control of the life cycle under artificial culture conditions is challenging and has not been achieved so far.

This study aims to evaluate the effect of the season and culture conditions such as light intensity and spectrum, nutrient concentration, and water movement on the reproductive status, and early stages of development of *C. tomentosum*. The ultimate goal is to define optimized culture conditions for sexual reproduction, which will form the basis for the future establishment of hatchery protocols for seedling production.

Material and methods

Specimens of *Codium tomentosum* were collected from two natural populations from Northern Portugal: Aguçadoura shore (41° 26′ N, 8° 47′ O), located in **Póvoa** de Varzim, and Viana- Norte (41°41'49.3"N, 8°51›03.4»W), located in Viana do Castelo. The biomass has been collected monthly or bi-monthly since January 2020 for evaluation of seasonal variation in the reproductive status.

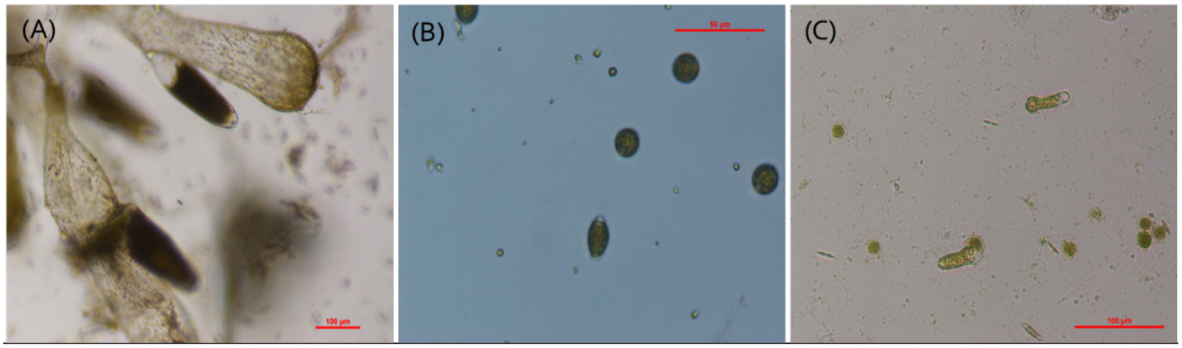


Figure 1. (A) Utricles with gametangia; Scale bar = 100 μm (B) Male and female gametes 24 hours after release; Scale bar = 50 μm. (C) Zygotes started to germinate forming filamentous germlings (4 days in culture); Scale bar = 100 μm. Pictures by Gonçalo S. Marinho

(Continued on next page)

The species identification, observation of reproductive structures, gamete release, zygote formation, and early stages of development were followed through microscopic observation. Images were recorded with a coupled camera, and post-processed (e.g. measurements) using ImageJ software.

The effect of the light spectrum (white, blue, green, and red) and intensity (20, 40, and 60 **µmol** m^{-2} s^{-1}), nutrient medium concentration (PES, PES/2, PES/10, and natural seawater), and water movement on the early stages of development of *C. tomentosum* was investigated.

Results

The absence of mucron in the apical part of the utricles indicates that the biomass collected from natural populations corresponds to *C. tomentosum*, and not the co-existing invasive *C. fragile*.

The presence of reproductive structures, gametangia (Figure 1A), which are indicative of the reproductive status of the specimens, was observed in the samples collected in January and March 2020, they were absent in the samples collected in May and June 2020, and then were observed again in the samples collected from August 2020 to March 2021. The reproductive status was further confirmed by the liberation of gametes, zygote formation and germination.

Both male and female gametes were successfully released and identified (Figure 1B), and after a few days in culture, the development of a zygote could be observed. After approximately 3 to 7 days each zygote germinated into a filamentous germling (Figure 1C). The germlings obtained from the specimens collected in the winter months (December 2020 and January 2021) presented a faster development than those obtained from specimens collected in any other month. Nevertheless, even after some months in culture, the formation of adult thallus could not be observed.

Regarding the culture conditions, the filamentous germlings had a faster development when exposed to a light intensity of 20 μmol m^{-2} s^{-1}, compared to 40 or 60 μmol m^{-2} s^{-1}, regardless of the light spectrum applied. Moreover, the green, red, and white light spectra resulted in faster development than the blue. The germlings performed better when grown in PES medium at normal concentration compared to those grown at lower concentrations (PES/2 and PES/10), or natural seawater alone. Moreover, preliminary results suggest that water movement promotes the development of the germlings compared to cultures kept without movement. The germlings developed (in length) for several weeks, and after some weeks some branching could be observed. However, even after some months in culture, the formation of adult thallus could not be observed.

Discussion

The results showed that *C. tomentosum* from the studied natural populations is reproductive most of the year with exception of a short period in spring-early summer, which matches a period in which an immature new generation of *C. tomentosum* is emerging for the rocky substrate to form the upright adult thallus replacing almost entirely the former one. This study will continue for another year to confirm the observed pattern.

Culture conditions that promote the development of germlings have been identified with respect to light intensity and spectrum, nutrient concentration, and water movement. Nevertheless, even after some months in culture, the development of upright adult thallus was not achieved. This suggests that the culture conditions required to induce the morphogenesis of germlings to form the spongy thallus may not be the same that favor the development of the filamentous germlings. Further multifactorial trials will focus on the specific condition required to induce the morphogenesis and thereby obtain seedlings of *C. tomentosum*.

Acknowledgments

This study/AquaVitae project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 818173.

DYNAMICS OF HARMUL ALGAE BLOOMS IN OFFSHORE AQUACULTURE AREAS BY OCEAN COLOUR REMOTE DETECTION

C. Silveira [1,*], P. Goela[2], S. Cristina[2]

[1] Universidade do Algarve [2] CIMA– Centre for Marine and Environmental Research of the University of Algarve, Campus de Gambelas, Faro, Portugal
*E-mail: catissilveira@gmail.com

Introduction:
Phytoplankton are unicellular organisms, primary producers and are the base of the marine food web are also important for the feeding of bivalves crustaceans, as well as larvae of commercially crustaceans and fish. Phytoplankton blooms are regular and seasonal in many marine areas, but in some situations, they can cause negative impacts, involving serious economic losses for aquaculture, fishing and tourism operations and have major environmental and human health impacts. These impacts can be caused by diatoms such as *Pseudo-nitzschia* spp., dinoflagellates such as *Dinophysis* spp. and other harmful algal blooms (HABs), which can produce toxins that include Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP) and Paralytic Shellfish Poisoning (PSP), due to the consumption of contaminated bivalves. The aim of this study was to detect the dynamics of HABs in offshore aquaculture areas using ocean colour satellite remote sensing.

Materials and methods:
Chlorophyll-a satellite images adquiredfrom the SW coast of Portugal has been collected from the sensors Moderate Resolution Imaging Spectroradiometer, aboard the Aqua satellite (MODIS-AQUA) and Visible Infrared Imaging Radiometer aboard the Suomi National Polar-orbiting Pathership (VIIRS Suomi-NPP) and crossed with algal bloom events in the region of study reported in literature (Danchenko et al., 2019) and from monitoring data from IPMA (Portuguese Institute for Sea and Atmosphere) in the L7a and L7c zones. It has been also made a remote sensing reflectance (R_{rs}) spectral shapes analysis were 3x3 pixel matrices were extracted from different coordinates between 2 km and 12 km of distance from the coast in both L7a and L7c zones.

Results:
Variations of Chlorophyll-a concentration during the occurrence of HABs in 2014
Ocean colour remote sensing was able to detect a bloom forming in the beginning of July, reaching 2.96 $mg.m^3$ of Chlorophyll a which started to fade after de 9th of July (Fig. 1). According to the literature (REF), on July 14, 2014, it was observed a bloom of *Dinophysis caudata* and *Dinophysis acuminata*, with a concentration of 520 cells L^{-1}. For the same day, the presence of *Gymnodinium catenatum* was also reported, with 2480 cells L^{-1}, which has resulted in the harvest banning of *Mytilus edulis*, for the 14/07/2014, in zone L7a. IPMA data (Site) shows that on July 16, for the L7a zone, the concentrations of *Pseudo-nitzschia* were 10800 cells L^{-1} and of *Dinophysis* was 240 cells L^{-1}.

Spectral analysis of phytoplankton communities during the occurrence of blooms
The R_{rs} (l) spectra extracted from 16.08.2014 (Fig.2a) and the R_{rs} (l) from 24.08.2014 (Fig.2b), which are the closest dates to the day of collection by IPMA, reveals differences in the spectral shapes, which may indicate a change in the phytoplankton community in the region close to aquaculture area in the region of study.

Discussion:
This study shows a DSP and ASP bloom initiated after a high Chorophyll a blooms detected with the use of ocean colour images and data. Further studies are needed to confirm that changes in spectral differences are due to ASP and DSP producing communities and not to other optical constituents in the water column.

References:
Danchenko, Sergei, et al. “Harmful phytoplankton diversity and dynamics in an upwelling region (Sagres, SW Portugal) revealed by ribosomal RNA microarray combined with microscopy.” Harmful algae 82 (2019): 52-71.

(Continued on next page)

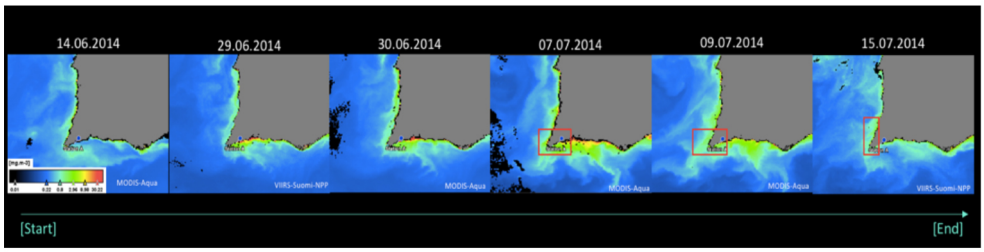


Fig.1: Chlorophyll-a satellite images of the VIIRS Suomi-NPP and MODIS-Aqua s in the L7a and L7c zone for period between June and July 2014.

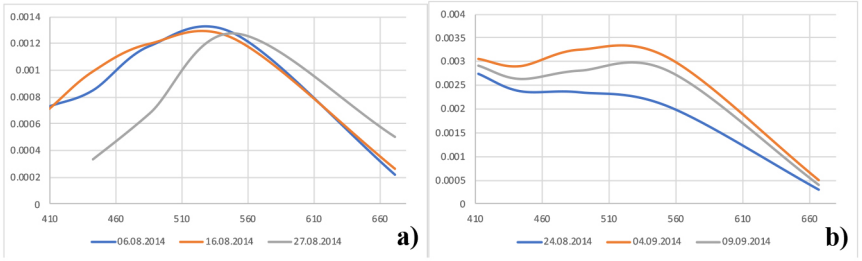


Fig.2: Remote sensing reflectance (R_{rs} (λ)) spectrum view from VIIRS Suomi-NPP sensor **(a)** and MODIS-Aqua **(b)** ocean colour sensors at 7 km from the coast in the L7c zone. .

CIRCULARITY AND WASTE VALORISATION FROM A (RAS) FISH FARMERS PERSPECTIVE

Paul-Daniel Sindilariu*, Jan Giebichenstein[1]

* Next Tuna GmbH, Londoner Strasse 3, 60327 Frankfurt am Main, Germany / Tropenhaus Frutigen, Tropenhausweg 1, 3714 Frutigen, Switzerland
paul@nexttuna.com
[1] Next Tuna GmbH, Londoner Str. 3, 60327 Frankfurt am Main, Germany / C-Feed AS Brattørkaia 17B 7010 Trondheim Norway

Introduction

In the framework of the European Green Deal, the EU commission has published its Aquaculture strategic guidelines. In these guidelines, the circular approaches, waste valorisation and recirculating aquaculture production are key elements to increase the needed EU aquaculture production.

Within the Horizon 2020 framework, the Commission financed three major research projects looking into novel approaches of aquaculture farming and circular economy in aquaculture (Gain, IMPAQT and iFishIENCi). These projects bring essential new impulses to the discussion on waste valorisation and circular concepts in aquaculture. However, the majority of presented circular solution take the approach from the perspective of the additional value creation, rather than from the need of the fish farmer. This presentation want's to bring back the focus of the discussion to the needs of the industry.

The Farmers Perspective

However, compared to the scientific approach to the topic, a fish farmer has a far more practical approach to waste management, waste valorisation and circularity.

At the moment all waste handling in aquaculture is a necessity due to legal/public requirements. Waste management is part of the production license of each aquaculture facility but the rules for waste management differ tremendously from country to country within the EU, from region to region within one country and even from site to site within one region.

For the fish farmer, waste management, in most cases, does not produce more fish, or makes the product more appreciated. Waste management only adds costs, in a very competitive business environment.

Thus, any new approach to waste valorisation to be accepted by the industry, it needs to have some key features:

1. Change the waste handling for the farmer from a cost factor, to a revenue stream, or at least to a lower cost factor.
2. Easy and simple solution, as the farmer wants to focus on fish production and not on waste valorisation.
3. Robust and reliable solution, the farmer never wants to come in a situation, where he has to stop the production, because the waste handling system is out of operation.

Potential solutions

In order to achieve the positive acceptance by the industry, solutions to waste valorisation need to have the following features:

1. Low mutual dependency and interconnection. None of the partners should be dependent of a well working process of the other partner. Thus, issues in the valorisation process do not affect the production capacity of the fish farm and vice versa.
2. No spatial dependency. The valorisation process should not absolutely need to happen next to the fish farm, as the required space, license, infrastructure might not be available. Ideally the valorisation of waste become just a question of smart logistics.
3. Mutual benefit. Both the aquaculture producer and the waste valorisation should profit from the valorisation process.

In conclusion new potential valorisation technologies need to go far beyond the actual applied industry standard of sludge thickening and subsequent biogas production or land application as fertilizer.

EFFECT OF FILAMENTOUS FUNGI *Neurospora intermedia* AND FEEDING PERIOD ON GUT MICROBIOTA OF RAINBOW TROUT (*Oncorhynchus mykiss*)

Aprajita Singh*, Sajjad Karimi, Aleksandar Vidakovic, Markus Langeland, Johan Dicksved, Jorge Ferreira, Mohammad J. Taherzadeh, Anders Kiessling and Torbjörn Lundh

Department of Animal Nutrition and Management, Faculty of Veterinary Medicine and Animal Sciences
Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
Email: aprajita.singh@slu.se

Introduction

Microbial proteins including filamentous fungal biomass contain noticeable levels of protein, fatty acids and other nutritious components (Karimi *et al*., 2018). Also fungi cell wall contains chitin and chitosan and other polysaccharides as their bioactive components having immunomodulatory and antimicrobial properties (Mario *et al*., 2008, Esteban *et al*., 2001). Thus, fungi may play a role in improving the health of farmed fish. Several studies has been reported that environmental (abiotic) and host (biotic) factors play important role in shaping the gut communities (Ingerslev *et al*., 2014a; Ringø *et al*., 2016; Yan *et al*., 2016; Sun *et al*., 2020). Most of these studies investigated the short or long term dietary effect of diets on gut microbiota. However, a study targeting the gradual change in microbial communities over time with the type of diet that to our knowledge has not been described earlier. Diet can adversely modulate the gut microbial composition leading inflammation of distal intestine as in case of Atlantic salmon fed with higher levels of soy protein (Gajardo *et al*., 2017). It is necessary to understand the interaction between host, gut microbiota, diet and feeding strategy for the development of novel diets ensuring better fish health and welfare. Hence, this study was conducted to observe role of *N. intermedia* in modulating the intestinal microbiota of rainbow trout under provided feeding periods.

Materials and Methods

Three experimental diets were prepared, a reference diet (RD), a non-preconditioned diet (NPD) and a preconditioned diet (PD). Diet RD was prepared with fishmeal as a major protein source without fungal biomass. Formulation for diet NPD and PD were created by mixing 30 percent of *Neurospora intermedia* biomass and 70% of diet RD according to (Cho, 1979). Formulation for diets PD and NPD were same, however, diet PD was conditioned in a convection oven (Electrolux Professional, FCE061) at 105°C for 5 min in order to increase the degree of gelatinization of starch and emulate temperature treatment during extrusion conditions. Twenty fish were distributed randomly in each experimental tank (mean weight: 127± 4 g) and were acclimated for 9 days on commercial diet. Feeding was provided in excess at 1.5% of body weight in excess. Gut microbiota were analyzed on day 0, 10, 20 and 30. Similarity Percentage Analysis (SIMPER) and Analysis of Similarity (ANOSIM), Principal coordinate analysis (PCoA) and Principal component analysis (PCA) were performed using Paleontological Statistics Software version 4.03 (PAST).

Results

The bacterial OTUs abundance were dominated by two phyla Firmicutes (58%) and Proteobacteria (15%). From day 0 to day 30, Firmicutes ranged from 38 % to 79% followed by Proteobacteria which ranged from 8% to 24% for different diets. *Peptostreptococcus* (9%), *Lactococcus* (*L. lactis,* 7%), *Brevinema* (6%), *Streptococcus* (5%), *Deefgea* (5%) and *Anaerotruncus* (4%) were the most abundant over 30 days. Diet and day has significant effect on shaping the gut microbial composition. The pairwise comparison of the treatments groups were significantly dissimilar within and between day intervals for top 6 OTUs. PCA plot reveals the occurance of *Peptostreptococcus* and *Streptococcus* are correlated. Their abundance was correlated to initial days of feeding in all diets whereas abundance of *Lactococcus* was more correlated to final days of feeding. One way ANOSIM confirmed that the microbial composition was similar at day 0 and day 30 whereas dissimilar at day 10 and day 20. The overall microbial composition for diet RD is different from PD and NPD at day 10.

Discussion

The analysis reveals that the core gut microbiota was dominated by Firmicutes followed by Proteobacteria which is similar with other studies on salmonids (Nayak, 2010; Gajardo *et al*., 2017). Bacterial composition for plant protein based diets or fish meal free diets show increase in abundance of Lactobacillales in rainbow trout and salmon respectively (Schmidt *et al*., 2016; Michl *et al*., 2017). Earlier studies suggested that change in microbiota with change in diet was observed in salmon, rainbow trout and brown trout after first feeding (Ingerslev *et al*., 2014b; Michl *et al*., 2017; Michl *et al*., 2019). No change in the gut microbiota with the time was observed suggesting after certain days or longer feeding gut microbiota gains stability. Similarly, it was observed from the analysis that gut microbiota were significantly dissimilar at day 10 and day 20 and were similar at day 30 of feeding suggesting that the microbiota might be approaching stability.

(Continued on next page)

Conclusion

- It can be concluded that diets with *N. intermedia* and duration of feeding shapes the gut microbial composition of rainbow trout.
- N. intermedia can be fermented in the gut by lactic acid bacteria.
- There is a shift in gut microbial composition with days of feeding and microbiota attains stability at 30 days. Therefore, minimum 30 days feeding trial is suggested for functional gut microbiome studies.

References

Cho, C. (1979). Apparent digestibility measurements in feedstuffs for rainbow trout. In: Halver, J., Tiews, K. (ed. *Proceedings of the world symposium on finfish nutrition and fishfeed technology* Heenemann, pp. 239-247.

Gajardo, K., Jaramillo-Torres, A., Kortner, T.M., Merrifield, D.L., Tinsley, J., Bakke, A.M. & Krogdahl, Å. (2017). Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic salmon (Salmo salar). *Appl. Environ. Microbiol.,* 83(5), pp. e02615-16.

Ingerslev, H.-C., Strube, M.L., von Gersdorff Jørgensen, L., Dalsgaard, I., Boye, M. & Madsen, L. (2014a). Diet type dictates the gut microbiota and the immune response against Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss). *Fish & shellfish immunology,* 40(2), pp. 624-633.

Ingerslev, H.-C., von Gersdorff Jørgensen, L., Strube, M.L., Larsen, N., Dalsgaard, I., Boye, M. & Madsen, L. (2014b). The development of the gut microbiota in rainbow trout (Oncorhynchus mykiss) is affected by first feeding and diet type. *Aquaculture,* 424, pp. 24-34.

Karimi, S., Soofiani, N.M., Mahboubi, A. & Taherzadeh, M.J. (2018). Use of Organic Wastes and Industrial By-Products to Produce Filamentous Fungi with Potential as Aqua-Feed Ingredients. *Sustainability,* 10(9).

Michl, S.C., Beyer, M., Ratten, J.-M., Hasler, M., LaRoche, J. & Schulz, C. (2019). A diet-change modulates the previously established bacterial gut community in juvenile brown trout (Salmo trutta). *Scientific reports,* 9(1), pp. 1-12.

Michl, S.C., Ratten, J.-M., Beyer, M., Hasler, M., LaRoche, J. & Schulz, C. (2017). The malleable gut microbiome of juvenile rainbow trout (Oncorhynchus mykiss): Diet-dependent shifts of bacterial community structures. *PLoS One,* 12(5).

Nayak, S.K. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture Research,* 41(11), pp. 1553-1573.

Ringø, E., Zhou, Z., Vecino, J.G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R., Dimitroglou, A., Foey, A. & Davies, S. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquaculture nutrition,* 22(2), pp. 219-282.

Schmidt, V., Amaral-Zettler, L., Davidson, J., Summerfelt, S. & Good, C. (2016). Influence of fishmeal-free diets on microbial communities in Atlantic salmon (Salmo salar) recirculation aquaculture systems. *Appl. Environ. Microbiol.,* 82(15), pp. 4470-4481.

Sun, S., Su, Y., Yu, H., Ge, X. & Zhang, C. (2020). Starvation affects the intestinal microbiota structure and the expression of inflammatory-related genes of the juvenile blunt snout bream, Megalobrama amblycephala. *Aquaculture,* 517, p. 734764.

Yan, Q., Li, J., Yu, Y., Wang, J., He, Z., Van Nostrand, J.D., Kempher, M.L., Wu, L., Wang, Y. & Liao, L. (2016). Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environmental microbiology,* 18(12), pp. 4739-4754.

Di Mario, F., Rapana, P., Tomati, U., & Galli, E. (2008). Chitin and chitosan from Basidiomycetes. *International journal of biological macromolecules*, *43*(1), 8-12.

Esteban, M.A., Cuesta, A., Ortuño, J. & Meseguer, J. (2001) Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish Shellfish Immunol*. 11, 303– 315.

A NEW TURBELLARIAN PARASITE INFLICTING SERIOUS MORTALITIES IN RED DRUM AQUACULTURE

F.E. Montero[1], I. Estensoro[2], L. Leria[3], M. Víllora-Montero[1], E. Planas Callao[4], M. Riutort[3], A. Sitjà-Bobadilla*[2]

[1]Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Paterna, Valencia, Spain
[2]Fish Pathology Group, Institute of Aquaculture Torre de la Sal, CSIC, Castellón, Spain
[3]Dpt. de Genètica, Microbiologia i Estadística, Facultat de Biologia, and Institut de Recerca de la Biodiversitat (IRBio), University of Barcelona, Spain
[4] Biofarm Team, Biomar Iberia, S.A., Spain
E-mail: ariadna.sitja@csic.es

Introduction

Turbellarian flatworms are controverted organisms with changing taxonomical adscription. Most of these platyhelminths are terrestrial and aquatic free-living organisms, but also include symbiotic species, and few cases of parasitic ones associated to fish, crustaceans and molluscs. Here, we report an epizootic due to a rhabdocoelan infection in cultured red drum (*Sciaenops ocellatum*) in a sea-cage farm in a tropical area. We describe the morphological, histological and molecular approaches for its identification.

Methods

Two subsequent outbreaks in 2018-2019 affected red drum in the first year after entering the farm (weight ranging from 12g to 180 g). Water temperature ranged from 23 to 29.5°C and salinity was 36 ‰. Different types of samples of fish presenting acute mortalities and clinical signs were taken. Fresh smears of gills were observed at light microscope on site. Necropsied gills were fixed in 10% neutral buffered formalin (NBF), processed routinely, embedded in Technovit7100-resin, sectioned at 1 µm and stained with Giemsa, PAS, and alcian blue-PAS. Some NBF-fixed specimens were dehydrated, cleared with dimethyl phthalate and stained with iron acetic carmine. Another sample set was stored in 70% ethanol for molecular identification. The ribosomal genes 18S and 28S were PCR-amplified (Giribet et al., 1996; Jovelin & Justine, 2001) and used concatenated to infer a phylogeny by Bayesian inference. Other representatives of Rhabdocoela present in GenBank were included in the analyses. Bacterial samples were taken for basic bacteriological analysis.

Results

Prevalence of infection was 100 % in some stocks, and mortality ranged from 5% to 60%. Clinical signs included anaemia, weight loss, pale and necrotic gills with mucous masses, desquamation and erosion of the skin, and asphyxiation. Bacteriological results were variable, from negative to opportunistic bacteria or septicaemia, often accompanied by splenomegaly. Microscopical observation of gill scrapings of affected fish revealed ciliated turbellarians with eyespots. When gravid adults were mounted in seawater under a glass coverslip, active swimming eyed juvenile emerged. Worms, with characteristic anterior eyes, were visible under low magnification (Fig. 1). The morphometric study of fixed worms showed that adults were elongated, piriform with pointed posterior end (854-1403 × 356-589 µm) and a short anterior distal projection. Eyespots were separated. Pharynx was subconical and anterior. Testes and ovary were small and follicular. Some large specimens exhibited numerous completely developed juveniles occupying most of the body. Based on the body shape, pharynx arrangement and the presence of juveniles, the worms were tentatively assigned to the family Graffillidae.

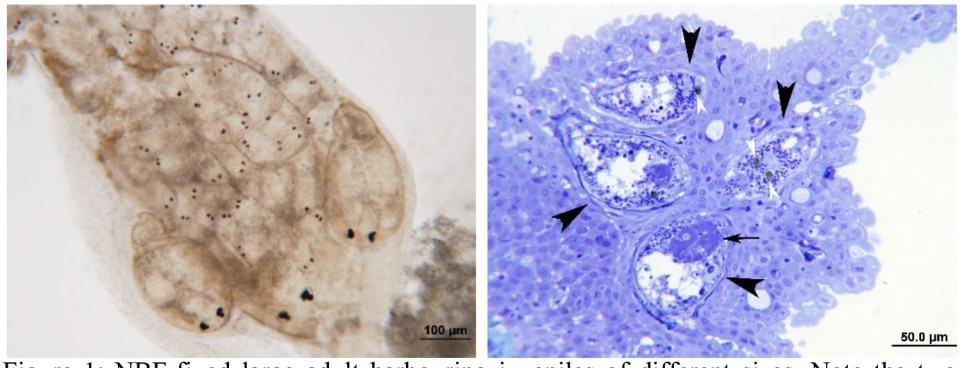


Figure 1: NBF-fixed large adult harbouring juveniles of different sizes. Note the two eyespots in all of them (left). Giemsa-stained gill section showing four worms (black arrowheads) buried in the tissue. Note the eyespots (white arrowheads) and the pharynx (arrow) (right).

(Continued on next page)

Histologically, worms were placed in shallow epithelial tunnels on secondary and primary lamella, and even on the cartilages of gill arches, and some small specimens were found free among gill filaments (Fig.1). The infection caused destruction of the normal gill architecture with minor histopathological reaction, lacking signs of necrosis or inflammation. Among the epithelial host cells forming the tunnel walls, little or no focal hyperplasia was observed. The primary direct effect of the turbellarian gill infection was the loss of respiratory function by impairment of gas exchange in parasitized lamella. In some sections, long filamentous bacteria covered the tissue and worm surface, suggesting secondary bacterial infections involved in the epizootic case. The worms enclosed within epithelial tunnels appeared to be covered by a sheath of cellular and mucous material.

The molecular study placed the parasite within the Order Rhabdocoela, Suborder Dalytyphloplanida and Infraorder Neodalyellida. However, it was highly divergent from all the deposited sequences of the group, indicating that it may belong to a new species not yet described. It did not match with the recent sequence of the old known *Pseudografillaria arenicola* (Meixner, 1938), and it could be similar to a turbellarian causing epitozootics (with mortalities > 60%) in the same fish species as well as in other cultured marine fish in China (Wang et al., 2002).

Attempts to treat the infections with formalin baths were unsuccessful.

Conclusions

The histological examination revealed the invasive nature of the worms infecting red drum, and the gill damage could easily explain the anaemia and the asphyxiation of the fish. According to the obtained molecular data, the available orphan sequences and morphological descriptions, the species could be new to science, but probably present in other far distant locations and hosts. Studies are ongoing for the full description of the species. The current study and the previous reports on turbellarians causing lesions on various marine fish from the Pacific, Caribbean, Chinese and Australian waters, suggest that these parasites may represent and emerging problem in aquaculture, as they are transmitted fish-to-fish, and topic treatments can be ineffective since they live within gill tissues. Future studies are needed to decipher if other reservoir hosts could be involved in its transmission to cultured fish, and which farming conditions favour its blooming.

References

Giribet G., Carranza S., Baguñà J., Riutort M., Ribera C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Mol. Biol. Evol.*, 13: 76-84.

Jovelin R., Justine J.L. 2001. Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *Int. J. Parasitol.*, 31: 393-401.

Meixner J. 1938. Turbellaria (Strudelwürmer). Die Tierwelt der Nord- und Ostsee. Allgemeiner Teil. In: G. Grimpe, E. Wagler, A Remane (eds). 33(IVb): 1-146, 220 f.

Wang, Y-Y. *et al*. 2002. Turbellarian parasitised in *Sciaenops ocellatus* and its control. *J. Fish. China*, 26: 379-381.

IMPACT OF SUPPLEMENTATION OF MICRO ALGAE *Schizochytrium sp.,* IN PLANT DIET ON REPRODUCTION OF FEMALE RAINBOW TROUT (*Oncorhynchus mykiss*) AND CONSEQUENCES ON PROGENY

E.Cardona[1,2]*, E.Segret[2,4], Y. Cachelou[3], T.Vanderesse[3], S.Lecou[3], C.Coigdarrippe[3], M.Rul[4], L.Larroquet[1], A.Hermann[1], A.Surget[1], F.Cachelou[4], J.Bobe[2] & S.Skiba-Cassy[1]

[1]INRAE, UMR1419 Nutrition, Metabolism, Aquaculture, F-64310 Saint-Pée-sur-Nivelle, France
[2]INRAE, UR1037 Fish Physiology and Genomic laboratory, F-35000, Rennes, France
[3]Viviers de Rébénacq, F-64260 Rébénacq, France
[4]Viviers de Sarrance F-64490 Sarrance, France
Email: emilie.cardona@inrae.fr

Introduction

In the last years, the increase in aquaculture production has forced a change in fish feed composition, with increasing substitution of fish meal (FM) and fish oil (FO) by more available plant sources. Despite the fact that their inclusion rates in compound feeds for aquaculture have shown a clear downward trend in the twenty past years (FAO, 2018), their total replacement by plant feedstuff is still not optimal, leading to reduced growth and reproductive performance (Lazzarotto et al., 2015)no whole breeding cycles of fish fed diets free from marine resources has been reported to date. We therefore studied the reproductive performance of trout after a complete cycle of breeding while consuming a diet totally devoid of marine ingredients and thus of n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFAs.

The broodstock diet, and in particular the lipid and fatty acid composition of the diet, is known to play a key role in reproductive efficiency and survival of the progeny (Izquierdo et al., 2001). And a major problem when replacing both FM and FO by plant sources is the lack of n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Materials & methods

For that purpose, we studied the effect, in rainbow trout female broodstock, of the plant diet supplemented with microalgae (MA) compared to a conventional commercial diet rich in FM and FO (C) on a reproductive performance and egg quality. The microalgae *Schizochytrium sp.,* chosen to supplement plant-based diet in this study, is known to be a rich source of DHA. An 18 months trial involving two reproductive cycles was carried out. During two spawning seasons, we followed the performance of female reproduction measuring fecundity and egg quality. We also investigated the consequences of maternal nutritional history on offspring after the second spawning season. Experimental design is presented in figure 1.

Results & discussion

Despite a significantly lower growth rate in female fed MA diet, the reproductive performances were not affected (egg weight, absolute fecundity). Moreover, in terms of egg quality, during the second cycle of reproduction, the egg diameter was significantly lower with the MA diet, but the eggs presented a significant better egg integrity (less white eggs after 24 hours of hydration) than with a C diet, which is a sign of a better egg quality. Regarding embryonic development, eggs from females fed MA diet had significantly higher hatching survival rate than eggs from females fed C diet. No effect was observed on fry weight at resorption. We linked this better egg quality to a better-balanced fatty acid profile.

Moreover, feeding female with the MA diet had no effect on the growth and survival of the progeny compared to feeding with C diet. However, consequences of maternal nutritional history were observed in the lipid metabolism of progeny. Progeny from female fed MA diet had increased fatty acid biosynthesis and cholesterol synthesis metabolisms. Genes involved in these metabolisms were up-regulated in progeny from female fed MA diet compared to progeny from female fed C diet, whether challenged with an MA diet or fed a commercial diet (figure 2).

Conclusion

To conclude, in the present study, we demonstrated that DHA-rich microalgae supplementation in a plant-based diet allowed to maintain reproductive performance and egg quality comparable to a conventional commercial feed rich in FM and FO. Moreover, this study confirms a reprogramming of the lipid metabolism of progeny in relation to the origin of maternal nutritional.

(Continued on next page)

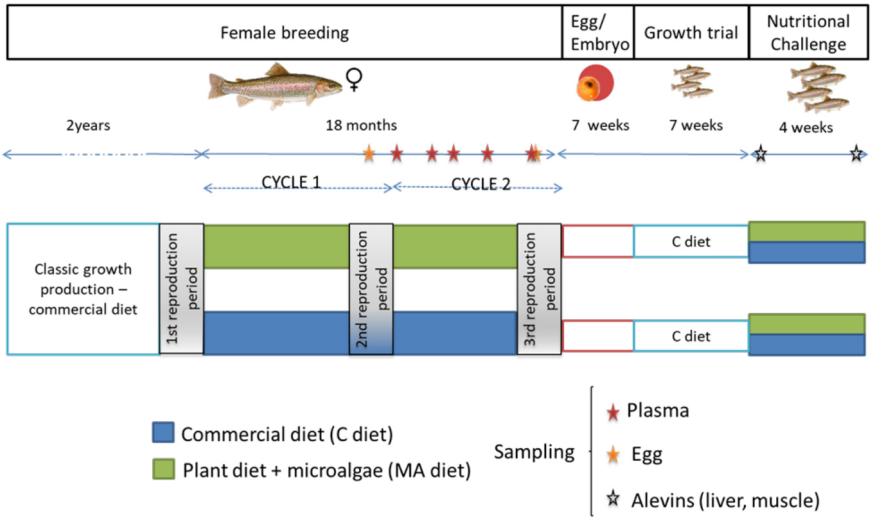


Figure 1 : Experimental design

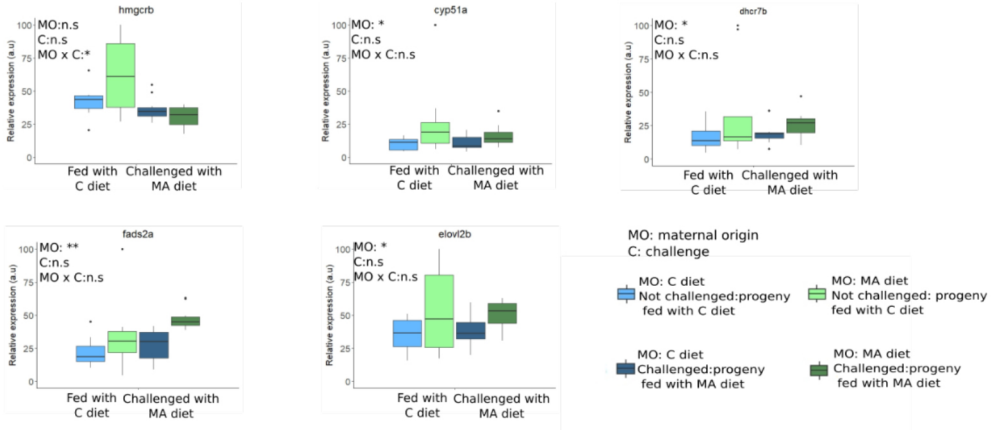


Figure 2:Relative expression of genes involved in fatty acid biosynthesis (elovl2b : fatty acid elongase2 and fads2a : fatty acid desaturase 2) and in cholesterol biosynthesis (hmgcrb : 3-hydroxy-3-methylglutaryl-CoA reductase ; cyp51a : lanosterol 14alpha-demethylase and dhcr7b : 7-dehydrosterol reductase) of progeny according maternal origin in response to a nutritional challenge (fed with C diet or challenged with MA diet).

Acknowledgements: This study is funded by FEAMP (NutriEgg N° PFEA470016FA1000002)

References

Food and Agriculture Organisation (FAO), 2018. The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence CC BY-NC-SA 3.0 IGO.

Izquierdo, M.S., Fernandez-Palacios, H., Tacon, A.G.J., 2001. Effect of broodtsock nutrition on reproductive performance of fish. Aquaculture 197, 25–42.

Lazzarotto, V., Corraze, G., Leprevost, A., Quillet, E., Dupont-Nivet, M., Médale, F., 2015. Three-year breeding cycle of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet, totally free of marine resources: Consequences for reproduction, fatty acid composition and progeny survival. PLoS One 10, 1–17. https://doi.org/10.1371/journal.pone.0117609

SEASONAL BROODSTOCK MANAGEMENT ALTERS THE EPIGENOME OF THE NEXT GENERATION OF ATLANTIC SALMON (*Salmo salar*)

K.H. Skjærven[1], T. Saito[1], Jorge M.O. Fernandes[2], P. Whatmore[1], P. Araujo[1], M. Mommens[3], M. Espe[1]

[1]Institute of Marine Research (IMR), Bergen, Norway
[2]Nord University, Bodø, Norway
[3]AquaGen AS, Trondheim, Norway

*E-mail: kaja.skjaerven@hi.no

Introduction

Atlantic salmon (*Salmo salar*) is an anadromous fish species, born in freshwater, spend most of the life in seawater and thereafter return to freshwater to spawn. For salmon aquaculture, female broodstock are kept in seawater for initial sexual maturation and transferred to freshwater few months prior to spawning. To keep the continuation during production cycle for aquaculture, it is important to have access to new generations of salmon throughout the year.

By adjusting the time for transferring sexually mature broodstock female from sea cages to land-based freshwater cages, the breeding companies have developed protocols to expand the spawning season. Water temperature, feeding and light regimes are abiotic environmental factors that can either accelerate or prolong the time until spawning. However, adjustments in production protocols might introduce both beneficial traits but also functional changes in tissues due to poor nutrition during organ development for the new generation according to the Barker hypothesis (Reviewed by Heindel and Vandenberg, 2015). This phenomenon is called intergenerational programming whereby the nutritional status of the parents influences the next generation (Reviewed by Heard and Martienssen, 2014).

The 1C metabolism, which includes choline, vitamin B6, vitamin B12, and folate, as well as the amino acid methionine, has previously shown to influence broodstock fecundity, but also liver lipid phenotype, metabolism and epigenetic gene regulation in mature zebrafish progeny (Skjærven et al., 2018). For salmon, a shift from normal spawning period by five months in RAS systems disturbs the nutritional status and gene expression in both the female broodstock and their offspring, which results in less allocated nutrients into RAS spawned eggs followed by a deprived growth by the time for first feeding (Skjaerven et al., 2020). Here, we continue by investigating the nutritional status in broodstock and offspring when transferring sexually mature female from sea cages to land-based freshwater cages but thereafter adjusting the abiotic factors to obtain two months earlier and later spawning than normal spawning in November. The aim of this project was to investigate if the offspring seasonal groups had differences in their epigenetic DNA methylation profile, gene expression regulation and nutritional status and also link these results with offspring growth.

Material and methods

Atlantic salmon broodstock normal spawning in November was compared with two months earlier or later spawning. We measured the nutritional status in broodstock liver and muscle, and the offspring nutritional profile during development. DNA and mRNA were extracted from offspring liver tissue to investigate the epigenetic DNA methylation profile (method: Reduced Representation Bisulfite Sequences (RRBS)) and gene expression regulation (method: RNA sequencing).

Results

The present study revealed that the seasonal changes significantly alter the nutritional level of the nutrients in both broodstock and offspring. Early spawning broodstock incorporated less nutrients into the eggs but the measures revealed a sufficient broodstock nutrient status. Late season spawners had similar nutritional status in offspring as normal spawners. Broodstock liver and muscle from late season broodstock indicate hunger. Enrichment analyses from mRNA sequencing revealed that genes controlling cell cycle and proliferation were significantly altered between spawning groups. Comparing early and normal spawning season revealed around 3500 differentially methylated cytosines. Especially interesting was the gene *ctl2b*, encoding the choline transporter, which had 9 significant hypermethylated CpG sites in promoter, 5 of which had 20% more methylation in the early spawning group.

(Continued on next page)

Discussion and conclusion

We believe our results provide an important understanding of the interplay between the abiotic environmental factors and nutritional status which together control the intermediary metabolism regulating growth and robustness from broodstock to next generation. Our in-depth study not just indicates a need for adjusting the broodstock feed to comply with the nutritional needs when changing abiotic factors, but also shows us how fragile and sensitive marine organisms are to a changing climate.

Acknowledgements

We are thankful to the technical staff at IMR, Nord University and AquaGen AS for assistance. This research was financed by IMRs Nutritional programming project and The Research Council of Norway (NutrEpi 267787).

References

Heindel and Vandenberg, 2015. Developmental Origins of Health and Disease: A Paradigm for Understanding Disease Etiology and Prevention, Curr Opin Pediatr. Doi: 10.1097/MOP.0000000000000191

Heard and Martienssen, 2014. Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157, 95-109. Doi: 10.1016/j.cell.2014.02.045

Skjærven, Jakt., Fernandes, Dahl, Adam, Klughammer, Bock, Espe, 2018. Parental micronutrient deficiency distorts liver DNA methylation and expression of lipid genes associated with a fatty-liver-like phenotype in offspring. Scientific Reports 8. Doi: 10.1038/s41598-018-21211-5

Skjærven., Oveland, Mommens, Samori, Saito, Adam, Espe, 2020. Out-of-season spawning affects the nutritional status and gene expression in both Atlantic salmon female broodstock and their offspring. Comp Biochem Phys A 247. Doi: 10.1016/j.cbpa.2020.110717

EPIGENETIC MECHANISMS OF IMPROVED POST-SMOLT GROWTH IN ATLANTIC SALMON FED A MODERATE SURPLUS PACKAGE OF METHIONINE, FOLIC ACID, VITAMIN B6 AND B12 THROUGHOUT SMOLTIFICATION

A.C. Adam*[1], T. Saito[1], P. Whatmore[1a], A.L.K. Putman[2], J.M.O. Fernandes[3], V. Vikeså[4], M. Espe[1], and K.H. Skjærven[1]

[1] Institute of Marine Research, Bergen (Norway)
[2] The Gurdon Institute, University of Cambridge, Cambridge (United Kingdom)
[3] Nord University, Faculty of Biosciences and Aquaculture, Bodø (Norway)
[4] Skretting ARC, Stavanger (Norway)
[a] Current address: eResearch Office, Queensland University of Technology, Brisbane (Australia)
*E-mail: Anne-Catrin.Adam@hi.no

Introduction

Changing feed raw materials from marine to plant-based alters the requirement levels of certain micronutrients for Atlantic salmon (*Salmo salar*). Recommendations have been re-evaluated through the EU-funded ARRAINA project (Vera et al., 2020, Hemre et al., 2016) and feeding plant-based diets through smoltification of Atlantic salmon needs verification of optimal levels of one-carbon (1C) nutrients. Recent research points to those 1C nutrients, which are methionine as a key micronutrient along with folic acid, vitamin B6 (pyridoxine) and vitamin B12 (cobalamin), for improved and healthy growth through smoltification (Espe et al., 2020). NRC* funded projects explore the 1C nutrient-responsive mechanisms in muscle, which are of metabolic, molecular and epigenetic nature and that can help to explain the improved growth observed. The availability of certain nutrients can affect histone tail modifications and DNA methylation that together regulate mRNA expression and thereby control metabolism, which is one underlying explanation for nutritional programming. Epigenetic changes during early life stages such as early impact in pre-smolts can program life-long consequences on physiology, robustness and growth. Understanding how growth is controlled by those non-genetic mechanisms becomes important for a rapidly growing aquaculture industry whose concerns are to optimize production, sustainability and quality.

Material and methods

Two experimental diets provided by Skretting ARC were fed to Atlantic salmon 6 weeks prior to smoltification until 3 months after saltwater transfer in triplicate tanks at Skretting's research station. A Control and a moderate 1C+ diet contained varying levels of methionine (6.7 and 9.5 g/kg), folate (2.6 and 4.8 mg/kg), vitamin B12 (0.15 and 0.18 mg/kg) and vitamin B6 (6.75 and 9.31 mg/kg), respectively. The formulation of the Control diet included 1C nutrients on requirement and recommended levels (NRC, 2011, Espe et al., 2014, Hemre et al., 2016), whereas 1C+ diet contained a moderate 1C nutrient surplus package to support maximal performance. Both diets contained 240 g/kg soy protein and 150 g/kg pea concentrates as main protein source, and smaller amounts of fishmeal (120 g/kg) and krill meal (20 g/kg). Lipid source was a mixture of rapeseed oil (81 g/kg) and fish oil (127 g/kg). Muscle samples from both dietary groups were taken in the end of the freshwater and in the on-growing saltwater period. Global metabolic profiling was performed on pooled muscle samples (five individuals per tank, n=3). RNA and DNA were extracted from single muscle samples (n=9) for gene expression (RNA-seq, n=9), DNA methylation (RRBS, n=9) and analysis of a histone tail methylation (H3K4me3) and acetylation (H3K27ac) mark (CUT&RUN, n=6).

Results

Salmon fed the 1C+ diet throughout smoltification significantly increased body weight and decreased liver weight in the seawater period, which was reflected in a higher condition factor and specific growth rate compared to the Control group (Espe et al., 2020). There were no differences in growth performance during the freshwater period. Feed conversion ratio and protein utilization were not different during either the freshwater or the saltwater period. Pre-liminary results from both metabolic and gene expression signatures in muscle revealed significant 1C nutrient-dependent changes already at pre-smolt, but differences intensified when analyzing post-smolt muscle (Adam et al., under review). 1C+ fed salmon showed less free amino acid and putrescine levels, and higher methionine and glutathione amounts in post-smolt muscle. Transcriptional differences between both dietary treatments revealed lower expression of genes related to translation, growth, and amino acid metabolization in post-smolt muscle when fed additional 1C nutrients. DNA methylation and histone tail modification data are under analysis and key results will be presented.

(Continued on next page)

Discussion and conclusion

Increased 1C nutrient levels given in the feed over the challenging smoltification period resulted in best growth performance in the saltwater period. The overall metabolic profile in muscle of salmon fed the Control diet suggests a lower amino acid utilization for protein synthesis, and increased methionine metabolization in polyamine and redox homeostasis, whereas gene expression profiles are indicative of compensatory growth regulation at local muscle tissue level. Linking both metabolic and gene expression profiles, our findings point to fine-tuned nutrient-gene-interactions fundamental for improved growth capacity through better amino acid utilization for protein accretion when salmon was fed additional 1C nutrients throughout smoltification. Following a multi-omics approach, integration of metabolic and gene expression with DNA methylation and histone tail modification profiles will reveal possible epigenetic mechanisms involved in improved growth. These results also provide documentation for adjustment of dietary nutrient levels in aquaculture and highlight the employment of nutritional programming strategies on healthy and robust growth.

References

Adam A.C., Saito T., Espe M., Whatmore P., Fernandes J.M.O., Vikeså V. and Skjaerven K.H. Metabolic and molecular signatures of improved growth in Atlantic salmon (*Salmo salar*) fed surplus levels of methionine, folic acid, vitamin B6 and B12 throughout smoltification. British Journal of Nutrition. *Under review.*

Espe M., Vikeså V., Thomsen T.H., Adam A.C., Saito T. and Skjærven K.H. 2020. Atlantic salmon fed a nutrient package of surplus methionine, vitamin B12, folic acid and vitamin B6 improved growth and reduced the relative liver size, but when in excess growth reduced. Aquacult Nutr 26, 477-489.

Espe M., S.M. Andersen, E. Holen, I. Ronnestad, E. Veiseth-Kent, J. E. Zerrahn, and A. Aksnes. 2014. Methionine deficiency does not increase polyamine turnover through depletion of hepatic S-adenosylmethionine in juvenile Atlantic salmon. British Journal of Nutrition 112: 1274-1285.

Hemre G.I., E.J. Lock, P.A. Olsvik, K. Hamre, M. Espe, B.E. Torstensen, J. Silva, A.C. Hansen, R. Waagbo, J.S. Johansen, M. Sanden, and N.H. Sissener. 2016. Atlantic salmon (*Salmo salar*) require increased dietary levels of B-vitamins when fed diets with high inclusion of plant based ingredients. PeerJ 4:e2493.

National Research Council (NRC). 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press. Washington DC, USA. 392pp.

Vera L.M., Hamre K., Espe M., Hemre G.-I., Skjærven K.H., Lock E.-J., Prabhu A.J., Leeming D., Migaud H., Tocher D.R. and Taylor J.F. 2020. Higher dietary micronutrients are required to maintain optimal performance of Atlantic salmon (*Salmo salar*) fed a high plant material diet during the full production cycle. Aquaculture 528.

* The Research Council of Norway (NFR-295118, NFR-267787).

FEEDEST: A TOOL TO ESTIMATE BASS AND BREAM FEEDING RATES

Filipe Soares*[1], Tomé Silva[1], Ana Nobre[1], Luís Conceição[1]

[1]SPAROS Lda., Olhão (Portugal)
Email: filipesoares@sparos.pt

Introduction

In fish farming, feeding is one of the management operations that can most impact overall production performance. This means that, to achieve a good performance in economic and environmental terms, fish farming companies have to equip themselves with the right tools to support the definition of good feeding practices (e.g., ration size). One of the most used tools to determine the ration size are feeding tables, which have shown to be effective in providing guidance in a simple, accessible and practical way.

Usually, feeding tables are constructed based on empirical data, and what varies between the multiple approaches used are the extrapolation methods employed (e.g., statistical models, mechanistic models, hybrid models) and criteria/targets defined (e.g., maximize growth, minimize feed conversion, minimize economic conversion). Each extrapolation method has its own advantages and disadvantages, depending on the situation in which it is being applied. The most important is to ensure that the chosen method is adequate to produce a robust and accurate feeding table (e.g., feeding rates per size and temperatures).

The objective of this work is to present a public decision-support tool (FEEDEST; www.webtools.sparos.pt/feedest), developed in the context of the PerformFISH project (www.performfish.eu) to allow fish farmers to estimate the feeding rates, for European seabass or gilthead seabream, that allow reaching the maximum fish growth potential, while ensuring minimal feed conversion and feed waste.

Methodological approach

The FEEDEST tool (Figure 1) was built on the top of a nutrient-based fish growth model. This model was developed based on a "energy-protein flux" concept (Nobre et al., 2019) and was calibrated for European seabass (*Dicentrarchus labrax)* and gilthead seabream (*Sparus aurata*) using data from a wide range of growth trials (both published and not).

One important aspect of this model, which is also common to most fish growth models, is that it requires feeding rates to be set as an *input* when operating in normal mode, while we consider it to be an *output* of the tool. To solve this inverse problem, we use a divide-and-conquer approach, where we split the difficult (multidimensional) problem into a large set of simple (unidimensional) problems which can be efficiently solved: for each possible input diet (only 3 options), we bin the input space and solve the problem for "feed amount" while keeping other variables fixed. Because the diets are pre-determined, we can pre-calculate approximately-optimal feeding tables, leading to very fast (real-time) re-optimization of the feeding tables for the user's conditions when using this tool.

Tool overview

FEEDEST is implemented as a web-application (R/Shiny framework). It presents a reactive interface and requires a minimum set of inputs (i.e., species, diet, 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 and feeding rates, but also estimates of environmental impact (in terms of nitrogen waste). Furthermore, the tool allows the user to export its results as a spreadsheet, which also contains the specific feeding table that the user should follow (see Figure 2).

Final remarks

In the context of the PerformFISH project (www.performfish.eu) the FEEDEST public decision-support tool was developed, aiming at support the implementation of better feeding management practices by the Mediterranean fish farming industry, and it is freely available at: https://webtools.sparos.pt/feedest/. This work illustrates how nutrient-based mathematical models can be used to build user-friendly practical decision-support tools to support efficient feeding management practices.

(Continued on next page)

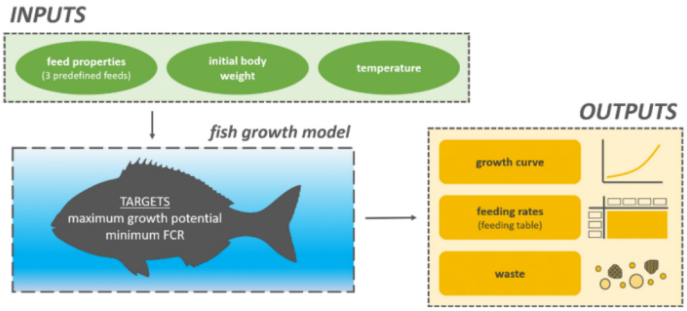


Figure 1 – Diagram detailing the overall concept of the tool, with the expected inputs in green and the outputs provided to the user in yellow.

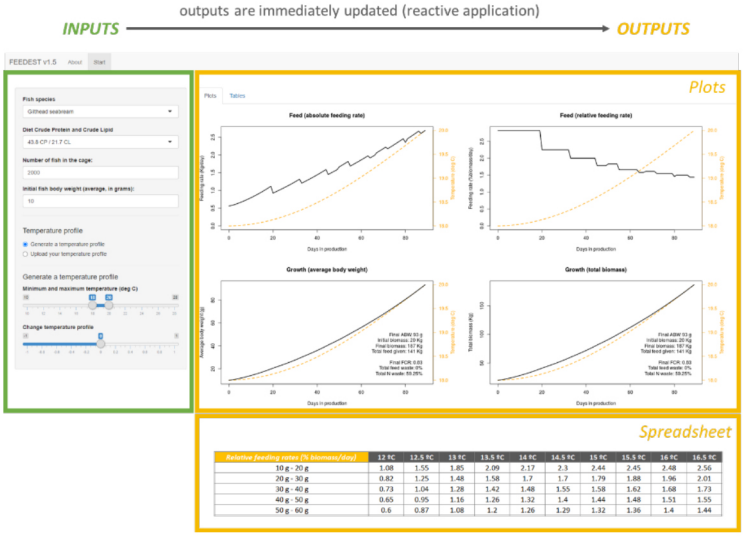


Figure 2 – Main page of FEEDEST, showing the typical workflow: inputs are introduced on the left-hand side and outputs (graphical or table format) are immediately given on the right-hand side.

Acknowledgements

This work is part of project 727610_PerformFISH supported by the European Union through Horizon 2020 research and innovation programme.

References

Nobre, A.M., Valente, L.M.P., Conceição, L., Severino, R., Lupatsch, I., 2019. A bioenergetic and protein flux model to simulate fish growth in commercial farms: Application to the gilthead seabream. Aquacultural Engineering. 84:12-22. doi:10.1016/j.aquaeng.2018.11.001

SYNERGISTIC ACTION OF BORON WITH VITAMIN D3 ON THE EARLY-STAGE SKELETAL DEVELOPMENT IN ZEBRAFISH LARVAE

Jerry Maria Sojan[1*], Manu Kumar Gundappa[2], Francesca Maradonna[1] and Oliana Carnevali[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona
[2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK
E-mail: j.m.sojan@pm.univpm.it

Introduction

Osteogenesis is a process which can be modulated by several factors including macro and micronutrients supplementation. Among the various proven and studied micronutrients, boron (B) has an important role in the maintenance and development of bone [1–3]"container-title":"FASEB Journal (Federation of American Societies for Experimental Biology. Bone related diseases are a common problem today and vitamin D deficiency is one of the main reasons for weak bones. Some earlier studies showed that the boron supplementation alleviates the problems associated with vitamin D3 deficiency such as disruptions in the mineral metabolism [4]supplementation of two levels (5 and 25 parts per million; ppm. Using zebrafish as a model, the effect of boron supplementation at two selected concentrations and its synergistic effects with vitamin D3 on skeletal development were analysed at a transcriptomic level defining the pathways modulated in the treated groups. The analysed data opens to greater understanding of the actions of these compounds to improve skeletal health which is either useful for aquaculture or biomedicine.

Materials and Methods

Zebrafish embryos were collected and divided into six groups each in triplicates- control group with ethanol (C), Vitamin D3 (VD), First concentration of boron (B10), Boron 10 with vitamin D3 (Sigma) (B10VD), Second concentration of boron (B100) and Boron100 vitamin D3 (B100VD). Boric acid (Sigma) was used to make the required concentrations of boron groups. At 7 DPH, larvae were sampled for RNA extraction and sequenced on Illumina platform to generate 150 x 2 paired end (PE) reads. ~30 million PE reads were generated across each experiment group in triplicates. Quality trimmed reads were then mapped to the reference genome assembly using STAR aligner. Read counts for the protein coding genes were retrieved using featureCounts. Differential gene expression analysis between different groups was performed using deseq2 package in R. Gene Ontology and KEGG pathway analysis was performed using clusterProfiler package in R. Plotting was done using ggplot package in R.

Results and Discussion

RNA-seq analysis identified several thousands of genes to be differentially expressed in at least one of the comparison groups (Fig1a). Clustering the data based on expression pattern revealed three different clusters (Fig1b) with one cluster clearly enriched for GO and KEGG pathways related to skeletal development. The outcomes revealed a clear evidence for boron and Vitamin D synergy contributing to enhanced bone physiology. Most affected pathway was the MAPK where many genes were seen to be highly expressed in the synergy groups than vitamin D. The calcineurin, denoted by the *ppp3cca* gene, which is a part multiple bone related pathways such as MAPK, WNT and Ca signalling, was seen upregulated in the B10VD synergy group and are known to be expressed in osteoblasts [5]. In the KEGG anlaysis of the TGF-β–BMP pathway, *Smad4* was significantly upregulated in the B10VD group and Smad family is associated with BMP activation of genes involved in mineralisation and osteoblast differentiation [9]. In brief, the synergistic effect of boron with vitamin D3 was clearly evidenced in the skeletal pathways by the transcriptome study using zebrafish larvae model. This information can be useful for aquaculture nutrition to enrich the vitamin D3 effect by the additional supplementation of boron to enhance the ossification and alleviate the vitamin D deficiency pathologies in the cultured fishes.

(Continued on next page)

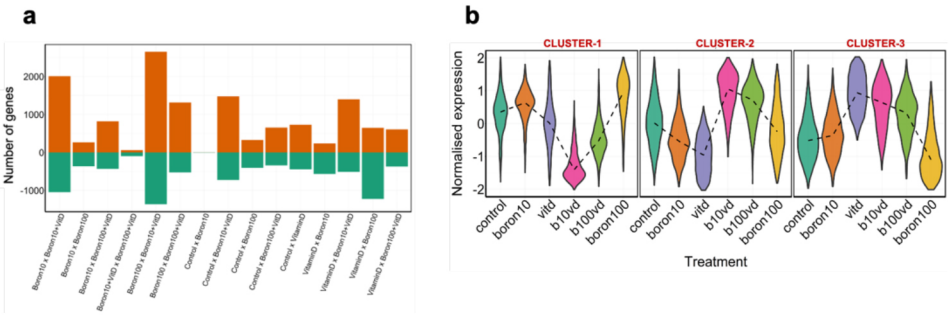


Fig.1 (a) Differentially expressed genes across different treatment categories ($p<0.05$) and log FC < -0.5 and > +0.5; several thousand genes differentially expressed in at least one group after deduplication; **(b)** General expression profile of genes (Normalised and VST transformed read counts) within three medoid clusters according to the treatment.

References

[1] H. McCoy, A. Irwin, M.A. Kenney, L. (Univ of A. Williams, Effects of boron supplements on bones from rats fed calcium and magnesium deficient diets, FASEB Journal (Federation of American Societies for Experimental Biology); (United States). 5:5 (1991). https://www.osti.gov/biblio/5213312 (accessed December 17, 2019).

[2] M.T. Gallardo-Williams, R.R. Maronpot, C.H. Turner, C.S. Johnson, M.W. Harris, M.J. Jayo, R.E. Chapin, Effects of boric acid supplementation on bone histomorphometry, metabolism, and biomechanical properties in aged female F-344 rats, Biol Trace Elem Res. 93 (2003) 155–169. https://doi.org/10.1385/BTER:93:1-3:155.

[3] F.H. Nielsen, Dietary fat composition modifies the effect of boron on bone characteristics and plasma lipids in rats, BioFactors. 20 (2004) 161–171. https://doi.org/10.1002/biof.5520200305.

[4] V. Kurtoğlu, F. Kurtoğlu, B. Coşkun, Effects of boron supplementation of adequate and inadequate vitamin D3-containing diet on performance and serum biochemical characters of broiler chickens, Research in Veterinary Science. 71 (2001) 183–187. https://doi.org/10.1053/rvsc.2001.0517.

[5] L. Sun, H.C. Blair, Y. Peng, N. Zaidi, O.A. Adebanjo, X.B. Wu, X.Y. Wu, J. Iqbal, S. Epstein, E. Abe, B.S. Moonga, M. Zaidi, Calcineurin regulates bone formation by the osteoblast, PNAS. 102 (2005) 17130–17135. https://doi.org/10.1073/pnas.0508480102.

[6] G. Chen, C. Deng, Y.-P. Li, TGF-β and BMP Signaling in Osteoblast Differentiation and Bone Formation, Int J Biol Sci. 8 (2012) 272–288. https://doi.org/10.7150/ijbs.2929.

[7] C.M. Karner, S.-Y. Lee, F. Long, Bmp Induces Osteoblast Differentiation through both Smad4 and mTORC1 Signaling, Molecular and Cellular Biology. 37 (2017). https://doi.org/10.1128/MCB.00253-16.

GENETIC PARAMETERS AND GWAS OF LIPID TRAITS IN MUSCLE AND LIVER OF EURPEAN SEABASS (*Dicentrarchus labrax*)

S.S. Horn*[1], G.F. Difford[1], B. Ruyter[1], M.L. Aslam[1], C. Diaz-Gil[2], M. López Belluga[2], M. Herlin[2], C. Peñaloza[3], R. Houston[3] and A.K. Sonesson[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-1433 Ås, Norway
[2]Culmarex, C/ Don Carnal, 13, P.I. El Labradorcico 30889 Águilas, Murcia, Spain
[3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, United Kingdom.

Email: siri.storteig.horn@nofima.no

Background

Lipid deposition is an important factor of production efficiency, product quality and health in animal production. The liver is a major lipid storage site in European seabass (Dias et al., 2005). However, excess lipid accumulation in the liver can negatively affect production efficiency and liver function and health, with onset of fatty liver disorder. The lipid content of muscle tissue affects organoleptic and processing qualities of fish fillets, and the omega-3 fatty acid content is important for the nutritional quality. As a significant part of the Mediterranean diet, seabass is an important nutritional source of the health-promoting essential omega-3 fatty acids EPA and DHA.

Substantial genetic variation has been reported for muscle lipid content in seabass (Besson et al., 2019; Saillant et al., 2009). Studies in other fish species have revealed a genetic component to the omega-3 levels of fillets (Horn et al. 2018; Wang et al. 2019). The genetic parameters of fatty acid composition traits and liver fat have not yet been explored in seabass. The aim of this study was to estimate genetic parameters of lipid and omega-3 fatty acid content in muscle and liver of European seabass. For the first time in seabass, a GWAS on lipid and fatty acid traits was performed.

Materials and methods

European seabass originating from ABSA-Culmarex were used in this experiment. Fish were fed commercial feed and kept in commercial sea cages in Murcia, Spain. Samples of liver (n = 87) and muscle (n = 313) were homogenized and total lipids were extracted using the Folch extraction method. The fatty acid composition of the total lipids was determined using the Mason & Waller method by means of gas chromatography.

The fish were genotyped using the 60K MedFish array (Peñaloza et al. 2020). Genotypic data was filtered using the Plink software. Approximately 21K SNPs passed filters and quality control. A genomic relationship matrix between the animals was generated with the "-grm" function implemented in GCTA software, and used for the estimation of the genetic parameters. The genomic relationship matrix was computed according to VanRaden (2008) as $\frac{ZZ\prime}{2*\sum_{i=1}^{Nsnp} p_i(1-p_i)}$; where p_i is the allele frequency of the second allele and $Nsnp$ is the total number of SNP markers.

(Co)Variance components and the corresponding heritability were estimated from bivariate and univariate restricted maximum likelihood (GREML) analyses, including effect of sex and batch as fixed effects. GWAS was performed using a linear mixed animal model implemented in GCTA program with the "–mlma-loco" function. With fixed effects including (phenotypic) sex, batch, and 5 principal components as covariates (only sex and batch for liver traits due to the small dataset). The following traits were analysed for both muscle and liver tissue: Total fat (%), DHA (%), EPA (%), DHA/ALA ratio, omega-3/omega-6 ratio.

Results and discussion

On average, the fish weighed 346 g and had a muscle fat content of 9 %, ranging from 2 to 21 %. The phenotypic and genetic correlation between muscle fat and liver fat was weak, suggesting independent regulation of the two lipid deposits. This was supported by the high heritability of muscle fat (0.59), which was within the range of previous estimates (e.g. Besson et al. 2019; Saillant et al. 2009), and the lower heritability of liver fat (0.17).

(Continued on next page)

We found high heritability for omega-3 related traits in both tissues, but for different specific traits, one exception being DHA, which had high heritability in both liver (0.45) and muscle (0.51). In liver, EPA and DHA/ALA ratio had high heritability, while in muscle omega-3/omega-6 ratio had a high heritability (0.41). The high heritability of DHA and omega-3/omega-6 ratio in muscle indicates a substantial potential to improve the fatty acid profile of fish fillets through selective breeding, by increasing the ratio of anti-inflammatory omega-3 to pro-inflammatory omega-6 fatty acids.

The proportional content of DHA was 6.4 % and 7.3 % in muscle and liver, respectively, which was higher than the proportion supplied in the feed (3.9 %). There was a positive phenotypic correlation between muscle DHA and liver DHA/ALA ratio (0.6), and both of these traits had a high heritability (>0.4). As the DHA/ALA ratio can be seen as a marker of omega-3 bioconversion of ALA (18:3n-3) to DHA (22:6n-3), these results may imply that European seabass has omega-3 bioconversion activity in liver that has a strong genetic component to it, and that impacts the DHA content of muscle.

The GWAS showed a clear signal on LG X with chromosome-wide significant peaks for the four traits muscle and liver omega-3/omega-6 ratio, muscle DHA and liver EPA. Although these traits did not have the same top significant SNPs, some were in the same region; 5,6-5,8Mb and 7-8Mb. One very strong candidate gene was identified here: *Peroxisome proliferator-activated receptor alpha*, a nuclear transcription factor central in regulation of genes involved in lipid metabolism, including mitochondrial beta-oxidation. Another signal was found for the SNP AX-172297229 on LG 11 for the two traits DHA and DHA/ALA ratio in liver. Three candidate genes involved in lipid and/or omega-3 metabolism were found ± 300Kb region of this SNP: ALOX5, PLCB3 and SPTLC3. ALOX5 is especially interesting as it can mediate the lipoxygenation of DHA.

Conclusions

Differences in genetic parameters between liver and muscle tissues reflect differences in lipid metabolism. There is a substantial potential to increase DHA content of seabream fillets through selective breeding. The results also suggest that there is omega-3 bioconversion activity in liver of seabass that has a strong genetic component to it, and that impacts the DHA content of fillets. GWAS results showed signals on LG X for omega-3 traits across tissues, in a region where the candidate gene PPARα is located.

Acknowledgements

This study was made possible by the EU project MedAID (H2020 grant agreement No 727315).

References

Besson, M., Allal, F., Chatain, B., Vergnet, A., Clota, F. & Vandeputte, M. (2019). Combining individual phenotypes of feed intake with genomic data to improve feed efficiency in sea bass. Frontiers in genetics, 10: 219.

Dias, J., Alvarez, M. J., Arzel, J., Corraze, G., Diez, A., Bautista, J. M. & Kaushik, S. J. (2005). Dietary protein source affects lipid metabolism in the European seabass (Dicentrarchus labrax). Comp Biochem Physiol A Mol Integr Physiol, 142 (1): 19-31.

Horn, S. S., Ruyter, B., Meuwissen, T. H. E., Hillestad, B. & Sonesson, A. K. (2018). Genetic effects of fatty acid composition in muscle of Atlantic salmon. Genetics Selection Evolution, 50.

Peñaloza, C., Manousaki, T., Franch, R., Tsakogiannis, A., Sonesson, A., Aslam, M. L., Allal, F., Bargelloni, L., Houston, R. D. & Tsigenopoulos, C. S. (2020). Development and validation of a combined species SNP array for the European seabass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata). bioRxiv: 2020.12.17.423305.

Saillant, E., Dupont-Nivet, M., Sabourault, M., Haffray, P., Laureau, S., Vidal, M.-O. & Chatain, B. (2009). Genetic variation for carcass quality traits in cultured sea bass (Dicentrarchus labrax). Aquatic Living Resources, 22 (1): 105-112.

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. Journal of dairy science, 91 (11): 4414-4423.

Wang, L., Chua, E., Sun, F., Wan, Z. Y., Ye, B., Pang, H., Wen, Y. & Yue, G. H. (2019). Mapping and Validating QTL for Fatty Acid Compositions and Growth Traits in Asian Seabass. Marine Biotechnology, 21 (5): 643-654.

THE EFFECT OF CODIUM SP. AND OSMUNDEA SP. INCLUSION ON GROWTH PARAMETERS OF JUVENILE TURBOT (*Scophthalmus maxima*)

M. Soula,* M. Ferreira, P. Frade, D. Méndez, L. Regueiro, Y. Melean, D. Costas

ANFACO-CECOPESCA, Division of Aquaculture. Estrada Colexio Universitario 36310 – Vigo, Pontevedra (Spain)
Corresponding author: mohamed@anfaco.es

Introduction

In recent years, with the growth of aquaculture and the interest in improving the health of fish during the breeding process (Asimi and Sahu, 2013), the search for natural products with a source of bioactive compounds has increased. Macroalgae offer a novel and value-added dietary ingredient in diets formulated for fish. Like terrestrial plants, the nutritional content of macroalgae can vary greatly among species, seasons, and locations. Beside from its basic nutritional value, algae contain several pigments, immunostimulant compounds, and secondary metabolites that may have beneficial effects on farmed fish (Wan et al., 2018; Cruces et al., 2012; Oliveira et al.2013). Macroalgae such as *Codium* and *Osmundea* abound in the area of Galicia (NW Spain) and the north of Portugal, where the interest of exploitation is increasing due to the bioactive compounds they contain. Within the ALGALUP project, an experiment has been carried out to evaluate the effect of 4 diets with inclusions of macroalgae (*Codium* spp. and *Osmundea pinnatifida*) and extracts of these rich in polysaccharides on growth and digestive physiology in juvenile turbot (*Scophthalmus maxima*).

Materials and methods

750 juvenile turbot from Nueva Pescanova with an average weight of 10.3±2.38 g were used. Fish were distributed in 15 tanks of 200 L with 50 fish per tank. During the following 60 days turbot were kept in a recirculation system with a daily renewal of 10% and were fed 3 times a day. 3 tanks were assigned to each one of the 5 diets formulated and manufactured with the support of SPAROS, Portugal. Four diets contained macroalgae soluble fiber obtained by enzymatic hydrolysis using a combination of protease, cellulase, hemicellulase and pectinase. Diets DC5% and DO5% contained 5 % of soluble fiber from *Codium* spp. and from *O. pinnatifida* respectively, whereas diets DC15 and DO15 contained 1.5 g/kg of soluble fiber from *Codium* spp. and from *O. pinnatifida* respectively. A commercial feed was used as control diet (DC). Water conditions were maintained at 16 ± 0.5 ºC, 35 ‰ of salinity and 7.2 ± 0.9 mg / L of dissolved oxygen. RAS parameters were stable throughout the experiment. Ammonia, nitrate, and nitrite were measured daily. Every 4 weeks fish were weighed, and the length was measured. Before handling, the fish were anesthetized with 200 mg / L of 2-phenoxyethanol. At the end of the experiment, fish were euthanised with 600 mg / L of 2-phenoxyethanol. Condition factor, specific growth rate (SGR), conversion index (FCR), protein efficiency index (PER) and relative growth rate (RGR), were calculated. Also 10 fish from each tank were dissected by separating and weighing liver and viscera and the hepatosomatic index (HSI) and the viscerosomatic index (VSI) were calculated.

Table 1. Initial and final weight, growth (SGR and RGR percentages) and feed conversation (FCR and PER ratios), HIS and VSI index of turbot juveniles fed with the experimental diets. Different letters indicate significant differences (ANOVA P<0.05)

	DC5	DO5	DC15	DO15	DC
Initial weight	10.74±2.41	9.94±2.77	10.82±2.3	9.60±2.2	10.41±2.2
Final weight	27.01±5.54	26.31±4.96	25.28±4.06	26.42±5.21	26.17±6.17
SGR	1.84±0.05^{a,b}	1.95±0.01^{a,b}	1.70±0.12^{a}	2.02±0.09^{b}	1.84±0.09^{a,b}
RGR	1.51±0.07^{a,b}	1.65±0.01^{a,b}	1.35±0.15^{a}	1.76±0.12^{a}	1.51±0.12^{a,b}
Feed intake	724.45±22	711.18±14	697.65±14	710.90±11	720.48±27
Biomass gain	846.39±61	851.18±41	752.20±67	874.49±47	819.75±79
FCR	0.86±0.04	0.84±0.02	0.94±0.13	0.82±0.07	0.89±0.11
PER	1.79±0^{a}	1.75±0^{a}	1.75±0^{a}	1.85±0^{b}	1.75±0^{a}
HSI	1.56±0.09^{a}	1.41±0.08^{a}	1.47±0.06^{a}	1.33±0.05^{a,b}	1.09±0.06^{b}
VSI	4.08±0.16^{b}	4.13±0.13^{b}	4.96±0.24^{a}	3.63±0.18^{b}	3.69±0.12^{b}

(Continued on next page)

Results

At the end of the experiment turbot juveniles had a mean weight of 26.29±0.65 g and a length of 11.8±0.61 cm. No significant difference was observed between diets neither in weight nor in length (P> 0.05). Although, in terms of growth, a difference was detected in the SGR and the RGR. The DC15 diet showed the lowest values in both rates with 1.7 and 1.35 respectively. These data were significantly different from those of the DO15 diet, which has driven the highest values in SGR (2.02) and RGR (1.76). The growth data of the diets including whole macroalgae and extracted polysaccharides did not show significant differences with the control. However, the trend has clearly shown that the DO5 and DO15 diets favor an increase in the SGR of 6 and 9.25%, respectively, compared to the control and 8 and 14%, respectively, in terms of the RGR.

During the experiment, fish fed the DC15 diet showed the lowest feed intake and biomass gain. Although no significant difference is observed, the FCR also follows the same trend with the worst data observed in the DC15 diet. It should be noted that the DO15 diet improved FCR by 8.2% and 13% respectively compared to the DC and DC15 diet.

The analysis of the hepatosomatic index data showed that the control diet had a significantly low value compared to the rest of the diets. In the case of the viscerosomatic index, the lowest values were observed in the DO15 diet and DC when the highest in the DC15 diet.

References

Asimi O. A., Sahu N. P. (2013). Herbs/spices as feed additive in aquaculture. Scientific Journal of Pure and Applied Sciences 2:284-292.

Cruces E, Huovinen P, Gomez I (2012). Proteınas de estres ycompuestos anti-estres auxiliares en algas marinas inter-mareales. Latin American Journal of Aquatic Research40: 822–834.

Wan, A. H. L., Davies, S. J., Soler-Vila, A., Fitzgerald, R., & Johnson, M. P. (2018). Macroalgae as a sustainable aquafeed ingredient. Reviews in Aquaculture. doi:10.1111/raq.12241

Oliveira, A.S., Sudatti, D.B., Fujii, M.T., Rodrigues, S.V., Pereira RC (2013). Inter- and intrapopulation variation in the defensivechemistry of the red seaweedLaurencia dendroidea (Cerami-ales, Rhodophyta). Phycologia52: 130–136.

Aknowledgements

This work was realized Within the ALGALUP project founded by Interreg POCTEP Spain-portugal with FEDER co-found

ADAPTING *Codium tomentosum* AND *Chondrus crispus* TO INDOOR CULTURE: ASSESSMENT OF LIGHT AND NUTRIENT CONDITIONS

M. Soula*, P. Frade, A. Pexegueiro, V. González, Y. Melean and M. Ferreira.

ANFACO-CECOPESCA, Division of Aquaculture. Estrada Colexio Universitario 36310. Vigo, Pontevedra (Spain)
Corresponding author: martina@anfaco.es

Introduction

Macroalgae cultivation is gaining interest in the EU, both as an alternative to imports from third countries and to the harvesting of wild biomass. Whereas cultivation in coastal waters may involve large areas and production volumes, land-based cultivation in tanks provides the opportunity of including seaweeds in IMTA schemes to remove inorganic nutrients from aquaculture effluents, thus reducing costs of water treatment and providing an additional value-added product. *Codium tomentosum* Stackhouse 1797 and *Chondrus crispus* Stackhouse 1797 are two autochthonous species of commercial interest in Europe, but information on their culture is scarce. In this work we provide some data regarding their acclimation to indoor culture, and their potential of biomass production and capacity of nutrient uptake.

Materials and methods

Experiment 1. *C. tomentosum* was cultured in aerated 5-l flasks. Light was provided by daylight white LED lamps (W) or a combination of daylight white plus red-enriched LED lamps (WR), yielding 330 µmol m^{-2} s^{-1} and 360 µmol m^{-2} s^{-1} respectively. Photoperiod was circadian, 14 h light: 10 dark. Culture medium consisted in filtered seawater supplemented with $NaNO_3$ and NaH_2PO_4. Three culture conditions combining light and nutrient conditions were tested: 19 W, 500 μM $NaNO_3$ + 50 μM NaH_2PO_4 (W50); 2) W, 500 μM $NaNO_3$ + 75 μM NaH_2PO_4 (W75) and 3), WR, 500 μM $NaNO_3$ + 50 μM NaH_2PO_4 (WR50). After 5 weeks of culture, part of the *C. tomentosum* biomass was removed from culture flasks to avoid self-shading, and experiment was continued for 3 more weeks. Cultures were run in triplicate.

Experiment 2. *C. crispus* was cultured in aerated 1-l flasks and lightened by daylight white LED lamps (81 µmol m^{-2} s^{-1}, photoperiod as in Experiment 1). Culture medium was filtered seawater supplemented with either 500 μM NH_4Cl (N cultures) or 250 μM NH_4Cl + 250 μM $NaNO_3$ (NN cultures) + 50 μM NaH_2PO_4. After 7 weeks of culture, part of the *C. crispus* biomass was removed from culture flasks and nutrient concentration was doubled. Experiment continued for 7 more weeks. Cultures were run in triplicate.

Results

Experiment 1. *C. tomentosum* grew continuously in both phases of the experiment and in all culture conditions tested (Figure 1A). WR50 cultures experienced the highest SGR, suggesting that the enrichment of light in red wavelengths is beneficial for this species (Figure 1B). *C. tomentosum* cultures fully consumed N and P supplied on a weekly basis. N uptake ranged between 0.030 and 0.045 g kg^{-1} fresh weight d^{-1}, with no statistical differences among culture conditions. P uptake ranged between 0.004 and 0.010 g kg^{-1} fresh weight d^{-1} in W50 and WR50 cultures. W75 cultures responded to the higher P concentration by a 20 % to 30 % increase of P uptake rate, but these differences were statistically significant in some cases only.

Experiment 2. No statistical differences were observed in the growth of *C. crispus* cultured either with NH_4^+ or a mix of NH_4^+ and NO_3^- as N source during the first phase of the experiment. Nevertheless, when nutrient concentrations were doubled in the second phase, growth was higher in N cultures (Figure 2A). This was also observed in the SGR (Figure 2B). Both N and P uptake significantly increased ($p<0.05$) with the doubling of nutrient concentration, from 0.070-0.106 to 0.127-0.280 g N kg^{-1} fresh weight d^{-1} and from 0.009-0.011 to 0.011-0.045 g P kg^{-1} fresh weight d^{-1}, but in general no differences were found between nutrient uptake among N and NN cultures.

Results suggest that both *C. tomentosum* and *C. crispus* can be adapted to indoor cultivation for long periods. Light quality was an important factor in *C. tomentosum* cultures, since the increase of the supply of red wavelenghts resulted on a higher growth rate. Self-shading must be avoided to ensure that light intensity is sufficient to promote growth. In the conditions tested, changes in nutrient concentrations were not relevant for the growth of *C. tomentosum*, but the growth of *C. crispus* decreased when a mixture of NH_4^+ and NO_3^- at high concentrations was used as N source, compared to NH_4^+.

Both light and nutrient quality and intensity are crucial factors for the cultivation of macroalgae and these results may provide useful indications for the establishment of larger-scale indoor cultures of *C. tomentosum* and *C. crispus*.

(Continued on next page)

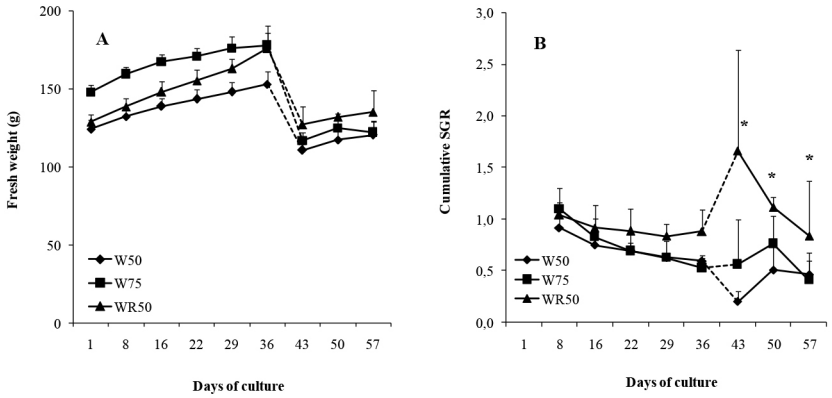


Figure 1. Increase of fresh biomass (A) and cumulative specific growth rate (B) in cultures of *C. tomentosum*. Asterisks mean statistically different values ($p<0.05$) among culture conditions on a given day. Dashed lines indicate when biomass was partially removed from culture flasks.

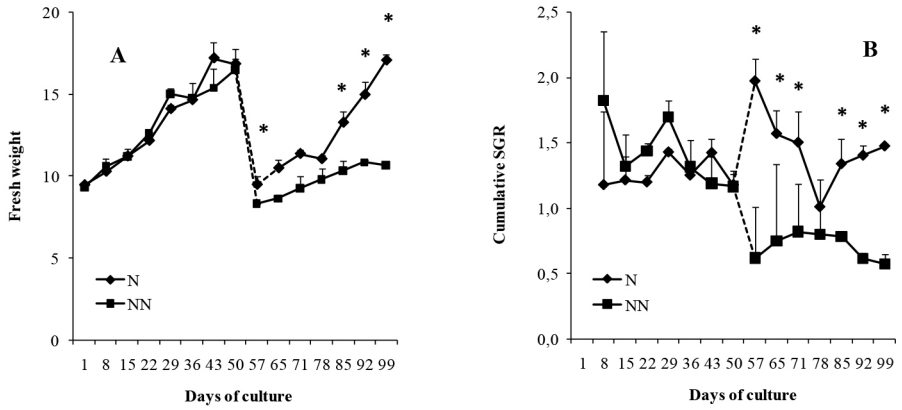


Figure 2. Increase of fresh biomass (A) and cumulative specific growth rate (B) in cultures of *C. crispus*. Asterisks mean statistically different values ($p<0.05$) among culture conditions on a given day. Dashed lines indicate when biomass was partially removed from culture flasks.

EATING ALGAE AS A REGULAR PART OF A EUROPEAN DIET? IMPROVING THE GENERAL PUBLIC'S LITERACY OF ALGAE TO ENCOURAGE A CHANGE IN CONSUMER EATING HABITS

Estelle Soulet[1]*, Iwona Gin[1], Lotte Bronswijk[2]

[1] Nausicaá, Centre National de la Mer, Boulevard Sainte Beuve, 62200 Boulogne sur Mer, France
[2] North Sea Farmers, Den Haag, The Netherlands
valgorize@nausicaa.fr

Introduction
Eating seaweed as a regular part of the diet? Not for most Europeans - not yet, anyway. The ValgOrize[1] research project co-funded by the Interreg 2 Seas European programme hopes to change that by stimulating seaweed production and consumption in Europe.

To feed the growing world population, 50 to 70% more food must be produced by 2050. The sustainable cultivation of marine organisms such as algae can play an important role in feeding the world.

Worldwide, approximately 90% of algae are used for food applications. The European Union only represents a really small part of the worldwide production (less than 1% of the global production volume) and market. A number of obstacles stand in the way of further introduction. One of them is a European consumer reluctance to eat algae because of the limited knowledge about their vitamin and mineral content, food applications, how to cook them, cultivation methods, prejudice about taste and smell and association with beach debris.

Our method
Before determining the communication campaign and educational tools to reach out to the general public, desk and market research[2,3] and expert interviews have been carried out to find out about public perception of algal consumption, consumer motivations and the food trends. The results have shown that the European consumers in the North Sea basin are motivated first of all by the practicality to find and cook algae, attractive pricing and adapted packaging. The study by the North Sea Farmers has further shown that adapting algae products to the western palate has an important role in the European product development and that the current European food landscape is subject to several trends and developments. The main trends that could help increase public awareness of seaweed and microalgae consumption have been identified: the globalised cuisine that seems to create awareness of algae as food, the overwhelming presence in the modern European diet of Asian seaweed products such as sushi or wakame salad, the consumer openness to try new types of food, the consumer awareness of the impact of food on their health and on the planet, the popularity of health-enhancing food with nutritional benefits, the search for alternative protein sources, the guarantee of high quality and sustainable cultivation methods[4,5].

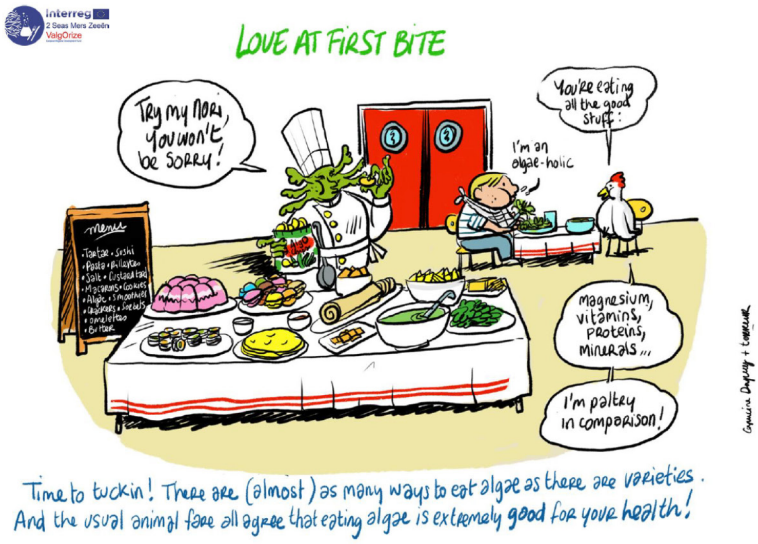
Our tools to improve consumer algae literacy
Based on these research findings, the ValgOrize project has been developing communication and learning tools to improve the European consumers' perception of seaweed and algae and the market acceptance. They are circulated on social media, in partner organisations newsletters and other public outreach channels.

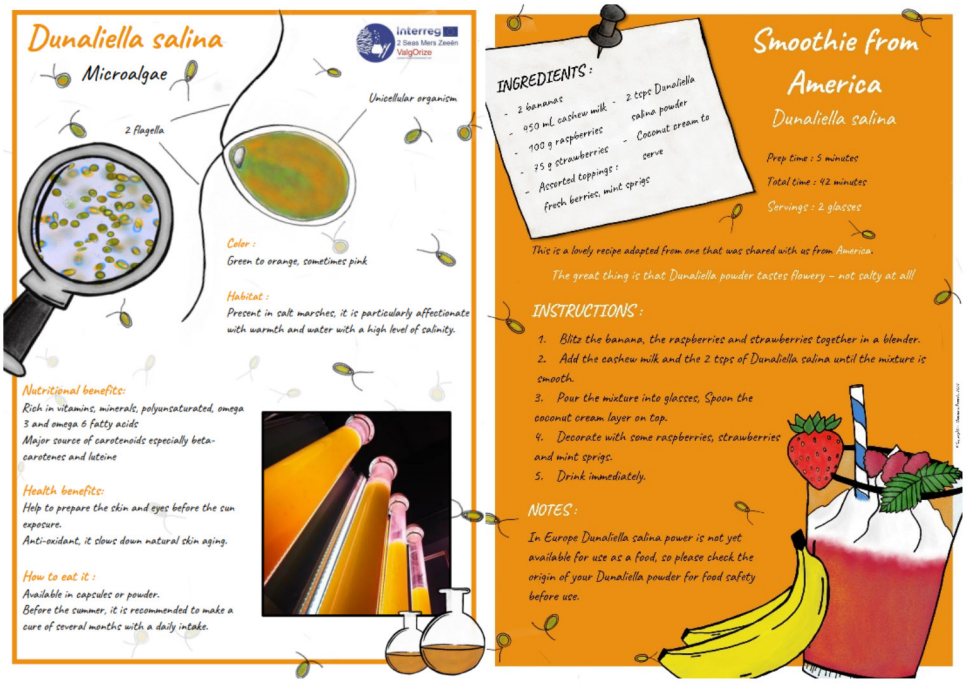
A comic strip was published to illustrate how algae arrives on a consumer' plate in stages: cultivation of micro and macro algae, harvesting, processing, consumption (culinary benefits and health benefits of seaweed), as well as the role of algae in the treatment of polluted water.

Moreover, as series of fact sheets about seaweed and microalgae is being developed to improve the consumer knowledge of their nutritional and health benefits, habitat, how to cook and eat them.

A sequence of tasting events for the general public have been scheduled on community major cultural events such as local festivals, The EU4Ocean Coalition festival – Celebrating the Atlantic Ocean and the North Sea at the European Maritime Day annual conference and the World Ocean Day.

(Continued on next page)





The ValgOrize project is subsidised by the Interreg 2 Seas programme 2014-2020 and co-financed by the European Regional Development Fund under subsidy contract Nr. 2S05-17.

Deliverable 4.1.1 Study on existing market for algal food applications, North Sea Farmers, September 2019.

Visitor survey, Nausicaá, Centre National de la Mer, 2019.

Deliverable 4.2.1 Market potential report for cultivated seaweeds in existing seaweed food markets, North Sea Farmers, January 2021.

Deliverables 4.2.2 Key success factors for transitioning to a sustainable seaweed supply in the 2 seas region, North Sea Farmers, January 2021.

HOW TO BEST TARGET NEW AQUACULTURE FISH PRODUCTS TO INTERNATIONAL CONSUMER SEGMENTS

Violeta Stancu*[1], Rikke Nyland Christensen[1], Athanasios Krystallis[2], Irene Peral[3], Sonia García Muñoz[3], Luis Guerrero[4], Karen Brunsø[1]

[1]MAPP Centre, Department of Management, Aarhus BSS, Aarhus University, Fuglesangs Allé 4 8210, Aarhus V, Denmark
[2]American College of Greece (ACG), Management and International Business, School of Business and Economics, 6, Gravias str., GR 153 42, Greece
[3]AZTI, New Foods, Bizkaiko Zientzia eta Teknologia Parkea, Astondo Bidea E609. 48160 Derio (Bizkaia) Spain
[4]IRTA, Finca Camps i Armet, Edificio A E-17121 Monells (Girona), Spain
E-mail: viost@mgmt.au.dk

Introduction

Consumption of fish and seafood has been increasing globally (FAO, 2020). A large part of the fish for consumption comes from aquaculture, with estimates ranging between a quarter and more than a half across the world (EUMOFA, 2020; FAO, 2020). Forecasts at the global level suggest that the market for aquaculture products will continue to grow (FAO, 2020). In order to be competitive in this evolving international market, new products can be developed to match consumers' preferences. Identifying consumer segments with international relevance for food-related products becomes increasingly important in this context. Consumers place value on certain value-added attributes of new aquaculture fish products (Banovic et al., 2019), however, they differ in terms of their food-related lifestyles and such differences can help explain which attributes are most sought after by different consumer segments. The present study aimed to identify how the importance of attributes valued by consumers when choosing new aquaculture fish products varies by their food-related lifestyle. Understanding the most relevant attributes for different consumer segments is the first step towards the successful development and marketing of new aquaculture fish products.

Materials and methods

An online survey that included a discreet choice experiment was used to collect data from consumers in three European countries (i.e. Spain, France and Germany) that vary in their fish consumption and household expenditure for fish and seafood products (EUMOFA, 2017). The survey included consumers' food-related lifestyle, their preferences in terms of attributes of new aquaculture fish products and several demographic and psychographic measures. Consumers' food-related lifestyle was measured using the modular food-related lifestyle instrument, which is a newly updated and cross-culturally valid scale (Brunsø et al., 2021). Consumer preferences for new aquaculture products (i.e. Sea & mountain meagre burger, Organic sea bream with couscous, Grilled sea bass with lemon) were assessed through a discreet choice experiment. People were asked to make 12 choices between three versions of the product varying in the following attributes derived from previous literature (Banovic et al., 2019, Cantillo et al., 2020): 1) ASC label, 2) NutriScore, 3) country of origin, 4) health claim, 5) price. Multilevel latent class cluster analysis that accounts for the fact that respondents are nested within countries was conducted in LatentGold using the three core dimensions of the modular food-related lifestyle (i.e. food involvement, food innovativeness and food responsibility) to segment the consumers. To identify the classes of consumers based on their choices, LatentGold choice analysis was performed. The food-related lifestyle segments were introduced as covariate in the choice analysis to assess their impact on choices.

Results

Based on their food-related lifestyles we identified four segments of consumers and one country group (i.e. all three countries belonged to a single group). The 'foodies' (11%) are those consumers who are highly involved in food, highly innovative and care about responsibility in food systems. The 'adventurous' (44%) consumers are also interested in all these dimensions, though to a lower degree than the 'foodies'. The 'moderate/conservative' (40%) segment represented those consumers that score about average on all these dimensions. The fourth segment consisted of those who are 'uninvolved' (6%) as they score very low on all food-related lifestyle dimensions.

(Continued on next page)

In terms of choice of new aquaculture fish products, we find that all attributes were significant and influenced people's preferences towards the chosen products. However, consumers differed in the importance placed on the different attributes and in this regard we identified five groups of consumers with similar preferences within the group but dissimilar between groups. There was a highly significant relationship between the consumer segments based on food-related lifestyle and the choice groups based on people's new aquaculture product choices. The 'foodies' are especially prevalent in the choice groups that value the ASC label and the NutriScore, but also domestic products with a health benefit and medium prices. The 'adventurous' prefer the ASC label as well as the NutriScore, but the former is of higher interest for them. They also prefer domestic products with a health benefit, but price is their top priority thereby products targeted towards them should be low to medium priced. The 'moderate/conservative' share some of their preferences with the 'adventurous' but in addition they are more prevalent in the group that does not value the ASC label but likes the NutriScore, EU products with a health benefit and especially products with low prices. Lastly, the 'uninvolved' consumers were more prevalent in the choice group that is mostly interested in domestic products that are medium priced, they dislike the NutriScore but place some value on the ASC label.

Conclusion

These results have important implications for new product development in the aquaculture market as they show the attributes that are most valued by different segments of consumers. As not all consumers seek the same value-added attributes from new products, our results are highly relevant for targeting new aquaculture products to the most promising segments of consumers in order to ensure their success on the market.

References

Banovic, M., Reinders, M. J., Claret, A., Guerrero, L., & Krystallis, A. (2019a). A cross-cultural perspective on impact of health and nutrition claims, country-of-origin and eco-label on consumer choice of new aquaculture products. Food Research International, 123, 36-47.

Brunsø, K., Birch, D., Memery, J., Temesi, Á., Lakner, Z., Lang, M., Dean, D., Grunert, K. G. (2021). Core dimensions of food-related lifestyle: A new instrument for measuring food involvement, innovativeness and responsibility. Food Quality and Preference, 91, 104192.

Cantillo, J., Martín, J. C., & Román, C. (2020). Discrete choice experiments in the analysis of consumers' preferences for finfish products: A systematic literature review. Food Quality and Preference, 84, 103952.

EUMOFA. (2017). EU consumer habits regarding fishery and aquaculture products. Annex 4 Country fiches.

EUMOFA. (2020). The EU fish market.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action.

Acknowledgments

This study has received funding from the European Union in the frame of Horizon 2020 - MedAID (Mediterranean Aquaculture Integrated Development), grant agreement number 727315 (http://www.medaid-h2020.eu/).

STABILISATION OF POLYCHAETE (*Nereis virens)* BIOMASS DURING FROZEN STORAGE

IB. Standal, R. Mozuraityte and R. Slizyte

Department of Fisheries and New Biomarine Industry, SINTEF Ocean, Trondheim 7010, Norway

Introduction

Cultivation of low trophic organisms, such as polychaetes, has been proposed as one mean for recycling nutrients from sidestreams, and at the same time producing omega-3 rich lipids and marine proteins, that are in high demand both for feed and human applications. Polychaete worms are presently cultivated in both Europe and Asia, but at relatively small scale and mainly sold in the premium feed market (e.g sold as live feed or freeze-dried). Live polychaete worms have particularly showed beneficial effects in shrimp broodstock diets, but the mechanisms responsible for the superior effect of live feed is not known. Marine raw materials are highly perishable, therefore proper handling and preservation methods are needed to maintain the valuable lipids, proteins and other components after harvesting. However, only few studies have investigated the effect of different storage/prosessing conditions on important quality parameters on polychaete biomass.

In order to up-scale and use polychaetes as an alternative aquafeed ingredient – the production and processing should be cost and energy efficient – while still maintaining the quality of the raw material. The goal of the present study was to evaluate the stability of the biomass during different frozen storage conditions, including effect of heat-treatment. Quality was evaluated based on both lipid and protein composition.

Materials and methods

Cultivated *Nereis virens* was procured from a commercial producer. Three different storage processes were tested namely 1) industrial frozen worms (vacuum packed and blast frozen (-38 °C) and further stored at – 27 °C, 2) frozen storage in plastic bags at – 18 °C and 3) pre-treated by blanching prior to frozen storage at – 18 °C. Quality was evaluated after 1, 6 and 12 months storage, and compared with control samples, i.e. worms sampled/analyzed directly after snap freezing by liquid nitrogen.

At each sampling point, lipid quality was evaluated based on, oxidative quality, amount of free fatty acids, fatty acid composition and lipid profile evaluated by Nuclear magnetic resonance spectroscopy. Changes in protein fraction were evaluated based on amount -and composition of amino acid composition, and changes in acid soluble metabolites, such as free amino acids and osmolytes.

Results

Frozen storage, both at – 18 °C and -27 °C, led to relative fast changes in lipid composition and quality. For example, the content of free fatty acid significantly increased after 6 months frozen storage compared to control samples. The results show that introducing heat-treatment prior to frozen storage was effective to prevent activity of endogenous enzymes leading to lower lipid hydrolysis during frozen storage (Figure 1).

Lipid oxidation took place during the storage, especially at a storage temperature of –18 °C, both in non-treated and blanched biomass (**Figure 2**). On the other hand, vacuum packed worms stored frozen at – 27°C, showed much lower lipid oxidation. Blanching led to reduction of minerals/salt, but also some leakage of lipids and low molecular weight metabolites. The leakage led to higher protein efficiency ratio (PER) of blanched worms – compared to biomass without this pre-treatment. There were only small changes in the profile of low molecular weight metabolites profile during frozen storage.

Discussion and conclusions

In order to use polychaetes as an alternative aquafeed ingredient - there is a need to find economically relevant handling and processing methods of large volumes of biomass, while maintaining acceptable quality. The results obtained in this study – shows that both high endogenous enzymatic activity and lipid oxidation is a challenge. Therefore effective measures to hinder such activity is important, e.g for preserving phospholipids. The results shows that prolonged frozen storage of un-treated biomass should be prevented. Processing or fractionation direct after harvesting – could be one solution to maintain valuable lipids and proteins. Blanching is one method for hindering enzymatic activity in biomass with high endogenous activity – and is also beneficial for salt reduction – however, some loss of lipids and free amino acids are anticipated.

(Continued on next page)

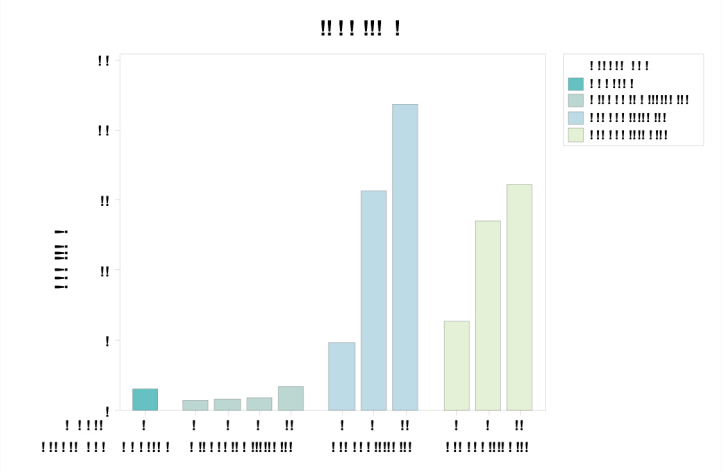


Figure 1. Content of free fatty acid (as weight % of total fatty acids) in polychaetes biomass during at different conditions.

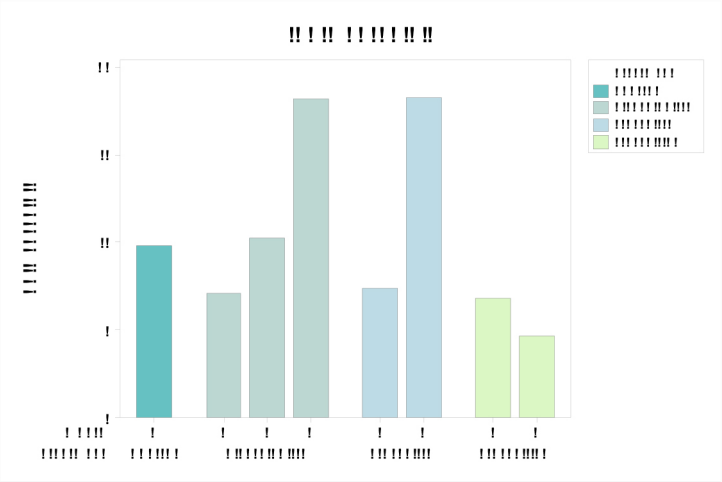


Figure 2. Peroxide value (PV) in lipid extracted from polychaete biomass during storage at different conditions.

UPDATING THE ORGANIC ACID STORY: NEW TECHNOLOGY FOR ADDED VALUE

B. T. Standen*, M. Spiegelhofer, N. Roth

*Benedict.Standen@dsm.com

Organic acids have been widely used in aquaculture, and their benefits have been well documented. However, there are certain misconceptions about their usage, one of the main being that are primarily used for an 'acidifying effect'. Whilst this can be true, in order to achieve this a large amount of organic (or inorganic) acids are needed to overcome the buffering capacity of high protein feeds or the gut. High dosage may come with higher costs and takes up valuable space in the diet, which is often limiting. Further, utilizing inorganic acids can damage equipment, adding unnecessary maintenance costs.

A better usage of organic acids, is for their direct antimicrobial effects which can be enhanced by other components, which act in synergy to boost said benefits. BIOMIN has developed a commercial acidifier product called Biotronic® Top3 which contains a unique Permeabilizing Complex™. This facilitates the transport of the organic acid blend (propionic, formic and acetic acid) and phytochemical (cinnamaldehyde) through the Gram-negative membrane of pathogens. Once inside the cell, the acids dissociate and disrupts cell metabolism and membrane functionality, slowing their growth and killing them.

Here we demonstrate the high efficacy and consistency of Biotronic® Top3, indicated by low MIC (minimum inhibition concentration) across a wide range of aquaculture pathogens. Benefits beyond conventional MIC testing was explored, demonstrating that the acid blend with Permeabilizing Complex™ negatively effects the pathogen physiology by disrupting membrane function, membrane potential and efflux pump activity. In the current study, the MIC value ranged from 0.1% for *Moritella viscosa* and *Tenacibaculum maritimum*, and 0.2% for *Yersinia ruckeri* and *Vibrio parahaemolyticus*. In a previous experiment, an MIC value <0.05% was also obtained for *Piscirickettsia salmonis*, the causative agent of Salmonid Rickettsial Septicemia (SRS).

In order to investigate pathogen physiology, representative pathogens from salmon and shrimp culture (*M. viscosa* and *V. parahaemolyticus*) were incubated with Biotronic® Top3, at high and low dose, for 30 minutes. Different dyes were used to indicate specific characteristic, which were validated using prokaryotic flow cytometry protocols. The stains used were propidium iodide, DiBAC4(3) and ethidium bromide to assess membrane integrity, membrane potential and efflux activity, respectively. In each case, low fluorescence was observed in control treatments (i.e. without Biotronic® Top3), indicating a healthy and viable population. However, when exposed to the enhanced acidifier, fluorescence was increased corresponding to increased membrane damage, depolarized membrane potential and decreased efflux pump activity. Interestingly, the latter might have important implications in reducing antimicrobial resistance. Importantly, there was a strong dose dependent effect, showing a clear product response.

Together, these results indicate that the Permeabilizing Complex™ adds value and complements organic acid blends, and can have strong antimicrobial effects at relatively low inclusion levels. *In vivo* studies (not described here) have also corroborated these effects in the field, reducing disease and contributing to sustainable aquaculture production.

BIOPROSPECTING FOR CYANOBACTERIA AND MICROALGAE IN PELOIDS – EXTREME ENVIRONMENT KNOWN FOR ITS HEALING PROPERTIES

A. Starcevic*, R.Coz-Rakovac, M.Roje, D. Vadlja, M.Bujak, L.Cizmek, A. Horvatic, D.Oros and J.Zucko

Affiliations: Institute Ruđer Bošković and University of Zagreb - Faculty of Food Technology and Biotechnology
BioProCro under the center of excellence for Marine Bioprospecting: BioProCro
E-mail: astar@pbf.hr

Seawater is an environment in which numerous organisms evolve, some with great potential for biotechnology. In recent years, however, many scientists have moved away from the assumption that the origin of life was in pools of water, and instead propose life on Earth probably originated in accumulations of warm, nutrient-rich mud. This mud, also called a peloid, has formed over many years through various processes and is a rich source of living organisms that, due to their adaptation to this unique environment, produce a wide variety of primary and secondary metabolites with numerous and diverse activities, including anti-cancer, anti-inflammatory, antiviral, and immunomodulatory ones. In this research, two important questions were addressed using collected samples of a localized peloid with demonstrated healing properties. First, whether it is the mineral (abiotic) or the secondary metabolic (biotic) component that contributes most to the healing properties of the peloid. Secondly, the biodiversity in this ecological niche was explored in order to build an ecological model of the microbial communities present in it, to gain insight into their dynamics and to identify key species.

Importance:
The use of peloids in medical therapy dates back to ancient times. Abiotic components such as clay and mineral water are believed to be the main components of the healing properties of natural peloids. The places where peloids are usually found are characteristically shallow and enclosed lagoons. The constant UV exposure and increased salt concentration could classify peloids as an extreme environment. The spectrum of claims for which peloid therapy provides relief ranges from purely cosmetic, skin-related, musculoskeletal to immunological problems. These claims can hardly be supported by mineral content and heat-retaining properties alone. However, organic compounds from decaying microorganisms as well as secondary metabolites from microbial communities could explain the observed range of health benefits. The fact that the relationship between the therapeutic activity of peloids and their composition outside the range of mineral and physicochemical properties has not been adequately studied indicates a real biotechnological potential. Since one of the main microorganisms discovered in peloids with therapeutic properties is microalgae, which produce a range of organic compounds, they should be the focus of study of this complex and promising natural resource.

PRELIMINARY DYNAMIC ENERGY BUDGET MODELS FOR STUDYING THE THERMAL TOLERANCE OF E. SEABASS AND MEAGRE

Orestis Stavrakidis-Zachou[1,2]*, Nikos Papandroulakis[1], Pavlidis Michail[2], Konstadia Lika[2]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, AquaLabs, 71500, Gournes, Heraklion, Greece
[2]Department of Biology, University of Crete, 71003, Heraklion, Greece
e-mail: ostavrak@hcmr.gr

Introduction

Developing tools to describe temperature induced effects on fish becomes increasingly important for aquaculture in the context of climate change. Dynamic Energy Budget models can describe the bioenergetics of individual fish as a function of temperature and food availability and, thus, offer means of studying the effects of temperature on relevant fish parameters and traits. In particular, temperature effects in DEB models are quantified via the parametrization and use of the Arrhenius function (Kooijman, 2010). Simulations can then be performed under different temperature conditions to study the effects on fish traits, while the patterns in parameter values may be used to compare the bioenergetics and the thermal tolerance of different species (Freitas *et al*., 2010). Here, we have parametrized DEB models with particular focus on higher temperatures for two aquaculture species, the European sea bass and the meagre, and developed a preliminary module for describing changes in body composition.

Materials and methods

The DEB models were parametrized according to Marques *et al*. (2018) using experimental data and data from literature and are provided in Stavrakidis Zachou *et al*. (2019, 2021). In addition, growth performance and respiration data at temperatures close to the edges of the species thermal tolerance range were included to parametrize the Arrhenius function and thus calculate a correction factor to describe changes in the various physiological rates across the entire thermal tolerance range.

Moreover, a module is being developed to model changes in the proximate composition of the fish reared under different temperature conditions. This is done by simulating temperature driven changes in the model state variables such as reserve (E) and structure (V), which in DEB context comprise the fish biomass, and subsequently translating them to changes in proximate composition.

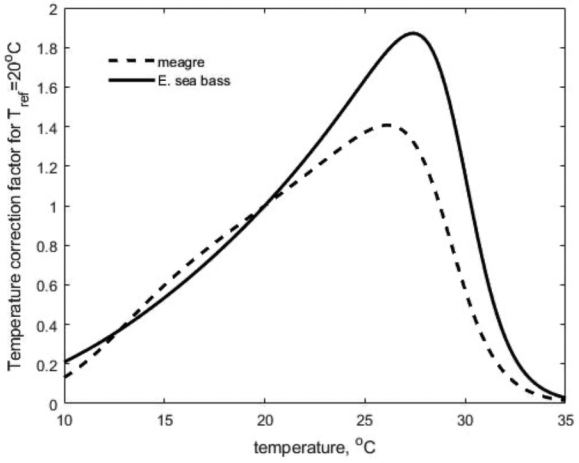


Figure 1 The temperature correction factor (for a reference temperature of 20 °C) across the thermal tolerance range for E. seabass and meagre

(Continued on next page)

Results and Discussion

The two models were parametrized and validated against datasets from farms as well as experimental data at high temperatures. The derivation of parameters for the Arrhenius function revealed differences in the thermal tolerance patterns of the two species in terms of the optimal temperature as well as the decline of performance towards the edges of the tolerance range. As depicted by the calculated correction factor, a factor that compares the various metabolic rates to a reference temperature (here 20 ^{o}C, where it equals 1) (figure 1), the width of the tolerance range was similar for E. seabass and meagre. However, the peak (which represents the highest metabolic activity) was higher for the former and also shifted to the right, occurring at a higher temperature compared to meagre. . Moreover, the reduction of performance at the upper end of the tolerance range was sharper for E. seabasss.

Compared to experimental data, the models could capture the decline in the voluntary feed intake and growth at temperature close to the upper end of the tolerance range, reasonably well. Provided that no shrinking occurs (reduction of structural mass) the energetic demands in DEB models are fueled only by the energy reserves. Consequently, preliminary simulations have shown that as temperature increases beyond the thermal optimum, the contribution of structure relative to that of reserve in the total biomass also increases. As a result, the model can depict some trends in body composition namely the increase in moisture content and the concomitant decrease in protein and lipid contents. Such trends are typically observed under low feeding or starvation conditions (Shirvan *et al.*, 2020), which is also the case for high temperature regimes due to the reduction in appetite. Therefore, application of such DEB models in aquaculture not only offers means of elucidating and comparing thermal tolerance patterns across species, but may also provide tools to quantify thermal effects on growth and body composition.

References

Freitas, V., Cardoso J., Lika, K., Peck, M., Campos, J., Kooijman, S.A.L.M, van der Veer, H. (2010). Temperature Tolerance and Energetics: A Dynamic Energy Budget-Based Comparison of North Atlantic Marine Species." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 365: 3553–65.

Kooijman, S.A.L.M. (2010). Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press.

Marques, G.M., Lika, K., Augustine, S., Pecquerie, L., Kooijman, S.A.L.M. (2019) Fitting multiple models to multiple data sets. *Journal of Sea Research,* 143, 48–56.

Shirvan, S., Falahatkar, B., Noveirian, H.A., Abbasalizadeh, A., 2020. Physiological responses to feed restriction and starvation in juvenile Siberian sturgeon *Acipenser baerii* (Brandt, 1869): effects on growth, body composition and blood plasma metabolites. *Aquaculture Research*, 51, 282–291.

Stavrakidis-Zachou, O., Lika, K., Anastasiadis, P., Papandroulakis, N. (2021). Projecting climate change impacts on Mediterranean finfish production: a case study in Greece. *Climatic Change,* 165, 67.

Stavrakidis-Zachou, O., Papandroulakis, N., Lika, K. (2019). A DEB model for European sea bass (*Dicentrarchus labrax*): parameterisation and application in aquaculture. *Journal of Sea Research*, 143, 262–271.

THE EFFECT OF *Hermetia ilucens* INSECT MEAL INCLUSION ON INTESTINE MICROFLORA IN PIKEPERCH *Sander lucioperca*

V. Stejskal[1]*, H. Q. Tran[1], M. Zare[1], M. Prokešová[1], T. Gebauer[1], I. Ferrocino[2], C. Caimi[2], F. Gai[3], L. Gasco[2,3]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters
[2]University of Torino, Department of Agricultural, Forest and Food Sciences, Largo Braccini 2, 1095 Grugliasco, Torino, Italy
[3]National Research Council Institute of Science of Food Production, Largo P. Braccini 2, 10095 Grugliasco, Torino, Italy

E-mail: stejskal@frov.jcu.cz

Introduction
Pikeperch (*Sander lucioperca*) currently drawing a great attention for aquaculture (Schulz et al., 2006) mainly in intensive recirculation aquaculture system. However, pikeperch have received little interests from feed manufacturers (Bochert, 2020). Though some commercial aquafeeds dedicated for percids have been available, salmonids-targeted feeds are widely used in practice. Insect meal produced from black soldier fly (BSFM) is rich in amino acids, lipids, vitamins and minerals. From these reason BSFM have been considered as potential alternatives to fish meal. The aim of this study was to investigate the effect of dietary defatted BSFM in diets for juvenile pikeperch on intestine microbiota.

Materials and methods
Four isonitrogenous and isolipidic experimental diets were formulated, comprising one fishmeal-based diet (CO), and three diets, where fishmeal was replaced at 25 (HI9), 50 (HI18) and 100% (HI36) by BSFM. Experimental extruded diets were prepared at a commercial feed producer (Exot Hobby s.r.o., Czech Republic). All groups were conducted in triplicate in 84 days trial. Pikeperch juveniles (68.7 ±7.1 g) were randomly allocated to twelve 250 L round conical tanks. Fish were fed to apparent satiation using combination of automatic feeders (EHEIM Twins) at 07.00, 09.00, 11.00, 13.00 and one hand feeding at 15.00.

At the end of main growing trial, three fish per each tank were randomly taken and euthanized with overdose anesthesia). Nucleic acid was extracted from the gut content (500 mg as starting materials). Total DNA from the samples was extracted using the RNeasy Power Microbiome KIT (Qiagen. Milan. Italy) following the manufacturer's instructions. One microliters of RNase (Illumina Inc. San Diego. CA) was added to digest RNA in the DNA samples with an incubation of 1 h at 37°C. DNA was quantified using the NanoDrop and standardized at 5 ng/µl. DNA directly extract from digesta samples was used to assess the microbiota by the amplification of the V3-V4 region of the 16S rRNA gene. The PCR products were purified according to the Illumina metagenomic standard procedure (Illumina Inc. San Diego. CA). Sequencing was performed with a MiSeq Illumina instrument with V3 chemistry and generated 250 bp paired-end reads according to the manufacturer's instructions.

Results and discussion
The result from OTUs analysis showed that significant increase was observed in HI18 compared to fishmeal diet, while two other insect-based diets did not differ in observed species from fishmeal diet. Feeding pike perch with dietary HI resulted in higher Chao 1 index than those with fishmeal diets, while no difference was observed for all HI-based diets (Fig. 1). Those difference were also observed by plotting the PCA at genus level. It was also possible to observed a degree of separation between the control samples CO quite near to the insect meal at 9% while the microbiota of fish feed with 18 and 36 % of HI well separated (Fig. 2). In agreement with previous studies on intestinal microbiota of percid fish and freshwater species, the present study reveals that *Firmicutes*, *Proteobacteria*, *Bacteroidetes* were the most dominant phyla in the intestine of pikeperch, regardless of the HI inclusion level. The inclusion of HI up to 18% or 50%

fishmeal replacement in pikeperch diets increased abundance of *Clostridium*, *Oceanobacillus*, *Bacteroides*, and *Faecalibacterium*, whereas the predominant bacterium, *Cetobacterium* was found in the control and HI36 groups.

(Continued on next page)

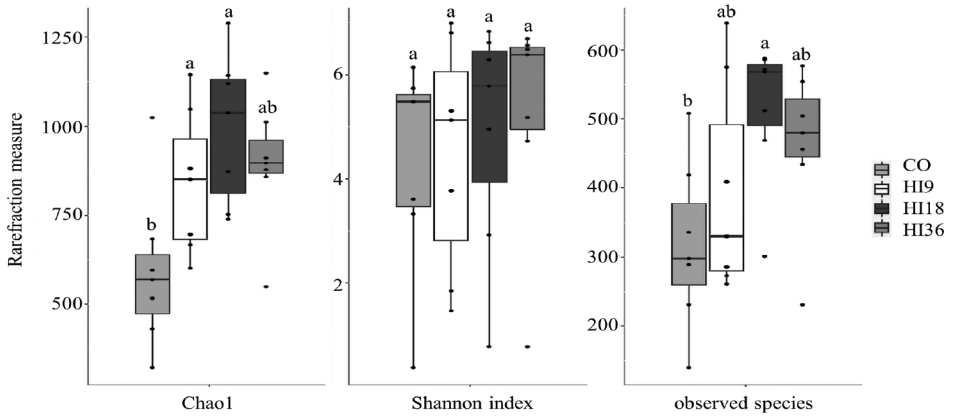


Fig. 1. Boxplots showing the alpha diversity rarefaction index in fish fed with 0% (CO), 25% (HI9), 50% (HI18) and 100% (HI36) of BSFM.

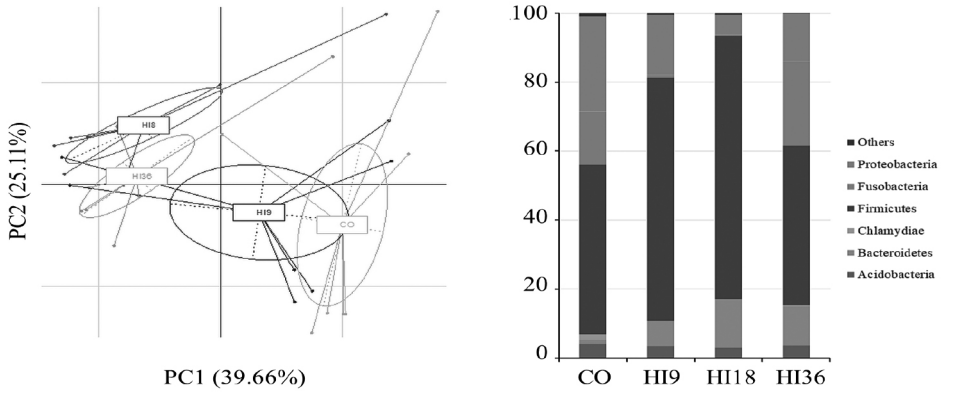


Fig. 2. Intestinal microbiota composition (PCA plots) in fish fed with 0% (CO), 25% (HI9), 50% (HI18) and 100% (HI36) of BSFM (A) and relative abundance (%) of the OTUs in the intestine of pikeperch fed experimental diets at phyla level.

Acknowledgements

The study was financially supported by the Ministry of Agriculture of the Czech Republic, project NAZV (QK1810296) and European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 652831 (AQUAEXCEL2020), the TNA project (ID AE070026)

References

Schulz, C., Günther, S., Wirth, M., Rennert, B., 2006. Growth performance and body composition of pike perch (*Sander lucioperca*) fed varying formulated and natural diets. Aquaculture International. 14, 577-586.

Bochert, R., 2020. Comparative performance, biochemical composition, and fatty acid analysis of Eurasian perch (*Perca fluviatilis*) during grow-out in RAS fed different commercial diets. Journal of Applied Aquaculture, 1-15.

Sparicotyle chrysophrii TRANSMISSION IN *Sparus aurata* FARMS: A MODELLING STUDY

Elisa Stella[1,*], Roberto Pastres[1], Damiano Pasetto[1], Lorenzo Mari[2], Enrico Bertuzzo[1]

[1] Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Scientific Campus, Via Torino, 155, 30172, Mestre-Venice, Italy
[2] Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Via Ponzio 34/5, 20133, Milan, Italy
E-mail: elisa.stella@unive.it

Introduction

The increasing rates of fish consumption require aquaculture to achieve sustainable solutions to maintain a healthy and productive environment, also for the surrounding ecosystems (UN Environment Programme 2017, FAO 2020). Among other issues, the spreading of parasites can represent a considerable challenge for fish farmers. In the Mediterranean sea, gilthead bream (*Sparus aurata*) farms are mainly challenged by the presence of the parasite *Sparicotyle chrysophrii,* which can cause lethal epizootics in sea cages. *S. chrysophrii* is a hermaphrodite parasite that attaches to fish gills; it releases eggs that can either remain attached to the host or be released in the environment, potentially attaching to cage nets (Antonelli et al. 2010, Repullés-Albelda et al. 2012). Eggs mature into miracidia that can eventually attach to other fish hosts, thus closing the life and transmission cycles of the parasite.

Epidemiological modelling represents a valid tool to help fish farmers understand parasite transmission and evaluate control measures. To this purpose, in the following we propose and analyse a novel epidemiological model that investigates *S. chrysophrii* transmission within gilthead bream farms.

Methods

We developed a stratified compartmental model to reproduce parasite distribution within fish hosts. The model accounts for eco-epidemiological and evironmental dynamics driven by aquaculture practices. To account for the major pathogenicity of adult S. chrysophrii, we distinguished between juvenile and adult parasites. To do this, we model the number of infected fishes considering both juveniles and adults parasites attached. The model also includes the parasite life cycle, which is essential to realistically reproduce the epidemiological dynamics.

Results

We run our model over wide ranges of critical parameters (as shown, as an example, in Figure 1 with reference to the transmission rate, β) to study transmission dynamics under different eco-epidemiological conditions. For some parameter combinations, the conditions for parasite invasion are not met, as shown also on the top panels in Figure 1. For other combinations, the model allows to track the progression of the outbreak and, potentially, to evaluate the best timing for interventions aimed at reducing or breaking parasite transmission.

Discussion and conclusions

We proposed a model to reproduce the transmission dynamics of *S. chrysophrii* within gilthead bream farms. We studied transmission dynamics for different environmental and eco-epidemiological conditions. Our approach can be a useful tool to help aquaculture farmers develop intervention strategies. Further developments of the model could include fish growth dynamics (Ferreira et al. 2021), which in turn depends on the hosts' infection status, to investigate how management practices could synergistically improve both fish health and aquaculture production.

References

Antonelli, L, Quilichini, Y., & Marchand, B (2010). Sparicotyle chrysophrii *(Van Beneden and Hesse, 1863) (Monogenea: Polyopisthocotylea) parasite of cultured gilthead sea bream* Sparus aurata *(Linnaeus 1758) (Pisces: Teleostei) from Corsica: Ecological and morphological study.* Parasitology Research, 107:389–398.

FAO (2020). *The State of World Fisheries and Aquaculture (SOFIA).* Sustainability in action. FAO, Rome.

Ferreira, J. G., Taylor, N. G., Cubillo, A., Lencart-Silva, J., Pastres, R., Bergh, Ø., and Guilder, J. (2021). *An integrated model for aquaculture production, pathogen interaction, and environmental effects*. Aquaculture, 536:736438.

Repullés-Albelda, A., Holzer, A. S., Raga, J. A., and Montero, F. E. (2012). *Oncomiracidial development, survival and swimming behaviour of the monogenean* Sparicotyle chrysophrii *(Van Beneden and Hesse, 1863)*. Aquaculture, 338:47–55.

UN Environment Programme (2017). *Mediterranean Quality Status Report*.

(Continued on next page)

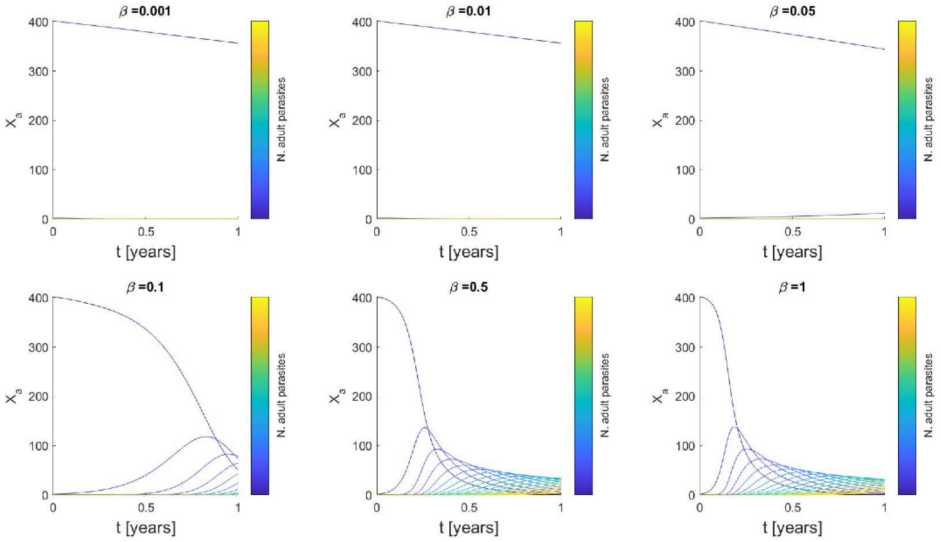


Figure 1 Time evolution of X_a (i.e. the number of fishes with a adult parasites), for different values of the exposure rate β.

INDUSTRIAL TESTING OF A NEW NORWEGIAN OZONE REACTOR AND SKIMMER SYSTEM

Kevin T. Stiller*, Stig Johansen, Michael Vadseth, Per S. Brunsvik

Nofima AS, Sjølseng, 6600 Sunndalsøra, Norway
Kevin.Stiller@nofima.no

The Mowi close containment smolt facility in Steinsvik was built by Krüger Kaldnes without an RAZONE treatment system of their process water. Steinsvik is ideal location to test a newly develop two phase ozone reactor tank- followed by a skimmer- system. The product of the Ålesund based company Normex is called RAZONE WATER CLARIFIER. The innovative approach based on preliminary findings to prolong the ozone exposer time in the RAS water through an additional reactor tank for a later so-called micro flock production that can be removed by the sea-/fresh- water "skimmer system" which is different build to a common protein skimmer that been normally only used in seawater. The ozone system was installed to the Smolt unit of Mowi Steinvik to treat around 10% of the reuse water.

In this project water quality and fish welfare was evaluated monthly over 3 production cycles in in Mowi Steinsvik. The first cycle was without an installed RAZONE system. The second cycle was done with a relative conservative Oxidation-reduction potential (ORP) that is used as a proxy of ozone dosage of around 280 mv and the second with around 300 mV.

The water quality obviously become clearer using ozone in the production sending water as bypass through the RAZONE system. Turbidity, color, Total suspended solids (TSS) and Total organic carbon (TOC) are significantly reduced. The reduction is even viable with normal eyes comparing the 3 smolt production cycles. The production can be influenced by abrupt appetite loss in fish. Feed spill events and decreasing water quality when the fish stop eating where highly reduced using the RAZONE system. The automatic feedback loop regulated through 2 OPR probes works secure and stabile.

Nitrite, Germ count, Copper, zinc, and cadmium reductions could be measured just directly behind the RAZONE system. If the 10% treated water is diluted with the untreated process water it is more difficult to detect smaller changes in these variables. Gill histology and welfare score could not detect any negative effect through the RAZONE system to the fish under the given conditions. However, it could be recognized that the smolt quality increased, especially in the last test run. The subjective scoring system made it difficult to provide a solid answer if the welfare status increased but it could be seen that the fin quality increased significantly in the last test run.

In summary using the RAZONE system provided healthy smolts as well as bettering and stabilizing the water quality during the production cycle.

MEGA DEVELOPMENT PROJECTS IN NORWEGIAN FISH FARMING: HANDLING LOCAL AND GLOBAL RISKS FOR THE COMMON GOOD?

K.V. Størkersen*[1], T. Thorvaldsen[2], T. Osmundsen[1], P. Almklov[1], M.S. Olsen[1], S. Afewerki[2], V.S. Amundsen[1], A. Misund[2]

[1]NTNU Samfunnsforskning, 7491 Trondheim, Norway
[2]SINTEF Ocean, Pb 4762 Torggården, 7465 Trondheim, Norway
Email: kristine.storkersen@samforsk.no

Introduction
Managing and regulating aquaculture has been described as a complicated issue because of lack of firm knowledge regarding risks such as diseases and environmental impact, as well as the dynamic nature of new solutions emerging (Osmundsen et al. 2017).

Development licensing is a policy instrument launched by the Norwegian government for technological innovation to reduce risk – especially regarding escape of fish, prevalence of salmon lice, as well as access to sea-based production localities. The development licensing aims for technical innovation of new farm concepts, rather than social, organizational, or biological innovation or research. These projects can be seen as fish farming in extreme, and due to their clearly defined aims and major risks, they can be studied as showcases of norms and notions in Norwegian fish farming.

Research gap
Through earlier research in Norwegian fish farming, there is knowledge of risk dimensions, risk handling in the fish farm organizations, (Fenstad, Osmundsen, & Størkersen, 2009; Gismervik et al., 2020; Holen, Utne, & Yang, 2018; Stien et al., 2020; Thorvaldsen, Holmen, & Moe, 2015) and governmental measures to control these risks (Gismervik et al., 2020) (Osmundsen, Olsen, & Thorvaldsen, 2020).

We know that several risks are insufficiently reduced, and aims not met. This have led the Norwegian government and public, to require a stop in growth. The development licenses are supposed to solve some of these issues. However, there have not yet been any studies about how the development licences contribute to or solve the risks and what goals the actors plan to solve.

Research question and methodological approach
We have studied six developing concepts, and asked: *What goals do they have and what risks have they considered to be vital to handle?* We have interviewed about 10 persons involved in each developing concept (so far 40 semi-structured research interviews). The actors have been representatives for fish farming companies, technology providers, biology and fish health consultants, other service operators, authorities, associations, verification companies, research institutes, and knowledge providers.

Findings and implications for the future fish farming industry and research
Through the interviews and case studies, we have been seen that the actors involved in development projects handle the same risks that all Norwegian fish farmers need to handle to stay in business – and some more. The most common risks are Lice; Mortality and disease; Emission (fish escape, pollution); Personal injuries; Bankruptcy, and Incompliance with regulations. In addition, in this data material, we find that the actors involved in the development concepts also aim to reduce local and global societal risks: Unemployment, food scarcity, and value creation for Norwegian fish farming. They underline their role to develop lice free, emission free, safe and healthy operations, and and to increase aquaculture shares in global food production. They develop new networks with new actors, but employ local and known third party companies.

Possible effects of development licenses in aquaculture globally, may be new industry structures, more global networks, and perhaps increased competence and reduced risks. However, regulation needs changes if the industry should further develop.

The findings of this study may guide policy makers and the regulation of the industry, thus contributing to framework conditions for sustainable innovation long-term. Industry stakeholders will get knowledge that may be used to further evaluate development licenses as a strategy for innovation.

(Continued on next page)

Literature

Fenstad, J., Osmundsen, T. C., & Størkersen, K. V. (2009). *[Danger on the netpen? Need for changed safety work at Norwegian fish farms]*. Trondheim, Norway: NTNU Samfunnsforskning.

Gismervik, K., Tørud, B., Kristiansen, T. S., Osmundsen, T. C., Størkersen, K. V., Medaas, C., . . . Stien, L. H. (2020). Comparison of Norwegian health and welfare regulatory frameworks in salmon and chicken production. *Reviews in Aquaculture, n/a*(n/a). doi:10.1111/raq.12440

Holen, S. M., Utne, I. B., & Yang, X. (2018). Risk dimensions of fish farming operations and conflicting objectives *Safety and Reliability–Safe Societies in a Changing World* (pp. 1425-1432): CRC Press.

Osmundsen, T. C., Olsen, M. S., & Thorvaldsen, T. (2020). The making of a louse-Constructing governmental technology for sustainable aquaculture. *Environmental Science & Policy, 104*, 121-128.

Stien, L. H., Tørud, B., Gismervik, K., Lien, M. E., Medaas, C., Osmundsen, T. C., . . . Størkersen, K. V. (2020). Governing the welfare of Norwegian farmed salmon: Three conflict cases. *Marine Policy, 117*, 103969.

Thorvaldsen, T., Holmen, I. M., & Moe, H. K. (2015). The escape of fish from Norwegian fish farms: Causes, risks and the influence of organisational aspects. *Marine Policy, 55*, 33-38.

STATUS AND PERSPECTIVES ON MARICULTURE PLANNING AND IMPLEMENTATION - THE MARINE SPATIAL PLANNING (MSP) AND MARINE FUNCTIONAL ZONING (MFZ) FRAMEWORKS

Ø. Strand*[1], H. Liu[2], JG. Ferreira[3], J. Grant[4], E.S. Grefsrud[1], P. Kupka Hansen[1], Q. Sun[5], J. Weitzman[6]

[1]Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway.
[2]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Rd, Qingdao, 266071, China
[3]Longline Environment Ltd., 63 St Mary Axe, London, EC3A 8AA, UK
[4]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada
[5]Second Institute of Oceanology, Ministry of Natural Resources, 36 Baochubei Road, Xihu District, Hangzou, 310012, China
[6]Marine Affairs Program, Life Sciences Centre, Dalhousie University, 1459 Oxford Street, Halifax NS B3H 4R2, Canada
e-mail:oivinds@hi.no

The policy and legal frameworks that regulate expansion of mariculture differs worldwide, and specifically between some of the major players in mariculture development, China, the European Union (EU), Norway and Canada. In China, marine functional zoning (MFZ) is the legal framework regulating use of marine space, while in the other nations marine spatial planning (MSP) is applied. We detail how mariculture is implemented and compare the development experience for MFZ in China, and MSP in the EU, Norway and Canada. A comparison of the stepwise processes of MFZ and MSP implementation clarifies the differences in status of mariculture among the countries.

China, the EU, Norway, and Canada all have governmental visions and objectives to develop their mariculture industries. They have established institutional frameworks for managing aquaculture planning although these are highly diverse, where a general concern and condition for further development and growth of the aquaculture industry is the environmental interactions between mariculture and the environment. We suggest a stronger recognition of the relationship between food security, intensification of mariculture and environmental sustainability, to gain a better understanding how mariculture development can become an important contribution towards sustainable sea food production.

In the prospects of future increase in competition for space and resources in the oceans and consequently the need for efficient governance, the apparent weak or receding position of mariculture in MFZ and MSP processes should be of considerable concern if the endeavors of providing more ocean food using mariculture is promoted. As mariculture is regarded as the most promising route to achieve a substantial increase in provision of food from the oceans, we conclude that there is a need for strengthening the position of mariculture and its implementation in maritime spatial planning frameworks like MFZ and MSP.

A FRAMEWORK FOR SUSTAINABILITY ANALYSIS OF LOW TROPHIC SPECIES AQUACULTURE ACROSS THE ATLANTIC

Å. Strand[*1], L. Bengtsson[1], D.L. Flickinger[3], A.D. Hughes[5], J. M. Kimpara[4], G. Marinho[2], P. Moraes-Valenti[3], M.F. Palma[3], I. Sousa Pinto[2], J. Strandberg[1], W.C. Valenti[3], E. Lindblom[1]

IVL Swedish Environmental Research Institute, Kristineberg 566, 451 78 Fiskebäckskil, Sweden
asa.strand@ivl.se
CIIMAR, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N 4450-208 Matosinho, Portugal
UNESP São Paulo State University, Aquaculture Center, Rod. Paulo D. Castellane s/n, 14884-900, Jaboticabal, SP Brazil
EMBRAPA – Brazilian Agricultural Research Corporation, Tropical Agri-industry, Rua Dra. Sara Mesquita, no. 2270, Bairro Planalto do Pici, 60511-110, Fortaleza, CE, Brazil
Scottish Association for Marine Science, Oban, Argyll PA37 1QA, UK

Introduction

There is clear evidence that low trophic aquaculture is one of the most sustainable food production system available globally. However, in Europe the growth of low trophic aquaculture is stagnating. AquaVitae (AV) is a research and innovation project funded by the European Union'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. This includes macroalgae, echinoderms, shellfish and low trophic finfish species as well as exploring the use of LTS in integrated multi-trophic aquaculture (IMTA) systems. In addition to research on specific value chains, a significant part of the research activities in AV aim to support a sustainable expansion of LTS aquaculture by addressing aspects in all sustainability domains, e.g. consumer attitudes and market potential as well as policy and governance in the social domain, profitability and socio-economic aspects in the economic domain, and environmental monitoring, risk assessment, and ecosystem services in the ecological domain. Much of this work is integrated into one of AV's four cross-cutting work packages i.e. WP6 (Environmental Monitoring, Risk Assessment and Sustainability).

The main objective of WP6 is to develop recommendations on how to increase LTS aquaculture production with a net positive impact on sustainability in and around the Atlantic Ocean under the principle of circular economy and to understand the possible impact of climate change on aquaculture in this area. To fulfil this objective, WP6 is organized into five different activities aimed at developing a framework for sustainability analysis, risk assessments, and environmental monitoring as well as performing the actual analysis in each of these topics. The results will be summarised as recommendations on how the sustainability of increasing LTS Aquaculture production can be optimized, including which positive sustainability aspects to exploit, which negative sustainability aspects to minimize, which risks to consider in relation to these aspects and how to monitor the system for early identification and mitigation of specific risks. In this poster, the structure, methods and expected impact of the work in WP6 will be presented more in detail together with the results from the first activity, a framework for sustainability analysis of low trophic aquaculture around the Atlantic.

Methods and results

The first task in WP6 aimed at providing a unified context for the following activities of WP6, enabling efficient co-operation and exchange of information within the WP. The developed framework, therefore, describes a desired state for LTS aquaculture in the Atlantic region and explains how this is linked to Agenda 2030 and the UN's Sustainable Development Goals (SDGs). Acknowledging that the SDGs are integrated and indivisible, the SDGs with the strongest and most direct links to the desired state and vice versa were prioritized. A set of indicators to evaluate sustainability was developed in relation to the desired state and the SDGs (Figure 1).

The description of the desired state and prioritization of SDGs was based on a combination of literature reviews and interactions with experts, industry and other aquaculture stakeholders (e.g. third sector, public policy makers, consumers) at workshops and meetings, using a combination of top-down and bottom-up methods. In addition to SDG 14, Life below water, which was identified as being the core SDG linking LTS aquaculture to sustainable development, the four SDGs with the strongest and most direct links to the AV objectives were found to be 2 (Zero hunger), 8 (Decent work and economic growth), 12 (Responsible consumption and production) and 13 (Climate action).

(Continued on next page)

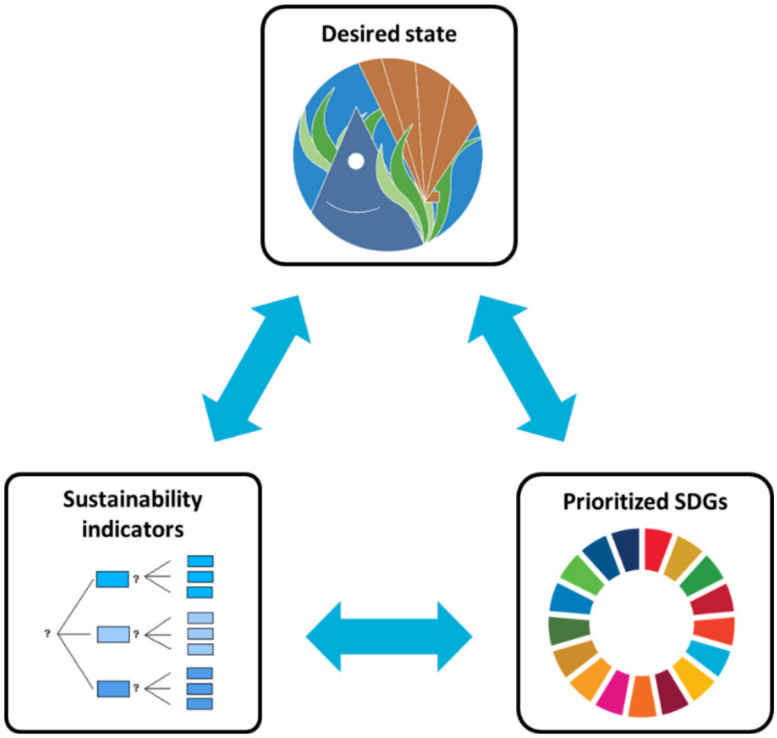


Figure 1. The interrelations of sustainability indicators, desired state and prioritized SDGs.

To compile the indicators, a broad screening of existing indicators was supplemented with the development of specific indicators. The screening covered, amongst other sources, the European Union Marine Strategic Framework Directive and Water Framework Directive, OSPAR, the SDGs, FAO and third-party certifications, and resulted in a database of 983 sustainability indicators. In addition to these 35 additional indicators were developed. Based on this, a set of 115 indicators was selected during a selection process, which included experts from within the AV consortium. The indicator set was developed to adequately describe, reflect and discern different types of LTS aquaculture, how they perform against a desired state and how they contribute to the SDGs most strongly linked to LTS aquaculture. The framework will be used to guide forthcoming sustainability analysis and risk assessments in WP6 with the aim to achieve the objective of the AV project and support the sustainable development of LTS aquaculture expansion around the Atlantic.

LESSONS LEARNED FROM STARTING AN INTEGRATED OFFSHORE AQUACULTURE-WINDFARM PROJECT IN TIMES OF A PANDEMIC (THE GERMAN NORTH SEA PILOT)

E. Strothotte*, M. Jaeger, J. Pforth, I. Drigkopoulou, E. Brouwers, N. Nevejan, A. Declercq, H. C. Søerensen, G. El Serafy

*R&D Centre Kiel University of Applied Sciences GmbH, Schwentinestr. 24, D-24149 Kiel
Email: eva.strothotte@fh-kiel-gmbh.de

Introduction

The ocean multi-use concept envisages to bundle natural, human and financial resources in the face of scarcity of space in order to enhance the viability of offshore operations (e.g. combining aquaculture with other offshore industries). Offshore projects, especially when implemented with a multi-use approach, are facing manifold challenges due to their different needs in logistics and operational intensity, particularly when extreme events offshore require different responses for each of them. To achieve an optimum of combined resource use, highly detailed, well thought-out planning of the work is needed. The organisation of complex logistics, preparation of infrastructure and pre-testing of materials and technologies are serious challenges while also assuring appropriate training of operational, multi-disciplinary personnel. Furthermore, the application of health and safety standards for people involved in operations are essential for a successful functioning of the multi-use system.

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 addresses the integration of a relatively new form of an old economic branch, namely aquaculture, into an already existing but relatively new set of offshore activities, to increase the potential for synergistic effects, enhancing the idea of multi-use co-locations (MUCL) at sea.

Within the UNITED project, five demonstration trials ("pilots"), were set up in different contexts (North- Baltic Seas, Mediterranean). 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 have to be more prominent than in all other pilots, although in principle needed by all.

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. This paper takes a special look at potential ways of solving unforeseen problems arising from the COVID19 outbreak with the focus on ad-hoc adjustments in the face of high uncertainty of their realisation. The described solutions demonstrate how intensified and rapid interactions and highly flexible and timely responses by all stakeholders can help to identify feasible alternatives to guarantee continued planning and subsequent operations

Materials and Methods

One of the major challenges due to COVID19 arose because of restrictions affecting the supply and service industries so that pre-test, and new design set-ups were seriously hampered. 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. The pre-testing and adjustment phase of the project is ongoing and provided most valuable information on adaptations to COVID19 in an innovative field, as there are no practical examples from which experience can be gained. Limited examples of successful MUCLs under these new conditions requires research inputs to find new solutions to arising questions in support of the development:

- What requirements are needed in terms of social, environmental, economic, legal and technological approaches when realizing a MU project in times of a pandemic of unknown duration and potential resurgence?
- What innovative approaches are needed to integrate offshore infrastructures and additional maritime activities with regard to adjusted health/safety standards?
- How can adaptations/ alternatives be made during the pre-operational phase to foster the development of MU activities in case a Pandemic re-appears?

(Continued on next page)

Results

One lesson learned from the pre-risk assessment was the need to always develop alternatives (e.g. plans B and C) at the onset of the project. This even for the most unlikely impairment. Several properly planned activities ready for timely implementation, had to be interrupted /postponed and subsequently to be cut short due to governmental ad-hoc decisions ("lock downs", restricting response options). Another major negative effect of the COVID19 situation has been the extremely prolonged and even uncertain delivery times for equipment and parts as well as services, including the purchase of aquaculture species. Alternatives had to be found to serve the original plans for data acquisition, particularly for monitoring instrument and their capabilities. Fortunately, comprehensive knowledge on the offshore engineering business contributed to timely find alternatives to specific suppliers. This partly allowed to keep schedules with limited delays. Forming a broad stakeholder register turned out to be most valuable. The pandemic led to an extensively expanded range of stakeholders which needed almost daily contacts to cope with newly arising risks. Stakeholders helped in reducing frustrations, fostering consensus building and gaining 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 pre-operational phase (first project year) has been used to determine the future-oriented skills for a safe operation of the pilots. The training objectives so far were not only 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 operational at all times.

Conclusion/Outlook

The pandemic caused delays with a terrible "domino-effect" downstream from the source to the end-user (the Pilot). The need for a time buffer to cope with unforeseen obstacles should be considered in manpower planning of future projects so that sufficient flexibility is available to cope with the problems adequately. The pandemic situation demanded an even broader and more flexible approach than ever anticipated. In the future, every possible flexible/alternative operational modus (including financial consequences) should already be in the drawer before being confronted with unforeseen events, even if no opportunity for testing occurred. This situation is likely to continue in all pilots as there is little hope that a return to the former "normal" will be restored within the duration of the UNITED project. Documenting these lessons is strongly recommended as additional benefits to multiple sectors may be generated, and infrastructural synergies may be created in the face of uncertainty. Translating the COVID19 pandemic conceptually to the aquatic environment, early response strategies might avoid damage to the aquaculture business by requesting regulations that prevent the transfer of exotic species and diseases via globalized trade and increased recreational boating. This while aquaculture locations near shipping routes are rapidly growing, this way increasing contact and risks entailed.

IN VITRO AND *IN VIVO* BIOLOGICAL ACTIVITY OF GREEN MICROALGAE *Desmodesmus spinosus*

I. Strunjak-Perović[a,b,*], L. Perković[a], A. Martić[a], E. Djedović[a], S. Babić[a,b], L. Čižmek[a,b], T. Paradžik[a,b] and R. Čož-Rakovac[a,b]

[a]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb (Croatia)
[b]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute
*E-mail: strunjak@irb.hr

Introduction

In recent years, studies of cellular extracts or isolation of intracellular and extracellular metabolites from different microalgae have been a high priority due to their diverse biological activity and possible discovery of novel nutraceuticals (Morais et al., 2015). A freshwater green microalgae *Desmodesmus spinosus* belongs to the family of Scenedesmaceae (Guiry, 2021). According to previous studies, genus of Desmodesmus showed relatively high antimicrobial and antioxidant activities due to the high content of pigments, phenolic compounds and tocopherols (Safafar et al., 2015). In this study, evaluation of biological activity of different polarity extracts from *D. spinosus* was conducted. Antioxidant activity was performed using both *in vitro* assays and *in vivo* zebrafish model, while an embryotoxicity test was performed in order to determine safety for non-target organisms.

Materials and methods

Cultivation of *D. spinosus* was carried out in an enclosed 2 L glass stirred tank bioreactor as a semi-continuous batch process. Temperature and pH values were kept constant at 25 °C and 9.2, respectively. The culture was stirred with an agitator at the rotation speed of 220 rpm. Air was provided from the bottom of the agitator at a flow rate of 120 L/h. The culture was illuminated with 2700 K warm white LED lamps. Microalgae cells were harvested in the exponential growth phase. The freeze-dried sample of microalgae *D. spinosus* was extracted by ultrasound-assisted extraction in four different solutions: water, water:methanol (1:1), methanol and methanol:dimethyl sulfoxide (1:1). Antioxidant activity was evaluated by implementing three *in vitro* assays: reduction of radical cation assay (ABTS) and ferric reducing antioxidant power assay (FRAP), while total phenolic content (TPC) was determined by Folin-Ciocalteu method. The toxicity of samples was determined using the zebrafish embryotoxicity test (OECD 236, 2013) up to 96 h of embryonal development. Upon establishing the concentration range of interest, zebrafish embryos were further employed to determine the protective effect of tested samples on H_2O_2-induced ROS generation *in vivo* (Jerković et al., 2021).

Results

Based on BLASTN results, microalgae used for this research was identified as *Desmodesmus sp.*, with the highest similarity to *Desmodesmus spinosus* (90% coverage, 96% identity). The highest *in vitro* antioxidant activity was obtained for methanol:DMSO cell extract solution using FRAP and Folin-Ciocalteu methods (Figure 1A). The lowest inhibitory concentration (IC_{50} = 0.876 mg/mL) obtained using ABTS assay was detected in methanol:DMSO solution extract which indicates the highest antioxidant activity, followed by MetOH>H_2O:MetOH>H_2O. The TPC showed a high positive correlation for both FRAP and ABTS assays with Pearson's correlation coefficients of 0.959 ($p < 0.05$) and 0.912 ($p = 0.09$), respectively. Additionally, a high positive correlation between FRAP and ABTS was found with Pearson's correlation coefficient of 0.967 ($p < 0.05$). Prepared extracts showed no embryotoxic potential at concentration of 0.5 mg/mL. Embryos pretreated with methanol and methanol:DMSO extracts of *D. spinosus* biomass showed a statistically significant decrease of fluorescence intensities for 52.75% and 50.06%, respectively, comparing to the group on H_2O_2 (Figure 1B).

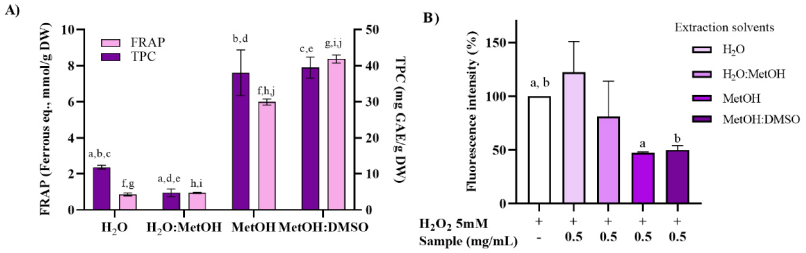


Fig. 1. Determination of antioxidant activity of *D. spinosus* cell extracts using: A) *in vitro* assays and B) *in vivo* zebrafish model. Columns sharing common letters indicate significant difference within each method. Results were presented as mean ± SD.

(Continued on next page)

Discussion

Conducted *in vitro* assays showed relatively high total phenolic content of *D. spinosus* MetOH and MetOH:DMSO extracts (38.05±6.33 and 39.47±2.93 mg GAE/g DW, respectively). Similar results were recorded by Patil and Kaliwal (2019) for *Scenedesmus bajacalifornicus* methanol extract (32.12 mg GAE/g DW). Although high antioxidant activity using *in vitro* assays was observed for two less-polar extracts (methanol and methanol:DMSO), all used methods showed significant correlations. Not statistically significant coefficient between TPC and ABTS suggest that the antioxidant activity of these extracts is, besides phenolic content, dependent on the presence of other compounds that exhibit different antioxidant modes of action. Results obtained on zebrafish embryos/larvae showed a protective effect of *D. spinosus* extracts against H_2O_2-induced oxidative stress, thus further confirming the high antioxidant potential of methanol and methanol:DMSO extracts. Overall, these findings indicate the potential of *D. spinosus* as a source of beneficial antioxidant agents in the functional food industry.

References

Guiry MD, Guiry GM 2021 *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 02 August 2021.

Jerković I, Cikoš AM, Babić S, Čižmek L, Bojanić K, Aladić K, Ul'yanovskii NV, Kosyakov DS, Lebedev AT, Čož-Rakovac R, Jokić S 2021 Bioprospecting of less-polar constituents from endemic brown macroalga *Fucus virsoides* J. Agardh from the Adriatic Sea and targeted antioxidant effects *in vitro* and *in vivo* (Zebrafish Model). *Marine drugs*, 19 (5), 235.

Morais MG, Silva Vaz B, Morais EG, Costa JAV 2015 Biologically active metabolites synthesized by microalgae. *BioMed Research International*. http://dx.doi.org/10.1155/2015/835761

OECD Guidelines for the testing of chemicals. Fish Embryo Acute Toxicity (FET) Test. 2013. Organization for Economic Cooperation and Development Publishing.

Safafar H, Van Wagenen J, Møller P, Jacobsen C 2015 Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine drugs*, *13*(12), 7339-7356.

Patil L, Kaliwal BB 2019 Microalga *Scenedesmus bajacalifornicus* BBKLP-07, a new source of bioactive compounds with in vitro pharmacological applications. *Bioprocess and biosystems engineering*, 42(6), 979-994.

INCREASING IRRADIANCES INCREASE ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND AFFECT PHOTOSYNTHESIS OF ECONOMICALLY IMPORTANT MACROALGA SEA GRAPES *Caulerpa lentillifera*

L. E. Stuthmann[1*], R. Achuthan[1,2], K. Springer[2], A. Kunzmann[1]

[1] Leibniz Centre for Tropical Marine Research, Fahrenheitstraße 6, 28359 Bremen, Germany
[2] Marine Botany, University of Bremen, Bibliothekstraße 1, 28359 Bremen, Germany
E-mail: lara.stuthmann@leibniz-zmt.de

Introduction

Caulerpa lentillifera is a green, macroalga of increasing economic interest, harvested as an aquaculture crop in several Asian countries (Chen et al. 2019). The alga is low-light adapted and its out-door culture takes place in shaded ponds (~ 50 μmol photons m^{-2} s^{-1}). In Europe, sea grapes are known as `green caviar´, due to the special texture of their edible fronds. Sea grapes contain significant amounts of polyphenols and vitamin C, resulting in high antioxidant activities (AOA, e.g. Matanjun et al. 2009; Nguyen et al. 2011)vitamin C, α-tocopherol, dietary fibers, minerals, fatty acid and amino acid profiles of three tropical edible seaweeds, Eucheuma cottonii (Rhodophyta. Antioxidants can avoid cell damage by functioning as scavengers of reactive oxygen species (ROS) and are therefore recognized as essential parts in human diets to counteract the metabolic syndrome (John et al. 2020). In plants ROS form as a byproduct of photosynthesis, especially under excessive light introducing photooxidative stress (Pospíšil 2016). In this study controlled light-stress is applied as a tool to trigger the antioxidant potential of sea grapes, while sustaining the healthy green color of the fresh sea grape fronds.

Material and Methods

Sea grapes were held in 2 L beakers (artificial seawater) in a water bath under irradiances of 50, 100, 200, 400 and 600 μmol photons m^{-2} s^{-1} for 21 days. Samples for measurements of AOA (ABTS assay, following Re et al. 1999), total phenolic content (TPC, Folin–Ciocalteu assay, following Ainsworth and Gillespie 2007), chlorophyll a and b content, maximum quantum yield of PSII (F_v/F_m) and pictures for color analysis were taken regularly over the run of the experiment. Data processing of chlorophyll content and color measurements (for a later processing using the image analysis software ImageJ) is still underway.

Results

Sea grape fronds were significantly enriched in AOA and TPC when exposed to excessive irradiances (≥ 200 μmol photons m^{-2} s^{-1}), compared to usual culture irradiances. However, AOA and TPC content of sea grapes were negatively correlated with F_v/F_m (Fig. 1 C), implying an overall decrease of the algae's physiological state with increasing antioxidant potential. Additionally, a partial de-coloration of sea grape fronds under irradiances ≥ 200 μmol photons m^{-2} s^{-1} has been observed over the experimental run.

Discussion

The results confirm, that antioxidative compounds accumulate in sea grape fronds under light-stress. Light irradiances ≤ 100 μmol photons m^{-2} s^{-1} seem to be too low to increase AOA and TPC significantly (Kang et al. 2020), however irradiances of ≥ 200 μmol photons m^{-2} s^{-1} more than doubled AOA and TPC, respectively. Decreasing F_v/F_m values and de-coloration, possibly induced by chloroplast relocation movement (Horstmann 1983) or chlorophyll degradation (Guo et al. 2015), might have a negative impact on the product quality. Therefore, carefully controlled higher irradiances could be applied as a post-harvest treatment in order to keep a balance between triggering AOA and sustaining healthy appearance of sea grape fronds.

(Continued on next page)

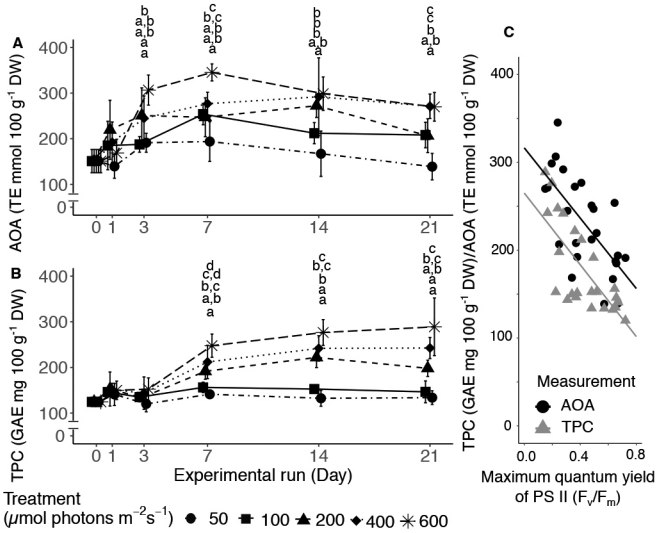


Fig. 1. (A) Antioxidant activity (AOA, as Trolox equivalents, TE) and (B) Total phenolic content (TPC, as Gallic acid equivalents, GAE) of *Caulerpa lentillifera* exposed to different irradiances. Data are means ± SD. Different letters indicate significant differences between treatments (One-factor ANOVA, post-hoc test, $p<0.05$). (C) Correlation of F_v/F_m with AOA and TPC (Kendall rank correlation, AOA: $R^2 = -0.46$, $p<0.001$; TPC: $R^2 = -0.55$, $p<0.001$). Presented are data of experimental days 3–21.

References

Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2:875–877. https://doi.org/10.1038/nprot.2007.102

Chen X, Sun Y, Liu H, et al (2019) Advances in cultivation, wastewater treatment application, bioactive components of *Caulerpa lentillifera* and their biotechnological applications. PeerJ 7:e6118. https://doi.org/10.7717/peerj.6118

Guo H, Yao J, Sun Z, Duan D (2015) Effect of temperature, irradiance on the growth of the green alga *Caulerpa lentillifera* (Bryopsidophyceae, Chlorophyta). J Appl Phycol 27:879–885. https://doi.org/10.1007/s10811-014-0358-7

Horstmann U (1983) Cultivation of the green alga, *Caulerpa racemosa*, in tropical waters and some aspects of its physiological ecology. Aquaculture 32:361–371

John OD, Du Preez R, Panchal SK, Brown L (2020) Tropical foods as functional foods for metabolic syndrome. Food Funct 11:6946–6960. https://doi.org/10.1039/d0fo01133a

Kang L-K, Huang Y-J, Lim W-T, et al (2020) Growth, pigment content, antioxidant activity, and phytoene desaturase gene expression in *Caulerpa lentillifera* grown under different combinations of blue and red light-emitting diodes. J Appl Phycol 32:1971–1982. https://doi.org/10.1007/s10811-020-02082-8

Matanjun P, Mohamed S, Mustapha NM, Muhammad K (2009) Nutrient content of tropical edible seaweeds, *Eucheuma cottonii, Caulerpa lentillifera* and *Sargassum polycystum*. J Appl Phycol 21:75–80. https://doi.org/10.1007/s10811-008-9326-4

Nguyen VT, Ueng J-P, Tsai G-J (2011) Proximate Composition, Total Phenolic Content, and Antioxidant Activity of Seagrape (*Caulerpa lentillifera*). J Food Sci 76:C950–C958. https://doi.org/10.1111/j.1750-3841.2011.02289.x

Pospíšil P (2016) Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress. Front Plant Sci 7:1–12. https://doi.org/10.3389/fpls.2016.01950

Re R, Pellegrini N, Proteggente A, et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

ULTRA-SENSITIVE SINGLE-CELL-ICP-MS MEASUREMENTS FOR STUDYING TiO_2 NANOPARTICLES INTERNALIZATION IN CELL LINES FROM SEA-BASS (*Dicentrarchus labrax*) AND CLAMS (*Ruditapes philippinarum*)

C. Suárez-Oubiña*[1], A. Moreda-Piñeiro[1], P. Herbello-Hermelo, P. Bermejo-Barrera[1], N. Mallorca[2], M. Vázquez[2], S. Cabaleiro[2]

[1] Trace Element, Spectroscopy and Speciation Group (GETEE), Strategic Grouping in Materials (AEMAT), Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain
[2] Ctr Tecnol Cluster Acuicultura, Cluster Acuicultura, Punta Couso S-N, Ribeira 15965, Spain
*cristiansuarez.oubina@usc.es

Introduction

Because of their outstanding physicochemical properties, metal and metal oxide nanoparticles (NPs) have been widely used. These particles will be released into the environment as a result of large-scale manufacture and consumption. The transfer and magnification of NPs in the trophic chain is one of the most serious risks associated with their use.

As a result, data are required to assess the environmental risk of these emerging pollutants, particularly in terms of uptake and biological impacts. In vitro experiments can reveal how these NPs are capable of being internalised in cells, which is a necessary step before determining bioaccumulation in fish and molluscs, as well as bioavailability in humans.

Single-cell inductively coupled plasma – mass spectrometry (SC-ICP-MS) has opened a new area of research, allowing for the sensitive detection of metals in single biological cells at ultra-low levels (attograms per cell) not possible with existing instrumental techniques. As a result, the aim of this study was to observe if SC-ICP-MS could be used to analyse TiO_2 NPs inside kidney cells from sea-bass (Dicentrarchus labrax) and clams (Ruditapes philippinarum) as a prelude to bioaccumulation investigations of TiO_2 NPs in these cultured species.

Methods and results

The internalization of TiO_2 NPs in kidney sea-bass and clam cells was assessed by SC-ICP-MS after they were exposed to TiO_2 NPs (5, 25 and 45 nm) at different NPs concentrations and exposure times.

Several SC-ICP-MS parameters were investigated in order to establish a unique, high-sensitivity, and accurate approach for determining TiO_2 NPs in single cells from aquaculture products. Multi-cell coincidence was avoided by optimizing cell concentration and dwell time.

The effect of washing stages (1% PBS) on removing non-internalised TiO_2 NPs and/or TiO_2 NPs deposited onto the cell surface was also studied. This approach helps to avoid high dissolved backgrounds, potential interferences, and excess particles that could hide the signal emitted by single cells. The rate of internalization of TiO_2 NPs of various size distributions was assessed using optimized SC-ICP-MS conditions based on concentration and exposure periods tested. The findings are now being used to develop TiO_2 NP bioaccumulation assays in cultured sea bass and clams.

Acknowledgments

The authors wish to acknowledge the financial support of the Ministerio de Economía y
Competitividad (project INNOVANANO, reference RT2018-099222-B-100), the European Union (Interreg POCTEP, project ACUINANO, reference 07-12-ACUINANO_1_E), and the Xunta de Galicia (Grupo de Referencia Competitiva, grant number ED431C2018/19; and Program for Development of a Strategic Grouping in Materials – AEMAT, grant number ED431E2018/08).

DETERMINATION OF THE QUANTITY OF SPERM REQUIRED FOR THE FERTILIZATION OF NORTHERN PIKE (*Esox lucius*) EGGS DURING HATCHERY BREEDING

T. Szabó[1*], J. Molnár[1], T. Müller[2], M. Nyitrai[1], G. Tóth[1], Z. Ugrai[3], K. Szabó[4] and B. Urbányi[1]

[1]Department of Aquaculture, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly Str. 1., H-2100 Gödöllő, Hungary
[2]Department of Freshwater Fish Ecology, Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly Str. 1., H-2100 Gödöllő, Hungary
[3]Danube Anglers Association of Ráckeve, Hungary
[4]Dinnyés Fish Farm, Dinnyés, Hungary
*E-mail: Szabo.Tamas@uni-mate.hu

Introduction

Northern pike (*Esox lucius*) is a piscivorous fish with a broad geographical and environmental range. It plays an important ecological role as a predator, and is highly regarded as a sport fish. To satisfy the needs of anglers, pike fry are stocked in natural waters. The number of fry required can only be produced in controlled hatchery conditions.

Since pike do not spawn in confinement (Huet 1986), ovulation and spermiation is induced by hormonal treatment (Szabó 2001). Eggs can easily be stripped after ovulation, however, only a small amount of milt can be stripped from the male fish. To enhance sperm yield, the testes are surgically removed and sperm is obtained by squeezing the organs through cheesecloth. This method requires sacrificing the males, so it is important to determine the amount of milt that is necessary for successful fertilization. For the above reasons, we aimed to determine the amount of sperm required to fertilize northern pike eggs under hatchery conditions.

Materials and methods

Experiments and routine hatchery work were conducted concurrently during the northern pike breeding season in the hatcheries of the Dinnyés Fish Farm and the Danube Anglers Association of Ráckeve in Hungary. Basic data of northern pike breeding at the two hatcheries are summarized in Table 1.

Both females and males received an injection of a crude preparation of dried carp pituitary at a dose of 3.5 mg kg^{-1} body weight (BW). Pituitaries were administered in a 2.5 % aqueous dispersion of Carbopol resin (Szabó 2006). Injections were applied intraperitoneally at a volume of 0.5 ml kg^{-1} BW. Stripping of females was conducted on the fourth day after injection. From the male fish, the testes were surgically removed and the sperm was obtained by squeezing the organs through cheesecloth. The number of males used for fertilization procedures was approximately 20 percent of the number of the ovulated females. The testes from different males were collected in one beaker. Milt extracted from this mixture of testes was used to fertilize the eggs from each female.

We aimed to determine the optimal amount of sperm needed to fertilize northern pike eggs. Our goal was to fertilize large batches of eggs in the tests so that the results would be informative for hatchery practices. In both farms, three egg batches from three females were used for the experiments (Figure 1). The sperm : egg ratios tested were as follows: 10 ml, 5 ml, and 2.5 ml sperm for 1,000 grams of eggs, respectively. Treatments were performed in duplicate.

For the experiments, we tried to select relatively large females from which at least 1,000 g of eggs could be gained. Egg batches of 300 g were weighed from each female into three bowls. For each batch, 3.0 ml, 1.5 ml, and 0.75 ml of sperm were used, respectively. The dry gametes were then mixed gently and activated by adding 100 ml of water. After fertilization, eggs were placed into Zuger jars for incubation at 11±1.0°C. Fertilization percentages were established 18 hours after fertilization, when eggs were in morula stage.

Results and conclusion

It was found that fertilization of 1,000 g of northern pike eggs with 5 ml and 10 ml sperm gave similar results (Figure 1). If the eggs were fertilized with 2.5 ml milt, the fertilization rate was somewhat reduced. However, if the number of males available for breeding is limited, a quantity of 2.5 ml of semen may be sufficient to fertilize 1000 g of eggs without a significant reduction in the fertilization rate.

(Continued on next page)

Place of propagation:	Time of treatment / WT	Time of stripping / WT	Amount of stripped eggs	Average FR
Dinnyés	3.24.2019 / 12 ºC	3.28.2019 / 10 ºC	18 kg	71 %
Ráckeve	3.21.2019 / 8 ºC	3.25.2019 / 12.4 ºC	30 kg	75 %

Table 1: Basic data of northern pike breeding at the Dinnyés and Ráckeve hatcheries. WT: water temperature, FR: fertilization rate

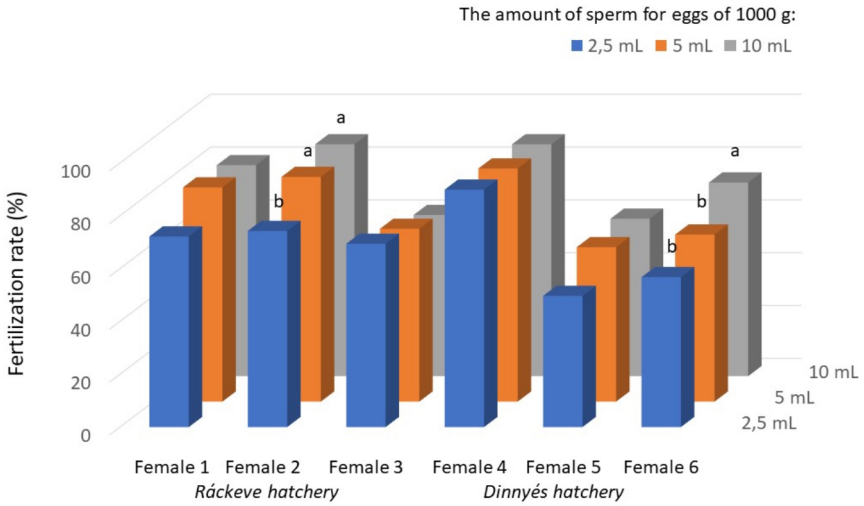


Figure 1: Fertilization rate data from three different "sperm : egg" ratios. Fertilization rates between egg batches fertilized with different amount of sperm were similar for females 1, 3, 4 and 5. In case of female 2 and 6, means that do not share a letter are significantly different (Kruskal-Wallis test, $p < 0.05$).

Acknowledgements

This work was supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. and by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Program 2020, National Challenges Subprogram (TKP2020-NKA-16).

References

Huet, M. 1986. Textbook of fish culture: breeding and cultivation of fish, 2nd edition. Fishing News Books, London.

Szabó, T. 2001. Sustained release vehicle improves the quality of northern pike (*Esox lucius*) eggs obtained by hormonally induced ovulation. North American Journal of Aquaculture, 63: 137-143.

Szabó, T. 2008. Use of Carbopol resin for carp pituitary administration improves the fertilization percentage of northern pike (*Esox lucius* Linnaeus) eggs in commercial hatcheries. Hydrobiologia, 601: 91–97.

B CELLS FROM THE RAINBOW TROUT ADIPOSE TISSUE SPECIFICALLY RESPOND TO PERITONEAL ANTIGENS

Rocio Simón[1], Alba Martín-Martín[1], Esther Morel, Patricia Díaz-Rosales, Carolina Tafalla*

Animal Health Research Center (CISA-INIA-CSIC), Valdeolmos 28130, Madrid, Spain
Email: tafalla@inia.es
[1] These authors equally contributed to this work

The immune response of the adipose tissue (AT) has been neglected in most animal models until recently, after investigations in human and mice linked obesity to chronic inflammation, highlighting the immune nature of this tissue. Thus, in mammals, the AT is increasingly seen as an active immune site, playing important roles in systemic and peritoneal responses. Despite this, in teleost fish, only a few studies have addressed the immune role of the AT. These studies revealed significant changes in the levels of transcription of a wide range of immune factors produced by the AT in response to diverse intraperitoneally delivered stimuli, and demonstrated the presence of B cells within the tissue. In the current study, we have continued the characterization of the immune role of rainbow trout (*Oncorhynchus mykiss*) AT, focusing on how the different B cell populations, that are present in the AT, react to an intraperitoneal stimulation. Initially, the B cell populations present in this immune tissue were characterized in comparison to B cells from other sources. As occurs in other rainbow trout tissues, IgM^+IgD^+, IgM^+IgD^- and IgD^+IgM^- B cell subsets were identified in the AT. Interestingly, AT IgM^+IgD^- B cells showed a transcriptional profile that seems to correspond to cells that have already committed to plasmablasts/ plasma cells, being this profile much more pronounced than that of blood IgM^+IgD^- B cells. Consequently, the IgM-secreting capacity of AT cells is significantly higher than that of blood B cells. Additionally, we established how these B cell subsets responded when rainbow trout were intraperitoneally injected with a model thymus independent antigen, TNP-LPS. Our results demonstrate that AT B cell differentiate to plasmablasts/ plasma cells that secrete specific IgMs against the antigen, as happens in the peritoneal cavity, the spleen, the head kidney and in peripheral blood. Although the presence of these antigen-specific IgM secreting cells was more abundant in the peritoneal cavity, these differentiated B cells were detected in the AT for long time periods at levels similar to those of spleen and head kidney. Our results provide new evidence regarding the immune role of the teleost AT, demonstrating that it functions as a secondary lymphoid organ that promotes immunity to peritoneal antigens.

CHARACTERIZATION OF B CELL RESPONSES IN THE RAINBOW TROUT (*Oncorhynchus mykiss)* GILL

J. German Herranz-Jusdado, E. Morel, P. Díaz-Rosales and C. Tafalla*

Animal Health Research Center (CISA-INIA-CSIC), Madrid, Spain
E-mail: tafalla@inia.es

Introduction

Most teleost B cells co-express surface IgM and IgD (IgM^+IgD^+ cells) and down-regulate IgD after encountering an antigen as mammalian B cells do (becoming IgM^+IgD^- cells). In addition to these populations, IgD^+IgM^- B cells have been detected in catfish (*Ictalurus punctatus*) peripheral blood and in different rainbow trout (*Oncorhynchus mykiss*) mucosal surfaces such as gills or gut. Thus, in both rainbow trout gills and gut, IgM^+IgD^+ cells are rare, with most B cells expressing just one surface Ig. In this context, in the current study, we wanted to further characterize these B cell populations found in the rainbow trout gill by determining their response to immune stimulation. Effects on B cell viability, Ig secretion and expression of CCR7 and CD38 were studied. CCR7 has been shown to condition the response of lymphocytes in peripheral immune tissues, whereas CD38 is known to be associated with an increased Ig secreting capacity.

Materials and methods

Gill leukocytes were isolated from adult rainbow trout by Percoll gradients. Leukocytes were then exposed *in vitro* to heat-inactivated viral hemorrhagic septicemia virus (iVHSV), to bacterial lipopolysaccharide (LPS) or were left untreated. After 48 h of incubation, the percentage of B cells from each subset was established through flow cytometry, also studying the surface expression of CCR7 or CD38 in each group through the use of specific rainbow trout antibodies. Finally, the number of IgM-secreting cells in these cultures was also determined by ELISPOT.

Results

The percentage of IgM^+IgD^- B cells decreased in gill leukocyte cultures exposed to iVHSV but significantly increased in response to LPS. Consequently, the number of cells secreting IgM also increased in LPS-treated cultures. In contrast, the percentage of IgD^+IgM^- cells was not significantly affected by stimulation. Nevertheless, the percentage of IgD^+IgM^- cells expressing CCR7 or CD38 significantly increased in response to both stimulations, whereas CCR7 expression decreased in IgM^+IgD^- B cells upon LPS stimulation.

Discussion and conclusions

Rainbow trout IgM^+IgD^- and IgD^+IgM^- B cells from the gill respond differently to immune stimulation. Our findings contribute to a deeper understanding of how fish B cells are regulated at mucosal surfaces. From a practical point of view, this information will be helpful for the future optimization of vaccines that are delivered through mucosal routes.

Acknowledgements

This work was supported by the European Research Council (ERC Consolidator Grant 2016 725061 TEMUBLYM) and by the *Comunidad de Madrid* (grant 2016-T1/BIO-1672).

GROWTH PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*) FED LOW FISHMEAL ORGANIC DIETS

A. Tampou[1*], S. Andreopoulou[1], I., Nengas[2], A. Vasilaki[2], I.T. Karapanagiotidis[1], E. Mente[1]

School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, N. Ionia
Magnisias,Greece
School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, N. Ionia
Magnisias,Greece
School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, N. Ionia
Magnisias,Greece
[1] School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessalyx, Fytoko Street, GR-38446, Volos, Greece
[2]Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Center for Marine Research, 46,7 Km Athens Sounio ave., Anavyssos, GR-19013, Attiki, Greece
E-mail: tampou@uth.gr

Introduction

Organic aquaculture seems to be a very well promising sector in the global ecology and economy (FiBL & IFOAM, 2020). Organic aquaculture reflects a specific production approach driven by the growing public interest in sustainable utilization of resources (Mente et al., 2011, 2012, 2019; Lembo & Mente, 2019). In the context of sustainability, the search for ingredients that are characterized by low FIFO ratios is of much interest. The aim of this study is to examine the growth performance of sea bream (*Sparus aurata*) fed diets with a low FIFO mix of ingredients for organic aquaculture.

Materials & Methods

The experimental trial was conducted at the Department of Ichthyology and Aquatic Environment, University of Thessaly. Sea bream individuals with initial mean weight 6.87±0.07g, were acclimated to the laboratory conditions for 15 days before they were randomly distributed into 250l tanks. Each diet was allocated to triplicate groups. The fish were fed three times per day *ad libitum* and the rearing water quality (21° C, DO>6.5 mg/l) remained stable during the experiment. Four experimental diets were formulated to be isonitrogenous (48.3%), isolipidic (16.2%) and isoenergetic (21.3 KJ/g). The control diet was designed to simulate the composition of a commercial diet incorporating organic fishmeal trimmings, organic fish oil and soybean meal (organic and concentrate). A low FIFO mixture replaced fishmeal at three different inclusion levels, 20, 25 and 30% and three experimental diets were formulated (LFiFo20, LFiFo25 and LFiFo30, respectively). The low FIFO mixture consisted of organic processing fish trimmings, bacterial protein and yeast protein. The duration of the experiment was 60 days. Fish were weighted individually at the beginning and end of the experimental trial under anaesthesia. Feed consumption was recorded daily to evaluate accurately values for feed utilization.

Results

Fish fed the replacement diets (LFiFo20, LFiFo25, LFiFo30) had similar ($p>0.05$) weight gain and SGR values that were significantly increased ($p<0.05$) compared to control group of fish. Fish fed the LFiFo20, LFiFo25 and LFiFo30 had also similar ($p>0.05$) FCR values that were significantly decreased compared to the control group. Voluntary feed intake and survival were statistically unaffected among the diets ($p>0.05$).

Table 1. Growth performance of gilthead sea bream (*S. aurata*) fed organic low fishmeal diets.

	Control	LFiFo20	LFiFo25	LFiFo30
Final weight (g)	14.65±0.46^a	19.44±0.48^b	19.86±0.49^b	19.37±0.45^b
Weight gain (g)	7.58±0.11^a	12.37±0.57^b	12.75±0.35^b	12.14±0.97^b
SGR (%/day)	1.21±0.02^a	1.68±0.07^b	1.69±0.04^b	1.63±0.08^b
FCR	1.28±0.07^a	1.05±0.02^b	0.99±0.03^b	0.99±0.04^b
Voluntary Feed intake (% BW/day)	1.49±0.10^a	1.63±0.09^a	1.56±0.09^a	1.51±0.11^a
Survival (%)	97.5±1.44^a	100±0.0^a	89.76±7.73^a	96.42±1.79^a

Values are presented as means ± standard error. Means sharing the same superscript are not significantly different from each other (P>0.05)

(Continued on next page)

Discussion

Since 2000, there has been an increasing demand for seafood farmed according to certified organic standards, notably in European countries (FGM, 2020). It follows, therefore, that like the terrestrial organic livestock production sector, the onus is on the aquatic nutritionist to formulate an organic feed close to the feed that each fish species is consuming in their natural environment. In the present study, a low FIFO mix with innovative ingredients and organic fishmeal trimmings improved the FCR and enhanced the growth performance of *S. aurata*. Microbial protein is an alternative source of high-quality protein able to replace animal protein like fishmeal in livestock nutrition and aquaculture (Matassa et al., 2016; Olmos et al. 2020). Furthermore, yeast meal contain appreciable crude protein (about 40–55%), and other bioactive components beneficiary to fish growth and development (Agboola et al., 2020)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 results suggest that wild sourced organic fishmeal trimmings can be successfully replaced by innovative ingredients of low FIFO that are based on microbial protein and yeast meal.

Future research using novel feed ingredients to replace fish meal in organic aquaculture should also take into account the quality of the raw ingredients, their process conditions and the fillet nutritional quality.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 Innovation Action, FutureEUAqua, under grant agreement No 817737.This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

References

Agboola, J. O., Øverland, M., Skrede, A., & Hansen, J. Ø. (2020). Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. Reviews in Aquaculture, September.

FiBL&IFOAM – Organics International (2020): The World of Organic Agriculture, Statistics and Emerging Trends 2020, p.26

FGM, Federation of Greek Maricultures (2020). Aquaculture in Greece 2020: Annual report, p.6

Matassa S., Boon, N., Pikaar, I., Verstraete W. (2016) Microbial protein: future sustainable food supply route with low environmental footprint, Microbial biotechnology, 9 (5), 568-575.

Lembo, P.and Mente, E. (2019). Organic aquaculture impacts and future developments. Springer -Nature Publishers. ISBN 978-3-030-05602-5

Mente, E. Jokumsen, A., Carter, C., Tacon A., Antonopoulou, E. (2019). Nutrition in relation to organic aquaculture: sources and strategies. Chapter 9 in: Organic aquaculture impacts and future developments (Lembo, P and Mente, E. (Editors), Springer-Nature Publishers.

Mente, E., Stratakos, A. Boziaris, I.S., Kormas, K.A., Karalazos, V., Karapanagiotidis, I.T., Catsiki, A.V., and Leondiadis, L. (2012): The effect of organic and conventional production methods on sea bream growth, health, and body composition; a field experiment. Scientia Marina.

Mente, E., Karalazos, V., Karapanagiotidis, I.T., Pita, C. (2011): Nutrition in organic aquaculture: An inquiry and a discourse. AquacultureNutrition 17, 798 – 817.

Olmos, J., Acosta, M., Mendoza, G., & Pitones, V. (2020). Bacillus subtilis, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. Archives of Microbiology, 202(3), 427–435.

RISK ASSESSMENT OF VIRAL ENCEPHALOPATHY AND RETINOPATHY INTRODUCTION AND SPREAD IN MEDITERRANEAN SEABASS FARMS

Saraya Tavornpanich*, Alain Le Breton, Nadia Chérif, Anna Toffan, Valentina Panzarin, Francesco Pascoli, Snježana Zrnčić, Panos Varvarigos, Bernardo Basurco, Hosam Saleh, Mona Dverdal Jansen, and Edgar Brun

Department of Aquatic Animal Health and Welfare
Norwegian Veterinary Institute
Ås, Norway 1431
saraya.tavornpanichetinst.no

The objective of this study was to use risk assessment to determine the biosecurity risk associated with Viral encephalopathy and retinopathy (VNN) introduction and spread in Mediterranean seabass production, and to identify the control measures to manage the risks.

Biosecurity plans for prevention of introduction and further spread of disease pathogens involve knowledge on risk assessment of disease hazards in the region, likelihood of introducing the infectious agent into the production system, epidemiology and transmission routes, critical points for introduction and control. VNN was identified as the most important disease of seabass in the western, central, eastern and southern Mediterranean areas in terms of production and economic impact due to factors such as the high mortality and morbidity by literature, survey, and expert opinion.

A group of 10 experts consisting of fish health specialists, veterinarians, biologists, and epidemiologists were asked to provide their opinion regarding disease hazards, likelihoods of disease introduction and spread in the Mediterranean seabass. Expert knowledge elicitation (EKA) approach was used to elicit expert opinion and perform the risk assessment. Risk matrix was used to present the overall risk estimates by integrating the numerical scores for the likelihood of disease introduction and the economic consequences. The experts to identify the points at which VNN introduction and spread could occur and be prevented. At each action point, experts gave a weight for potential biosecurity measures concerning its feasibility and effectiveness for controlling VNN

The main risk pathways differ by type of production for which the likelihoods of introduction are similar for hatchery and pre-growing, but different from the likelihoods for on-growing. Intake of water, live fish and eggs, vehicle transporting live fish, human, equipment, and high-risk purchasing were identified by the experts as the risk pathway for all types of production. This illustrates a clear recommendation to encourage focusing on introductory risk.

The economic consequences of VNN depend on the type of production of the facility. The consequences of VNN introduction were regarded most devastating in economic terms for hatcheries and pre-growing units and the risk estimate was in general high or very high for these productions. For on-growing the risk estimates were regarded medium to high. Measures to comply with introduction entail requirements of reliable health certificates and quarantining newly acquired fish upon arrival. The measures for disease management entail removing dead fish daily, preventing direct contact between quarantined fish and the other fish on the facility, separate water flow between quarantined fish and the other fish on the facility, and follow-up investigation of disease outbreaks.

TOWARDS PRECISION AQUACULTURE: A HIGH PERFORMANCE, COST-EFFECTIVE IOT APPROACH

Rafael R. Teixeira, Juliana B. Puccinelli, Vinícius M. Oliveira

Fundação Universidade Federal do Rio Grande
rafael@furg.br
julianapuccinelli@furg.br
vinicius@ieee.org

Introduction

Activities in aquaculture have seen a global annual growth rate of 7%, highlighting their increased relevance to animal protein production worldwide. As a result, the North and South Atlantic production has received considerable government and policymakers attention from the '90s onwards. For instance, Brazil's government stimulus and private investments helped increase the *per capita* fish consumption in this country, thus leading to a net production of 707 thousand tons in 2015. Such tailored interventions have taken Brazil to 12th place in the world's aquaculture league. On a global scale, aquaculture fish production reached 82.1 million tons worldwide in 2018 according to an FAO report. The substantial social and economic impacts of aquaculture farming cannot be underestimated as a catalyst for new employment posts throughout the whole value chain. Such benefits reach the small producers located on both sides of the Atlantic, including impoverished areas in low-to-middle income countries (e.g. communities in South America and Africa). Given the demand rise for high-quality animal protein to feed the world population, marine aquaculture is under tremendous pressure to accelerate production in a responsible, eco-friendly manner. A crucial enabler is the technology for real-time monitoring of aquatic organisms in an operational setting. Rigorous control of the environment's physical-chemical parameters is the first step towards an economically viable production. Unbalanced parameters can directly affect species' growth, generate pathogens and environmental conditions that may hinder animal reproduction and healthy growth. Aquaculture is an unpredictable biological system in which adverse outcomes cannot be ignored in the management of a production system. The European Commission adopted the term *Aquaculture 4.0* to contemplate the transfer of new technologies investigated in other industries, including Internet of Things (IoT), distributed computing, data analytics and artificial intelligence. The Internet of Things (IoT) is a rapidly growing technological field, providing social and economic benefits for all geographical areas but particularly low-cost technology delivered to low-to-middle income countries. For example, traditional water monitoring in aquaculture follows a very laborious and time-consuming procedure whereby essential variables are measured. The high cost of the needed human resources severely limits the monitoring and control of the aquaculture facilities.

IoT-driven monitoring and control in aquaculture is a challenging scenario in itself because of a number of constraints:

1. Site area: anything 5 and 30 hectares;
2. Difficult access (boats or off-road vehicles);
3. Power supply: none in open-water, none to limited in land-based facilities
4. Communication coverage: limited to none;
5. Climate and adverse weather conditions;
6. Lack of reliable and suitable monitoring technology;

Aquaculture 4.0 offers tools that can be leveraged and applied to level up production systems' quality and effectiveness. Previous limitations can be overcome when reliable technologies monitor and identify any undesired condition or event of interest learnt from data. A key question to ask is the following one: *how feasible is the design and deployment of a water quality sensor network that communicates data to an online platform for real-time visualisation and user decision making?*

AquaGreen Design

In order to answer that question we propose the *AquaGreen* system, an end-to-end IoT solution comprising a functional set of sensor nodes, an embedded network gateway, a cloud server software and a mobile app for real-time data visualization.

Our solution is based on a network architecture divided in a local and an external layer. The local layer addresses the communication from sensor nodes to the gateway over a Low Power Wide Area Network (LPWAN). On this layer, nodes sample specialised water quality sensors. Collected sensor data are encoded in a LoRa packet and transmitted to an in-range gateway node. Received sensor data (encoded in a JSON template), are forwarded via HTTP to an external

(Continued on next page)

cloud server. In the external layer Gateway nodes receive external connectivity either through an internet-connected WiFi router or cellular network, whichever is present at the aquaculture facility. In a few remote sites where there is limited to no internet access,it is anticipated that the gateway node enters into a delay tolerant network mode. This particular DTN setup requires end-user intervention to decide how to forward the stored data through some type of device-to-device transfer data off-loading via a smartphone cellular interface. In this work, gateway nodes communicate sensor data to external domain servers located in a commercial cloud infrastructure(e.g. AWS EC2 instances). A commercial ISP provides internet connectivity to the gateway node. The external cloud server offers a customised sensor data API that receives JSONformatted data and stores them into a NoSQL database for improved scalability in volume handling. The server API also provides endpoints for clients to consume the stored sensordata using a customised scheme with a regular expression scheme for data searching.

Implementation and results

LoRaWAN is a widely adopted solution in IoT networks due to its capability to connect to a wide area with many devices and still offer a low power communication. In this setup, LoRa is used as the physical layer protocol of a LPWAN network. A pair of TTGO LoRa32 v1.0 development boards were used to implement the sensor nodes. Each board includes an ESP32 MCU, an SX1276 LoRa Transceiver, a 3dBi antenna and a 0.96inch OLED display. The system has a JST battery plug and a battery charging unit. A Li-ion 3.7V battery powers up the sensor nodes. The sensor nodes also have a DS18b20 temperature sensor connected to monitor water temperature. The gateway node uses the same development board, but the temperature sensor is not attached and it is powered by a wall power outlet.

Initially, the implementation would be tested at FURG's Marine Aquaculture Facility (FURG-EMA) but due to local access restrictions to the aquaculture facility imposed in February 2021 by the worsening of the COVID-19 pandemic in Brazil the planned experiment scope was limited. The *AquaGreen* system had no other option but to be tested in an outside swimming pool. This lab setup still offered rigorous conditions to validate technical design assumptions.

To obtain the maximum battery life, the sensor node is kept in deep sleep mode most of the time. During this period, the temperature sensor is also powered down. On deep sleep mode, the overall system power consumption is reduced to around 10μA. The chosen system duty cycle contemplates that every 10 minutes, the sensor node wakes up from deep sleep to sample the sensors. The sample data is encoded in a LoRa packet and sent to the gateway node. The LoRa packet holds four key-value pairs of data samples (i.e. real-clock timestamp, sensor node id, instantaneous water temperature sensor sample and battery voltage level). Due to local regulations, only a maximum of 1\% duty cycle is permitted in LoRa communication. The system can send a packet (30 bytes payload plus 15 bytes header) every 10 seconds. To comply with the local rules, the actual packet rate is one every 10 minutes. As the final setup step, the sensor node was placed above the water in the swimming pool but having the temperature sensor probe under the water in a 1.2 water depth approximately.

The *AquaGreen* system ran for a non-stop period of 66 hours up to the point of fully discharging batteries in the sensor nodes. A total of 392 successful sensor readings were stored on the database during this time interval. Unfortunately, a packet loss of 2.3\% has been observed. Although this has not impacted the application, it calls for further investigation into whether a reliable communication protocol should be used. Our experimental results shed some light on the feasibility of a water quality sensor network that communicates data to an online platform for real-time visualisation and user decision making. This work presented preliminary results of a real-world pilot in a challenging environment for sensor and actuator networks. In addition, design principles and best practices for IoT aquaculture application development were presented. However, there are still many improvements to be made to the overall system.

HEALTH-PROMOTING EFFECTS OF DIETARY B-GLUCANS AND CURCUMIN ON GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES FOLLOWING INTESTINAL INFLAMMATION

C. Teixeira[1,2,3,*], D. Peixoto[1,3], M. Hinzmann[1], P. Santos[1,3], I. Ferreira[1,3], G. Pereira[2], J. Dias[2], B. Costas[1,3]

[1] CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal.
[2] SPAROS Lda., Olhão, Portugal.
[3] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.
*E-mail: cteixeira@ciimar.up.pt

Introduction

The gilthead seabream (*Sparus aurata*) production has scaled up in the last few years due to its high demand and commercial value (Ashry et al., 2021). The intensification of aquaculture can translate into high stocking densities for farmed fish, which usually results in stressful conditions leading to reduced growth, immunosuppression, higher fish mortality, and huge economic losses (Ashry et al., 2021). Therefore, improving fish health and disease resistance of cultured fish is crucial (Župan et al., 2018). Numerous feed additives have proved to be beneficial in eliciting fish health. For instance, β-glucans are polysaccharide immunostimulants known to stimulate growth performance, improve general health and enhance disease resistance in fish (Župan et al., 2018). Curcumin is a feed additive with growth-promoting and immunostimulant potential known to enhance growth, stimulate immunity, improve the antioxidative status, and increase disease resistance in fish (Ashry et al., 2021). The present study mainly aimed to evaluate the effects of dietary β-glucans and curcumin on gilthead seabream health condition following an intestinal inflammatory stimulus.

Materials and methods

Gilthead seabream juveniles (55.5 ± 0.5 g) were randomly distributed in 12 tanks (250 L) and maintained in a seawater recirculating system (temperature: 20.6 ± 0.5 ºC; dissolved oxygen: 6.3 ± 0.5 mg L^{-1}; salinity: 35.3 ± 0.5 g L^{-1};). Fish were fed three dietary treatments in triplicates: a practical commercial-type diet was regarded as control (CTRL), whereas the CTRL diet supplemented with β-glucans extracted from algae (GLU) or supplemented with curcumin (CUR). The feeding trial lasted for 30 days. At the end of the trial, four fish per tank (n=12) were randomly collected and samples were taken to evaluate growth and health condition. After the 30 days of feeding, a dextran sodium sulphate (DSS) induced inflammation in the intestine was performed. Thereafter, four dietary treatments were used in triplicates. A group of fish continued to be fed on the CTRL diet (CTRL), whereas the remaining groups where exposed to DSS: CTRLDSS (CTRL + DSS), GLUDSS (GLU + DSS), and CURDSS (CUR + DSS) for 6 days. At the end of the trial, four fish per tank were randomly collected and samples were taken to evaluate health and inflammatory conditions at both systemic and local (i.e. gut) levels.

Results

No changes were observed regarding growth performance at the end of both trials. After the feeding trial, no major alterations were observed in the haematological profile. While plasma anti-protease activity decreased in the GLU group compared to the CUR and CTRL groups, plasma peroxidase activity increased in GLU group compared to the CUR group. Catalase activity in the liver was improved in the CUR group compared to the GLU group. After the DSS induced inflammation, CTRL group fed with DSS increased RBC (Figure 1) and white blood cells (WBC) (Figure 2) counts compared to unstimulated fish. The GLUDSS group presented a strong response to the elevated RBC numbers induced by the DSS treatment, while CURDSS group presented a better response to the increased WBC counts induced by the DSS treatment compared to the other DSS groups.

Discussion and conclusion

The present study suggests that dietary algae β-glucans or curcumin at the levels tested did not induce major effects in the parameters evaluated at the end of the feeding trial. However, these feed additives seem to improve the fish responses to the DSS inflammatory challenge. While β-glucans helped to adjust the effects of DSS in the RBC profile, curcumin, presented a strong response against the DSS effects in the WBC profile.

(Continued on next page)

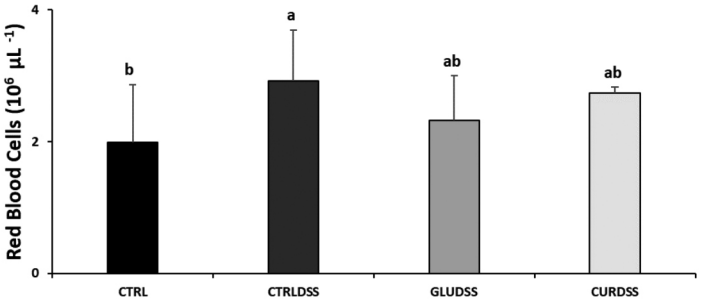


Figure 1: RBC counts after the dietary treatment with DSS. The letters indicate differences between the dietary treatments.

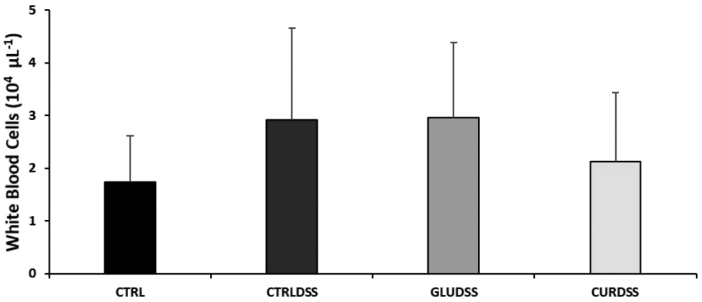


Figure 2: WBC counts after the dietary treatment with DSS. The letters indicate differences between the dietary treatments.

Acknowledgements

This work is part of project FICA_047175 supported by COMPETE 2020, CRESC Algarve 2020, Portugal 2020 and the European Union through ERDF.B. Costas and C. Teixeira were supported by FCT - Foundation for Science and Technology grants (IF/00197/2015 and PD/BDE/135541/2018), respectively.

References

Ashry, A. M., Hassan, A. M., Habiba, M. M., El-Zayat, A., El-Sharnouby, M. E., Sewilam, H., & Dawood, M. A. O. (2021). The Impact of Dietary Curcumin on the Growth Performance, Intestinal Antibacterial Capacity, and Haemato-Biochemical Parameters of Gilthead Seabream (*Sparus aurata*). Animals (Basel), 11(6). doi:10.3390/ani11061779

Župan, I., Tkalčić, S., Čož-Rakovac, R., Strunjak-Perović, I., Topić-Popović, N., Babić, S., … , Šarić, T. (2018). Biochemical parameters in the blood of gilthead sea bream (*Sparus aurata* Linnaeus, 1758) supplemented with commercially available β-glucan-based product (IMUNO-2865®). Aquaculture Research, 49(2), 786-792. doi:10.1111/are.13509

CHRONIC EXPOSURE TO NANOPLASTICS DOES NOT INDUCE HEMATOLOGICAL CHANGES IN GOLDFISH: A PILOT STUDY

M. Cánovas[1], I. Brandts[1,2], M. Mesalles[3], N. Roher[1,2], J. Pastor[3] and M. Teles[1,2]

[1]Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
[2]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
[3]Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
E-mail: mariiacr6@gmail.com

Introduction

Microplastics (MPs) and nanoplastics (NPs) pollution presents a threat to marine organisms and ecosystems (Guerrera et al., 2021). Several studies have focused on the effects of NPs on different aquatic species but most of them consist of short-term exposures, while studies concerning long-term exposures (>28 days) are scarce. In fish, health and welfare can be assessed with the analysis of relevant hematological parameters, such as hematocrit (HCT), red blood cell count (RBC) and white blood cell count (WBC). Automated analyzers are not yet routinely used to assess fish hematology parameters (Rebl *et al.,* 2021), but they represent several advantages when compared to the manual methods, such as an increased precision and exactitude of the results. The objective of the present study is to evaluate the hematological effect of a chronic exposure to polystyrene NPs (PS-NPs) in *Carassius auratus* (goldfish), using an automatic blood cell analyzer.

Material and methods

Goldfish (N=18) were exposed to 100 µg/L PS-NPs during 30 days. Blood was collected using a heparinized syringe and placed in tubes containing heparin. Some samples were discarded due to the formation of blood clots. All samples were analyzed within 1 - 2 hours from sampling. An automated laser flow blood cell analyzer (Sysmex XN1000V) with veterinary software version 3.04 and a betta version for bird blood analysis was used. HCT, RBC, WBC, hemoglobin concentration (HGB), mean corpuscular cell volume (MCV) and thrombocyte count (PLT) were counted automatically. Differential leukocyte count (lymphocyte, monocytes neutrophils and eosinophils) per 100 leukocytes were evaluated manually in blood smears stained with panoptic fast staining. All parameters followed a normal distribution, thus the Student's t-test was used to compare control with treated group.

Results and Discussion

In the present study we do not observe statistical differences between control and PS-NPs exposed group as it can be seen in Table 1.

To the best of our knowledge there are no previous available studies concerning the effects of NPs on fish hematology. However, in a previous study with MPs a significant decrease in RBC, HGB, HCT, PLT and WBC was found in juveniles of *Oreochromis niloticus* exposed to MPs during 15 days (Hamed *et al.,* 2019). Considering these previous findings, we could expect changes in blood parameters of goldfish in our study, since NPs are smaller than MPs and thus their probability to cross biological barriers and interact with cells is higher (Mattsson *et al.,* 2017). However, under the present studied conditions this was not the case, and our preliminary results show that NPs did not induce changes in any of the studied parameters. This absence of effects may be due to several differences between the studies, such as the type-size of the particle fragments, time of exposure, dynamic of the particles inside the organism, or animal age (adults *vs.* juveniles). Another limitation of the present study may be the low number of animals in each group. Further studies are needed to better understand the interaction between NPs and blood parameters in fish.

(Continued on next page)

Table 1: Hematological parameters of control and treated groups

Parameters	Control		Nanoplastics		P-value
	Mean -	n	Mean	n	
WBC (x10^3/uL)	3.05 ± 1.07	8	3.72 ± 2.10	4	0.4683
RBC (x10^6/uL)	1.43 ± 0.46	8	1.23 ± 0.51	5	0.5094
HGB (g/dL)	6.40 ± 0.48	8	6.38 ± 0.92	5	0.9593
HCT (%)	24.68 ± 3.63	8	22.96 ± 3.52	5	0.4197
MCV (fL)	143.6 ± 8.98	8	142.1 ± 7.43	5	0.7524
PLT (x10^3/uL)	23.50 ± 11.69	8	32.75 ± 10.78	5	0.2427
Differential leukocyte					
Lymphocyte (%)	78.00 ± 7.56	8	79.83 ± 2.48	6	0.5815
Monocyte (%)	8.75 ± 3.01	8	7.667 ± 2.42	6	0.4846
Neutrophils (%)	11.50 ± 6.23	8	11,67	6	0.9506
Eosinophils (%)	1.75 ± 1.58	8	0.8333	6	0.2167

Acknowledgements

This research was supported through Plan Nacional de Investigación (PID2020-113221RB-I00). MT is supported by a Ramon y Cajal contract (ref. RYC2019-026841-I) and IB is supported by a PhD grant from Generalitat de Catalunya (2018FI_B_00711).

Bibliography

Guerrera, M. C., Aragona, M., Porcino, C., Fazio, F., Laurà, R., Levanti, M., & Germanà, A. (2021). Micro and Nano Plastics Distribution in Fish as Model Organisms: Histopathology, Blood Response and Bioaccumulation in Different Organs. Applied Sciences, 11(13), 5768.

Hamed, M., Soliman, H. A., Osman, A. G., & Sayed, A. E. D. H. (2019). Assessment the effect of exposure to microplastics in Nile Tilapia (*Oreochromis niloticus*) early juvenile: I. blood biomarkers. Chemosphere, 228, 345-350.

Mattsson, K., Johnson, E. V., Malmendal, A., Linse, S., Hansson, L. A., & Cedervall, T. (2017). Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Scientific reports, 7(1), 1-7.

Rebl, A., Seibel, H., & Baßmann, B. (2021). Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. Frontiers in Veterinary Science, 8, 194.

INCREASING THE SUSTAINABILITY OF TILAPIA PRODUCTION THROUGH DIET OPTIMISATION

R. Teodósio[1,2]*, S. Engrola[1], M. Cabano[1], R. Colen[1], K. Masagounder[3], C. Aragão[1,2]

[1] Centre of Marine Sciences (CCMAR), 8005-139 Faro, Portugal
[2] Universidade do Algarve, 8005-139 Faro, Portugal
[3] Evonik Operations GmbH, 63457 Hanau-Wolfgang, Germany

*Presenting author: rteodosio@ualg.pt

Introduction

The economic viability and the environmental sustainability of the aquaculture sector is closely entangled with the optimal use of dietary protein content and the utilisation of more sustainable ingredients to produce aquafeeds. Protein is the most expensive nutrient in the diets, therefore, reducing excess dietary protein inclusion while following the ideal protein concept, has a direct positive economic and environmental impact. Supplemental amino acids help to partially reduce the dependency on intact protein sources in meeting the requirements for the most limiting amino acids. It is essential that the dietary amino acid profile is balanced so that fish growth is maximised while lowering nitrogen excretion into the environment.

Commercial diets for Nile tilapia juveniles contain high levels of plant protein sources. Soybean meal has been utilised due to its high protein content and relatively well-balanced amino acid profile. However, soy-based diets are limited in methionine and require its supplementation to fulfil fish requirements. DL-methionine (DL-Met) and calcium bis-methionine hydroxyl analogue (MHA-Ca) are synthetic sources of methionine that are commonly supplemented in fish diets. However, differences in biological efficiency as a source of methionine may arise due to structural dissimilarities.

In this context, two nutritional studies were performed to optimise growth and diet utilisation in Nile tilapia juveniles. The first study aimed to reduce dietary protein levels in diets for juvenile tilapia and to minimise diet environmental impact while maximising biological efficiency. The second study evaluated the effect of different methionine sources (DL-Met and MHA-Ca) on the metabolism and growth of tilapia.

Generating data on growth, diet utilisation and metabolism in a commercial relevant species such as the Nile tilapia is of paramount importance for the Aquaculture industry.

Materials and Methods

Nile tilapia juveniles (initial body weight ± 5.9 g) were allocated into 15 tanks and were randomly assigned one of the five diets varying in crude protein (CP) levels: 36, 34, 32, 30 and 28% (D36, D34, D32, D30 and D28, respectively). Diets were formulated to be isoenergetic and meet the minimum amino acid requirements for tilapia. Fish were fed to apparent satiety, three times a day, for 8 weeks. At the end of the growth trial, nutritional indicators were determined. Moreover, metabolic trials were performed to obtain an *in vivo* snapshot of dietary protein utilisation in fish from the higher, intermediate, and lower protein dietary treatments (D36, D32 and D28). Random fish from each dietary treatment were tube-fed with the respective experimental diet labelled with ^{14}C-amino acid mixture (AA mix). Amino acid utilisation was determined as a function of the dietary protein content.

For the second trial, tilapia (initial body weight of ± 2.3 g) were distributed into nine tanks and were randomly assigned one of the three experimental diets (REF, DLM and MHA). The REF was a basal diet with no methionine supplementation, while DLM and MHA diets were supplemented with 0.11% of DL-Met and 0.13% MHA-Ca (equal on molar basis to DL-Met), respectively. Diets were formulated to be isonitrogenous (32% CP) and isoenergetic, using only plant ingredients as protein sources. Fish were fed to apparent satiety, three times a day, for 8 weeks. At the end of the trial, nutritional indicators as well as the content of hepatic free methionine and one-carbon metabolites were determined in fish fed control and supplemented diets. After the growth trial, a time-course metabolic trial was performed to understand how the different methionine sources were absorbed and metabolised in tilapia juveniles. The DLM and MHA diets were labelled with ^{14}C-DL-Met and ^{14}C-MHA, respectively, and tube-fed to fish from the correspondent dietary treatment. The metabolic fate of the ^{14}C-DL-Met and ^{14}C-MHA was analysed at 1, 2, 3, 4 and 6 h after diet ingestion.

(Continued on next page)

Results and Discussion

In the first study, tilapia from all treatments showed similar growth performance, while feed conversion ratio (FCR) was only significantly lower in fish fed D28 than in those fed the D36 diet. Fish fed the D30 diet presented higher protein retention and lower nitrogen (N) losses than those fed the D36 diet, suggesting an improved protein utilisation. Fish from the D36 treatment presented an increased protein utilisation for energy purposes, while protein retention in the muscle was maintained at the same level as in fish fed the low-protein diets. Considering all these results, this study demonstrates that it is possible to reduce protein levels in juvenile Nile tilapia diets to 30% CP without hindering fish growth and FCR, while reducing N losses to the environment.

In the second study, DL-Met supplementation significantly increased final body weight and improved FCR and protein efficiency ratio compared with the REF diet. DLM fed fish presented the highest N gain and the lowest N losses, indicating that these fish were more efficient in retaining N than REF or MHA fed fish. Methionine from the different dietary sources appeared to follow distinct metabolic pathways; while methionine from DLM diet seems to be transsulfurated, methionine from MHA and REF diets is probably remethylated to maintain the free methionine pool. Moreover, the metabolic trial revealed that ^{14}C-DL-Met was absorbed faster and more retained than ^{14}C-MHA, resulting in a greater availability of free methionine in the tissues when fish are fed the DLM diet. In the long term, dietary DL-Met supplementation improved growth performance and N retention in Nile tilapia, reducing the environmental impact.

The optimisation of diets that promote fish growth while reducing nutrient outputs to the environment is essential to achieve high sustainability standards in the Aquaculture industry.

Acknowledgments

This work was supported by Evonik Operations GmbH (Germany) through project IsoMet, by national funds from FCT – Foundation for Science and Technology (Portugal) through project UIDB/04326/2020 to CCMAR and European Social Funds contracts IF/00482/2014/CP1217/CT0005 to SE and DL 57/2016/CP1361/CT0033 to CA.

ASSESSMENT OF MICROPLASTICS REMOVAL IN *Ostrea edulis* L. THROUGH LEGALLY REQUIRED DEPURATION FOR COMMERCIAL SHELLFISH

C. J. Thiele*[1], M. D. Hudson[1], A. C. Jensen[2] and A. E. Russell[3]

[1] Centre for Environmental Science, Faculty of Environmental and Life Sciences,
[2] Ocean and Earth Science, Faculty of Environmental and Life Sciences,
[3] Chemistry, Faculty of Engineering and Physical Sciences,
University of Southampton, University Road, Southampton SO17 1BJ, UK.
Email: c.j.thiele@soton.ac.uk

Introduction

Microplastics are ingested by many marine organisms, including bivalves. Laboratory evidence, especially from the blue mussel *Mytilus edulis*, suggests that not all microplastics pass quickly through the digestive system and are egested but may become stuck on gills or pass into the circulatory system (Browne et al., 2008; von Moos et al., 2012)the potential for ingestion by animals increases. The consequences of macroplastic debris for wildlife are well documented, however the impacts of microplastic (< 1 mm. Human exposure to microplastics through bivalve consumption is likely since microplastics have been found in the soft tissue of shop-purchased bivalves (Li et al., 2018). Commercial bivalves are generally depurated prior to being marketed to reduce microbiological contamination. Depuration in a laboratory setting has shown to reduce microplastic concentrations in mussels *Perna perna* (Birnstiel et al., 2019). The present field study aims to assess the removal efficiency of microplastics in commercial depuration facilities.

Materials and methods

Ostrea edulis were obtained from two UK shellfish merchants in November 2017 (n = 20; five untreated and five depurated specimens per merchant). Samples were stored at -20°C and subsequent microplastic extractions performed based on Thiele et al. (2019). Briefly, soft tissue was digested in 10% potassium hydroxide for 48 hours at 40°C and digestates filtered over 1.2 μm after neutralisation with citric acid. Contamination mitigation included working in a clean air cabinet (Bassaire 03VB, BS EN ISO14644, class 5, with additional cover), use of glass and metalware whenever possible, furnacing of filter papers at 500°C for two hours prior to use and wearing of 100% cotton clothing including a previously lint-rolled laboratory coat. Laboratory microplastic contamination was assessed using procedural blanks and dampened filter papers as airborne controls. Counts of potential microplastics in blanks were used to estimate the limit of detection (LOD) by using 3x standard deviation per particle type and colour (Macdougall et al., 1980). Results were adjusted using the LOD. Light microscopy (magnification ≤ 160x) was used to enumerate and characterise potential microplastics and Raman spectroscopy (Renishaw inVia, 785 nm) to ascertain plastic composition. Microplastics were reported as median concentrations ± 1 standard deviation. A two-sample two-tailed t-test for unequal variances was performed.

Results

Frequency of occurrence of specimens with microplastics was 20% in untreated and 40% in depurated samples. Median concentrations in specimens with microplastics were 1.0 ± 0.0 microplastics in undepurated oysters and 1.5 ± 0.96 microplastics in depurated oysters (maximum 3 microplastics). Microplastic concentrations were not statistically different between untreated and depurated specimens (t-test, p = 0.19). By category, 55.6% of microplastics were fibres and the remainder fragments/film. All but two microplastics were 12 - 18 µm in size (fibre diameter or largest fragment dimension). One fragment measured 52 and other approximately 400 µm. Microplastics were identified as polyester/PET, polypropylene, polyethylene and acrylic.

(Continued on next page)

Discussion

Little information about microplastic concentrations in *O. edulis* exist, but values are similar to concentrations in the Pacific oyster *Crassostrea (Magallana) gigas* at other locations (Martinelli et al., 2020; Phuong et al., 2018)we quantified the presence of microparticles in wild populations of Pacific oysters (Crassostrea gigas. Human exposure to microplastics through bivalve consumption is a concern. It has been suggested that bivalve depuration decreases microplastic contamination based on depuration experiments in controlled laboratory settings with mussels *P. perna*, *M. edulis* as well as *C. gigas* (Birnstiel et al., 2019; Van Cauwenberghe and Janssen, 2014). However, our work shows that depuration in commercial facilities does not reduce microplastic contamination in *O. edulis*. While concentrations are low, most microplastics are of a size that raises concern about human health. Microplastics ≤ 150 μm have the potential to cross the human gut tissue barrier and therefore absorb into the body (Wright and Kelly, 2017). Further work is needed to evaluate microplastic removal potential at other facilities and to assess how processes could be improved to aid microplastic reduction in bivalves prior to human consumption.

Conclusion

Our trial — performed at two commercial depuration facilities — shows that depuration aimed to reduce microbiological contamination prior to human consumption does not reduce microplastic concentrations in *O. edulis*. While frequency of occurrence and concentrations are low, the size of microplastics found warrants further investigations aimed at reducing human microplastic exposure through bivalve consumption.

References

Birnstiel, S., Soares-Gomes, A., da Gama, B.A.P., 2019. Depuration reduces microplastic content in wild and farmed mussels. Mar. Pollut. Bull. 140, 241–247.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). Environ. Sci. Technol. 42, 5026–5031.

Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.

Macdougall, D., Francis, D.P.-C., Amore, J., Cox, G. V, Crosby, D.G., Estes, F.L., Freeman, D.H., Gibbs, W.E., Gordon, G.E., Keith Manager, L.H., Lai, J., Chemical, D., Nina, U.S.A., Mcclelland, I., Phillips, W.F., Pojasek, R.B., Sievers, R.E., Wimert, D.C., Libby, R., 1980. Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry ACS COMMITTEE ON ENVIRONMENTAL IMPROVEMENT Technical Vice President Chemical Manufacturers Association, Anal. Chem.

Martinelli, J.C., Phan, S., Luscombe, C.K., Padilla-Gamiño, J.L., 2020. Low incidence of microplastic contaminants in Pacific oysters (Crassostrea gigas Thunberg) from the Salish Sea, USA. Sci. Total Environ. 715, 136826.

Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., Zalouk-Vergnoux, A., 2018. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? Mar. Pollut. Bull. 129, 664–674.

Thiele, C.J., Hudson, M.D., Russell, A.E., 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. Mar. Pollut. Bull. 142, 384–393.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.

von Moos, N., Burkhardt-Holm, P., Koehler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 327–335.

Wright, S.L., Kelly, F.J., 2017. Plastic and human health: A micro issue? Environ. Sci. Technol. 51, 6634–6647.

PHOTOTHERMAL CONTROL OF SEXUAL MATURATION IN LUMPFISH (*Cyclopterus lumpus*)

F.T. Mlingi*, V. Puvanendran, E. Burgerhout, M. Mommens, Ø. J. Hansen, E. Guercini, H. Tveiten, J. Tomkiewicz, E. Kjørsvik

Norwegian University of Science and Technology, Brattørkaia, 7010 Trondheim
Nofima, Muninbakken 9, 9019 Tromsø
AquaGenN-7462 Trondheim
The Arctic University of Norway, Breivika, 9010 Trømso
Technical University of Denmark, 2800 Kgs. Lyngby
E-mail: frank.t.mlingi@ntnu.no

Introduction

The production of lumpfish (*Cyclopterus lumpus*) juveniles which largely relies on wild broodstock collection has continued to increase and there is very little knowledge on its reproductive biology (Powell et al., 2018). To close the life cycle of lumpfish in captivity, successful control of sexual maturation is necessary. Photoperiod and temperature are manipulated to control sexual maturation and ensure a year-round availability of other temperate fish juveniles (Wang et al., 2010)that is, induction (initiation of oogenesis. Although photoperiod can alter spawning in lumpfish females (Imsland et al., 2019, 2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm, the speculated confounding effect of temperature (Imsland et al., 2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm is still unknown, further, there is no information on the long term gonadal development in relation to environmental manipulations. The monitoring of sexual maturation in many cultured fish species involves invasive methods, crowding and extensive handling, which negatively affect the fish health and reproductive performance (Næve et al., 2019)sex hormone analysis, and histological analysis of spermatogenesis. There were significant correlations ($R2 = 0.68$, $P < 0.01$. The use of ultrasound is noninvasive, and it has been applied successfully in monitoring sexual maturation in for example Atlantic salmon (*Salmo salar*) (Næve et al., 2019)sex hormone analysis, and histological analysis of spermatogenesis. There were significant correlations ($R2 = 0.68$, $P < 0.01$. Therefore, this study was aimed at determining the effects of different photothermal regimes on gonadal development and plasma sex steroid levels and evaluating the efficiency of ultrasound in monitoring sexual maturation in lumpfish.

Materials and methods

Two experiments were conducted:

Experiment 1: two groups of lumpfish ($n = 300$, body weight = 697 ± 364.1 g) in four tanks, were exposed to either a continuous or a short autumn-spring signal photoperiod (two tanks each).

Experiment 2: two groups of lumpfish ($n = 4000$, body weight = 157 ± 32.2 g) also in four tanks, were exposed to either a natural annual photoperiod or a nine-month compressed annual photoperiod. Using external appearances, gonadosomatic index and histomorphology, in both experiments, temperature in one tank from each photoperiod was elevated by 3 °C during final gonadal maturation in females. There were four and sixteen sampling points in the first and second experiments, respectively, during which body weight and length were registered. Ultrasound was tested in sexing and assigning the fish to different maturation categories. Blood for radioimmunoassay of sex steroids was collected. Gonads were excised, their weights registered, and tissues were collected for histological analyses of gametogenesis.

Results

Experiment 1: gonadal development in females was more synchronized and advanced in the short-autumn signal photoperiod. Temperature elevation resulted in accelerated final maturation and ovulation in both photoperiod regimes. In males, gonadal development appeared to be less affected than it was in females.

Experiment 2: fully matured males were observed seven months before matured females were recruited. Gonadal development in females exposed to the compressed annual photoperiod was advanced. Spawning in the compressed annual photoperiod was advanced, and more so with elevated temperature. In males however, gonadal development was not different between the photoperiod and temperature regimes. The ultrasound categorization of sexual maturation in both females and males successfully followed the progression of gonadal development, and blood plasma levels of sex steroids.

(Continued on next page)

Discussion and conclusion

Our findings showed that compressing the natural photoperiods caused temporal shifts in levels of sex steroids that are responsible for advanced gonadal development and leading to earlier spawning. Similar findings were reported for wolffish populations; temporal shifts in sex steroid profiles were observed and, sexual maturation and spawning were advanced under exposure to an eight-month compressed annual photoperiod (Dupont Cyr et al., 2018). In lumpfish, a more predictable spawning and peaks in spawning were observed under a compressed annual photoperiod and a short autumn-spring signal, respectively (Imsland et al., 2019, 2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm. The results obtained from accelerated sexual maturation and spawning under elevated temperatures in the present study, is similar to a study on rainbow trout where elevation of winter-spring temperatures caused an increased maturation rate (Wilkinson et al., 2010). Although ultrasound was successful in maturation monitoring, an ultrasound-based quantification of maturity in place of the conventional gonadosomatic index is still unavailable (Næve et al., 2019)sex hormone analysis, and histological analysis of spermatogenesis. There were significant correlations ($R2 = 0.68$, $P < 0.01$. Our findings demonstrated that the photoperiod and temperature combination was better than photoperiod alone in controlling sexual maturation in lumpfish. We also showed that ultrasound is a simple and non-invasive technique that could be used to assess sexual maturation in lumpfish.

References

Dupont Cyr, B.A., Tveiten, H., Maltais, D., Vandenberg, G.W., Le François, N.R., 2018. Photoperiod manipulation for the reproductive management of captive wolffish populations: Anarhichas minor and A. lupus. Aquac. Int. 26, 1051–1065. https://doi.org/10.1007/s10499-018-0267-x

Imsland, A.K., Hangstad, T.A., Jonassen, T.M., Stefansson, S.O., Nilsen, T.O., Hovgaard, P., Elvegård, T.A., Lindberg, K.S., Mikalsen, B., Urskog, T.C., Norberg, B., Andersson, E., Spetland, F., Reynolds, P., 2019. The use of photoperiods to provide year round spawning in lumpfish Cyclopterus lumpus. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 228, 62–70. https://doi.org/10.1016/J.CBPA.2018.11.004

Imsland, A.K., Jonassen, T.M., Hangstad, T.A., Stefansson, S.O., Elvegård, T.A., Lemmens, S.C.A., Urskog, T.C., Nytrø, A. V., Reynholds, P., 2018. The effect of continuous light and compressed photoperiods on growth and maturation in lumpfish Cyclopterus lumpus. Aquaculture 485, 166–172. https://doi.org/10.1016/J.AQUACULTURE.2017.11.053

Næve, I., Mommens, M., Arukwe, A., Virtanen, J., Hoque, M.E., Kjørsvik, E., 2019. Ultrasound as a noninvasive tool for monitoring reproductive physiology in male Atlantic salmon (Salmo salar). Physiol. Rep. 7. https://doi.org/10.14814/phy2.14167

Powell, A., Treasurer, J.W., Pooley, C.L., Keay, A.J., Lloyd, R., Imsland, A.K., Garcia de Leaniz, C., 2018. Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Rev. Aquac. 10, 683–702. https://doi.org/10.1111/raq.12194

Wang, N., Teletchea, F., Kestemont, P., Milla, S., Fontaine, P., 2010. Photothermal control of the reproductive cycle in temperate fishes. Rev. Aquac. https://doi.org/10.1111/j.1753-5131.2010.01037.x

Wilkinson, R.J., Longland, R., Woolcott, H., Porter, M.J.R., 2010. Effect of elevated winter-spring water temperature on sexual maturation in photoperiod manipulated stocks of rainbow trout (Oncorhynchus mykiss). Aquaculture 309, 236–244. https://doi.org/10.1016/j.aquaculture.2010.08.023

ZEBRAFISH AND CRISPR/CAS: A MODEL TO ELUCIDATE GENETIC EFFECTS ON THE MICROBIOTA

Eiríkur Andri Thormar*[1], Louise von Gersdorff Jørgensen[2], Moonika Haahr Marana[2], Cecilie Grønlund Clausen[1], Jacob Agerbo Rasmussen[1], Miyako Kodama[1], Morten T. Limborg[1]

[1]Center for Evolutionary Hologenomics, GLOBE Institute, University of Copenhagen, 1353 Copenhagen K., Denmark
[2]Section for Parasitology and Aquatic Pathobiology, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C., Denmark
Email: mrc419@alumni.ku.dk

Introduction

With the global population growing, the need for high-quality sustainably produced protein sources for human consumption is bound to increase. It is therefore important to develop novel, innovative and sustainable solutions to produce food and promote growth. Aquaculture will play a prominent role in that aspect. While probiotics for use in aquaculture have emerged as one possible solution for modulating the microbiota to promote growth, their effects remain inconclusive and uncertain. This may lie in the fact that microbiota research has largely been focused on the effects of microbiota on its host, rather than the potential effects host genetics have on its microbiota.

Elucidating potential host genetic effects on its microbiota has obvious biotechnological applications in the aquaculture industry. For example, designed feed to suit and promote growth of a particular genotype or genetic engineering of the host to promote a healthy microbiome. The field of applied hologenomics using zebrafish (*Danio rerio*) as a model organism provides a promising framework to study such effects.

Here, we applied a 16S metabarcoding approach in combination with a CRISPR/Cas mediated knockout of the gene coding for tyrosinase (tyr), the rate-limiting enzyme in melanogenesis, to provide a proof-of-principle that zebrafish can be used to study the effects of host genetics on its microbiota.

Methods

We generated a F0 generation of zebrafish by using CRISPR/Cas-9 to knock-out the *tyr* gene in the zebrafish genome. Phenotypes were classified into two groups based on pigmentation levels: albino and mosaic. The phenotypic groups were housed in separate tanks. The microbial community of faeces samples collected from the tanks containing the different pigmentation phenotypes was assessed with the bacterial 16S rRNA gene. The microbial communities of the two mutant pigmentation phenotype groups were compared to the microbial community of a third group with a wild type pigmentation pattern (figure 1).

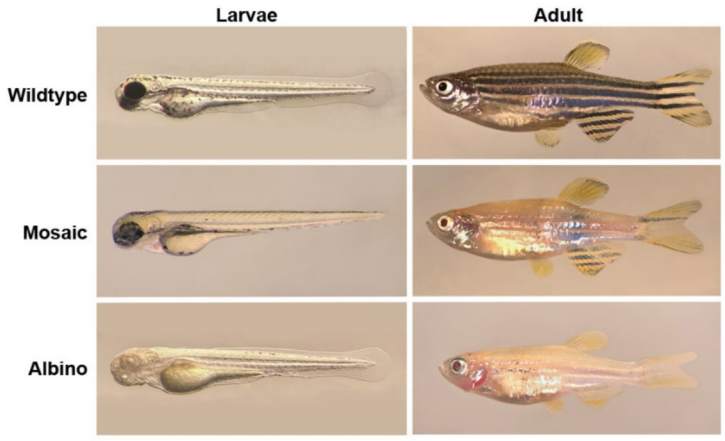


Figure 1. The three zebrafish pigmentation phenotypes generated, compared in their larval and adult stages. Photo by: Louise von Gersdorff Jørgensen and Moonika Haahr Marana

(Continued on next page)

Results
The knock-out of a single gene, *tyr*, using CRISPR/Cas-9, resulted in changes in pigmentation and in the zebrafish intestinal microbiota. Both alpha-diversity and beta-diversity differed significantly among the pigmentation phenotypes. The differences were mainly driven by the relative abundance of two genera: *Cetobacterium* and *Aeromonas*. Along with these genera, *Pseduomonas* and *Vibrio* were detected as differentially abundant among the pigmentation phenotypes. These genera may be implicated in immune- and metabolic-related mechanisms.

Conclusions
The results suggest an effect of tyrosinase function on the intestinal microbiota community of zebrafish. Overall, the study shows that the zebrafish presents a satisfactory model to study host-genomic effects on the microbiota and that further studies can shed even more light on such effects. Future studies should include a more multi-omic approach to gain more functional insights from the interactions between the host genetics and its microbiota. Moreover, such studies could prove highly valuable to the aquaculture industry. This could be in the form of probiotics tailored to the genotypes of broodstock fish, or perhaps the selection of genotypes that code for a healthy microbiota community.

SPERMATOZOA MORPHOLOGY AND REPRODUCTIVE POTENTICAL IN F1 HYBRIDS OF COMMON CARP AND GIBEL CARP

Tomáš Tichopád, Lukáš Vetešník, Andrea Šimková, Marek Rodina, Roman Franek, Martin Pšenicka

Sterility and semi-sterility often occur as a result of interspecific hybridization; however, the exact cause of sterility varies across the all animal kingdom; therefore, it is in the interest of scientists to examine every hybrid species separately. In the present study, the morphology of the spermatozoa of diploid F1 hybrids of *Cyprinus carpio* and *Carassius gibelio* was examined and compared with their parents. Hybrid spermatozoa showed great variability in head and flagellum lengths. These spermatozoa were diploid and triploid often with morphological abnormalities such as multiple flagella and nuclei. They were motile within two minutes after water activation which was almost two times longer than their parental species. We also analysed the reproductive output of F1 hybrids together with parental species in both parent positions via artificial spawning. Overall, the reproductive output of the hybrid × hybrid combination was the lowest; however, the hybrids were able to produce a small number of offspring documenting the viability of the F2 generation.

OCEAN LITEARCY AND HOW SERIOUS GAMES CAN PLAY A PART: THE CASE OF THE JELLYFISH AND MICROPLASTICS GOVERNANCE GAME MOREGOJELLY!

Tiller R*[1], Almås H, Ahlquist IH, Dankel D, Hakvåg M, Liu Y, Tiller W, Javidpour J

[1]SINTEF Ocean, 7465 Trondheim, Norway
*E-mail: rachel.tiller@sintef.no

***Introduction**:*

*The term Ocean Literacy, defined as "…*the understanding of the ocean's influence on humans and of our influence on the ocean*…" – was first discussed in 2004 after a group of scientists brough to the surface a concern that the general public had a lack of understanding about the importance of the Ocean which could hinder the uptake of knowledge around its importance* [1]*. We have now entered the UN decade of Ocean Science, where we will work global mobilization of the ocean community towards "The ocean we need for the future we want" – directly contributing to the implementation of the Sustainable Development Goals (SDGs) – even beyond Nr 14 – Life Below Water.* The term "Serious Games" dates back only half a *decade* [2], but the logic behind comes from military strategic applications called *wargaming* [3], which has a long and rich past. Although non-military games used for learning generally, and ocean literacy specifically, has developed gradually since the 70s, it was not until the popularization of digital games, around the turn of the millennium, that serious games rose to prominence in *academia* [4, 5]. Since then, vast amounts of serious games-research have been published each *year* [6] – chiefly on digital games, but also increasingly for hybrid and analog *games* [7]. In this article, the potential benefits of serious games for learning and motivation *within the context of ocean literacy, the UN decade of ocean science and the sustainable development goals* will be presented alongside an approach to using serious games as a tool for unintrusive data collection.

Material & Methods:
In 2020-2021, high school students from three different cities in Norway participated in a total of six Serious Game sets (two in each city) called MoreGoJelly! The game was developed through a collaboration with game developer House of Knowledge and the research institute SINTEF Ocean in Norway. The game was played live, with game boards for the participants in Trondheim and Tromsø, Norway, and it was played digitally with participants from Bergen, Norway. The latter was a pandemic need, because of demands for social distancing. The digital version was developed to simulate the board, game pieces and game setting as close as possible. The logic of the game centred on an assessment of a Serious Game as a communication tool for ocean literacy, with an emphasis on assessing similarities and differences between groups of future generation representatives from three different geographical regions in Norway (western-, mid- and northern- Norway) and diverse backgrounds. The game was to be played as a multi-player game with no more than four players, to ensure that all players were given ample time to discuss and participate in the game. They first had to fill in a personal questionnaire, and then as a group, they were presented with four sustainable development goals (SDGs 3 – good health and well-being, 8 – decent work and economic growth, 12 – responsible consumption and production and 14 – life below water) that they had to rank the top three in terms of importance within the game context of assessing microplastic pollution and jellyfish blooms in terms of how either and both affect coastal communities in Norway.

Because of this contextual setting, the game was set up around a map of Norway, and with game cards that first gave background information, preparing the students to play. The game board also had three fields for where they had to place the SDG goals they chose to prioritize – and in which order. This first step was followed by game cards that first gave the players a scenario where they had to assess how this event would affect social, economic and environmental sustainability of the region. They were then presented with three governance options, where they also had to rank how their governance choice would affect the three sustainability pillars. All in all, they were presented with nine events and nine governance cards (total of 27 governance options). They were given three opportunities throughout the game to change the order of their SDG cards.

(Continued on next page)

Results:
In the first ranking, all groups in all cities placed SDG 3 as either number one or number two. Further, all but one of the groups from Bergen had positioned SDG 12 as either number one or two. The group from Bergen chose SDG 14 instead. In the second round of ranking, only one of the groups from Trondheim changed their order of the SDGs. On the last card, when they for the third time were given the choice to re-rank - one of the two groups from each city (3 out of 6 groups) changed their positioning of the SDGs. Two of the three groups changed the position of two goals, while the last group moved all of the goals. All groups ended with SDG 12 on either first or second place, except for one group from Bergen who instead chose SDG 14. All but one of the groups from Tromso ended with SDG 3 in either first or second place. The group from Tromso had chosen SDG 14 instead of SDG 3 as the others. SDG 8 was positioned last in four out of six groups, and in third by the two remaining groups.

Discussion & Conclusion:
Consciously designing for each of these concepts around SDGs for ocean literacy can help leverage the potential benefits of serious games. However, it is the interaction between them that creates a lasting experience for players. For instance, social interaction is a crucial aspect of situated learning [8] especially for tacit knowledge [9], connected to the base need of relatedness [10], and as a potential strength when employing experiential learning – drawing on sharing of and learning from varied experiences [11]. Experiential learning can also be further strengthened when mapped to the antecedents of flow [12] – both of which can be seen as related to the self-determination theory need for competence [10]. Furthermore, despite the perceived importance of these concepts, there are of course several other potential benefits a game can have over traditional learning, such as multimodality, self-explanation [13], personalization, and adaptivity [14] to name a few. Ensuring the ocean science community can also contribute towards the implmenetation of the SDGs will take moving beyond traditional methods and including future geneartions in the discussions. Part of that move includes ocean literacy – ensuring "…the understanding of the ocean's influence on humans and of our influence on the ocean…" – and that this understanding is reached at a younger age so that the future decision makers already have a thorough understanding of the importance of the Ocean.

References:
[1] Costa, S., & Caldeira, R. (2018) Bibliometric analysis of ocean literacy: An underrated term in the scientific literature, Marine policy
[2] Abt, C. (1970) Serious games, University Press of America
[3] Caffrey, M. B. (2019) On wargaming: How wargames have shaped history and how they may shape the future, Naval War College Press
[4] Gee, J. P. (2003) What video games have to teach us about learning and literacy, Palgrave Macmillan
[5] Maier, F. H., & Größler, A. (2000) What are we talking about?—A taxonomy of computer simulations to support learning
[6] Zhonggen, Y. (2019) A meta-analysis of use of serious games in education over a decade, Intl journal of Computer Games Technology 2019
[7] Bayeck, R. Y. (2020) Examining board gameplay and learning: a multidisciplinary review of recent research, Simulation & Gaming
[8] Lave, J., & Wenger, E. (1991) Situated learning: Legitimate peripheral participation, Cambridge University Press
[9] van Haaften, M. et al. (2020) Do gaming simulations substantiate that we know more than we can tell?
[10] Rigby, S., & Ryan, R. M. (2011) Glued to games: How video games draw us in and hold us spellbound, ABC-CLIO
[11] Kolb, A. Y. (2002) The evolution of a conversational learning space, In Conversational learning: an experiential approach to knowledge creation
[12] Kiili, K. (2006) Evaluations of an experiential gaming model, Human Technology
[13] Mayer, R. E. (2019) Computer games in education, Annual Review of Psychology
[14] Wouters, P., & van Oostendorp, H. (2017) Overview of instructional techniques to facilitate learning and motivation of serious games.

MALDI-TOF MS IDENTIFICATION OF *Shewanella baltica* ISOLATED FROM FISH AFFECTED BY THE WASTEWATER TREATMENT PLANT

N. Topić Popović[1,2]*, S. Kazazić[3], B. Bilić[3], I. Strunjak-Perović[1,2] and R. Čož-Rakovac[1,2]

[1]Laboratory for Aquaculture Biotechnology, Ruđer Bošković Institute, Bijenička 54, Zagreb,Croatia
[2]Centre of Excellence for Marine Bioprospecting-BioProCro, Ruđer Bošković Institute, Zagreb, Croatia
[3]Laboratory for Mass Spectrometry and Functional Proteomics, Ruđer Bošković Institute, Zagreb, Croatia

*Email: ntopic@irb.hr

Introduction

Wastewater treatment plants (WWTP) perform primary and secondary biological treatment of municipal and related waters, and sometimes tertiary treatment for agricultural irrigation and wetlands restoration. The complex microbial community found in the treated effluent of WWTPs, although significantly reduced, might still contain pathogenic bacteria which present a threat to fish living downstream. If used for human consumption, such fish may pose a potential public health risk (Topić Popović et al, 2019). *Shewanella baltica* is a relatively rare finding amongst the bacteria withdrawn from native Prussian carp (*Carassius gibelio*). It is less known as a fish disease agent, although it was associated with outbreaks in farmed and ornamental fish (Kozińska and Pękala, 2004; Sicuro et al, 2020). In order to investigate the sensibility and accuracy of MALDI-TOF MS identification of *S. baltica* strains from Prussian carp, the isolates were grown on two culture media enriched by various NaCl concentrations, incubated at different temperatures and incubation times.

Materials and methods

Shewanella baltica strains were cultured on Tryptone Soy agar (TSA) and on TSA additionally supplemented with 0.5, 1.0, 1.5, and 2.0 % NaCl. They were also cultured on MacConkey agar (MCA), and on MCA additionally supplemented with 0.5, 1.0, 1.5, and 2.0 % NaCl (Oxoid Ltd, England UK). All plates were incubated at 22 °C and at 37 °C for 24 and 48 hours before further analyses.

The taxonomic position of the isolates was determined by the MALDI Biotyper using MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) Mass Spectrometry (Bruker Daltonik GmbH, Bremen, Germany). The on-target extraction method was applied for MALDI TOF MS sample preparation as described in Topić Popović et al. (2015a, b). Recorded mass spectra were processed with the MALDI Biotyper 3.0 software package (Bruker Daltonik), using standard settings.

Results and discussion

Although all MALDI-TOF MS measurements identified the tested strains as *S. baltica*, the type of the culture medium affected identification results. The TSA medium enabled a correct identification against the database in 84.53% of all measurements, if observing irrespective of NaCl supplementation, time of incubation, and incubation temperature, while the MCA medium allowed a correct identification in 56.59% of cases. The best identification scores were for strains cultivated on TSA for 24h at 22 °C for 75% of tested *S. baltica*. Contrarily, if assessing the impact of supplemented NaCl on these strains, the 1.5% NaCl was the optimum concentration for 91.67% of strains under these conditions.The strains cultured on MCA had lower identification results, and when incubated for 48h at 37 °C, they had unreliable identification scores. The number of unreliables was high also for *S. baltica* isolates cultured for 24h at 37 °C (1.381 ± 0.193). Best identification scores on MCA were after 48h at 22 °C for 43.33% of tested strains. The optimum concentration of supplemented NaCl under these conditions was 2% NaCl where 66.66% of strains had probable species identification. To summarize, the reliability of MALDI-TOF MS identification of bacteria from TSA significantly outperformed identification from MCA. The impact of media type on MALDI-TOF MS identification accuracy was also established for *Photobacterium damselae* (Kazazić et al, 2019b) and *Staphylococcus aureus* (Walker et al, 2002). The impact of temperature on identification sensibility and accuracy was even more adverse, particularly for MCA. The growth of *S. baltica* requires sodium chloride (Austin and Austin, 2016), thus the 1.5% and 2% NaCl supplemented TSA and MCA enhanced MALDI-TOF MS identification accuracy, respectively.

(Continued on next page)

References

Austin B, Austin D (2016) Bacterial fish pathogens: Disease in farmed and wild fish. 6th edition, Springer International Publishing Switzerland

Kozińska A, Pękala A (2004) First isolation of Shewanella putrefaciens from freshwater fish – a potential new pathogen of the fish. Bulletin of the European Association of Fish Pathologists, 24, 199-203.

Sicuro B, Pastorino P, Barbero R, Barisone S, Dellerba D, Menconi V, Righetti M, De Vita V, Prearo M (2020) Prevalence and antibiotic sensitivity of bacteria isolated from imported ornamental fish in Italy: a translocation or resistant strains? Preventive Veterinary Medicine, doi: 10.1016/j.prevetmed.2019.104880

Topić Popović et al, (2019) Aquatic bacterial contamination associated with sugarplant sewage outfalls as a microbial hazard for fish. Chemosphere, 30802779 doi: 10.1016/j.chemosphere.2019.02.110

Topić Popović N, Kazazić S, Strunjak-Perović I, Barišić J, Sauerborn Klobučar R, Kepec S et al (2015a) Detection and diversity of aeromonads from treated wastewater and fish inhabiting effluent and downstream waters. Ecotoxicol Environ Saf 120:235-242.

Topić Popović N, Strunjak-Perović I, Sauerborn Klobučar R, Barišić J, Babić S, Jadan M, Kepec S, Kazazić SP, Matijatko V, Beer Ljubić B, Car I, Repec S, Stipaničev D, Klobučar G, Čož-Rakovac R (2015b) Impact of treated wastewater on organismic biosensors at various levels of biological organization. Sci Total Environ 538:23-37.

Walker J, Fox AJ, Edwards-Jones V, Gordon DB (2002) Intact cell mass spectrometry (ICMS) used to type methicillin-resistant Staphylococcus aureus: media effects and inter-laboratory reproducibility. Journal of Microbiological Methods, 48, 2-3, 117-126.

REPLACEMENT OF FISH MEAL BY ANTARCTIC KRILL MEAL IN DIETS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*): GROWTH PERFORMANCE, FEED UTILIZATION AND LIVER LIPID METABOLISM

S. Torrecillas[1*], D. Montero[1], M. Carvalho[1], T. Benitez-Santana[2], M. Izquierdo[1]

[1]Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, Telde, Las Palmas, Canary Islands, Spain
[2]Aker BioMarine Antartic AS, Oksenøyveien 10. PO Box 496 NO-1327 Lysaker, Norway
Email: silvia.torrecillas@giaqua.org

Introduction

Fish meal (FM) can be partially replaced in the diets of many fish species, but in most cases, high or complete replacements have detrimental effects on fish performance and health. This side effects are mainly due to imbalanced amino acids and micronutrients, the presence of antinutritional factors or to a direct reduction of feed intake because of decreased diet palatability as the level of alternative protein sources increases. To the date, there is no information on the effect of dietary krill meal (KM) on European sea bass performance and liver lipid metabolism, being the main objective of the present study.

Materials and methods

Three isoproteic and isolipidic experimental diets were formulated containing different KM levels: 0% (control group), 5% and 7.5% KM (Qrill^{TM} Aqua; Aker BioMarine Antarctic AS, Norway). European sea bass juveniles were randomly distributed in 12 indoor 500 L fiberglass tanks (3 tanks/diet) at an initial stocking density of 1.5 kg·m^{-3}. Fish average initial weight and length were 22.54±0.30 g and 11.4±0.1 cm (mean ± SD) and were manually fed until apparent satiation for 90 days (3 times a day, 6 days a week). At the end of the feeding trial, growth performance parameters and food conversion ratio were calculated. Livers, dorsal muscle and whole-body samples from 5 fish per tank were sampled for chemical composition, fatty acid and lipid classes analyses. Livers of 3 fish per tank were collected for morphological and gene expression analyses.

Results

After 90 days of feeding, fish fed KM5 and KM7.5 diets presented higher final weight and improved SGR than fish fed control diet ($p<0.05$) (Fig. 1). FCR was improved from 60 days of feeding onwards when diets were supplemented with KM (Fig. 1). Whole-body, muscle and liver proximate composition were not affected by the experimental diets. Regarding tissue fatty acid composition, whole-body and muscle seemed to be more affected by diet composition than liver, which only presented an effect of KM on Σn-6 HUFA, being the highest in those fish fed KM5. Fish fed KM showed a tendency to present lower liver cholesterol levels than fish fed control diet. KM dietary content was positively correlated with the pigmented material content (*r= 0.84, p=0.01*). Fish fed KM diets (Fig. 2B, 2C) presented smaller hepatocytes area with a more regular-shaped morphology around sinusoidal spaces compared to fish fed the control diet (Fig. 2A). This effect was particularly noticeable in fish fed KM7.5, pointing to a dose-dependent effect of KM on these parameters. Sea bass fed KM5 and KM7.5 diets showed decreased *mRNA* levels of *fads2* ($p<0.1$) and *hmgr* ($p<0.05$). Despite that no significance differences in *fabp7* relative expression were detected among sea bass fed the different dietary KM contents, fish fed KM5 and KM7.5 diets expressed slight lower *mRNA* levels, which were significantly correlated with liver cholesterol. (Pearson (Pearson correlation= 0.99, p=0.04). *lpl* mRNA levels were linearly correlated with liver lipids (Pearson correlation= 0.99, p=0.03).

Conclusions

The results of the present study showed that dietary KM at 5% and 7.5% as partial replacer for FM promotes European sea bass juvenile's growth performance, feed and nutrient utilization. Besides, KM modulates liver lipid metabolism and reduces liver hepatocytes vacuolization. Therefore, presenting KM not only as an alternative protein source in practical diets for European sea bass with low FM/FO content, but also as a potential functional ingredient capable of promoting liver health status.

(Continued on next page)

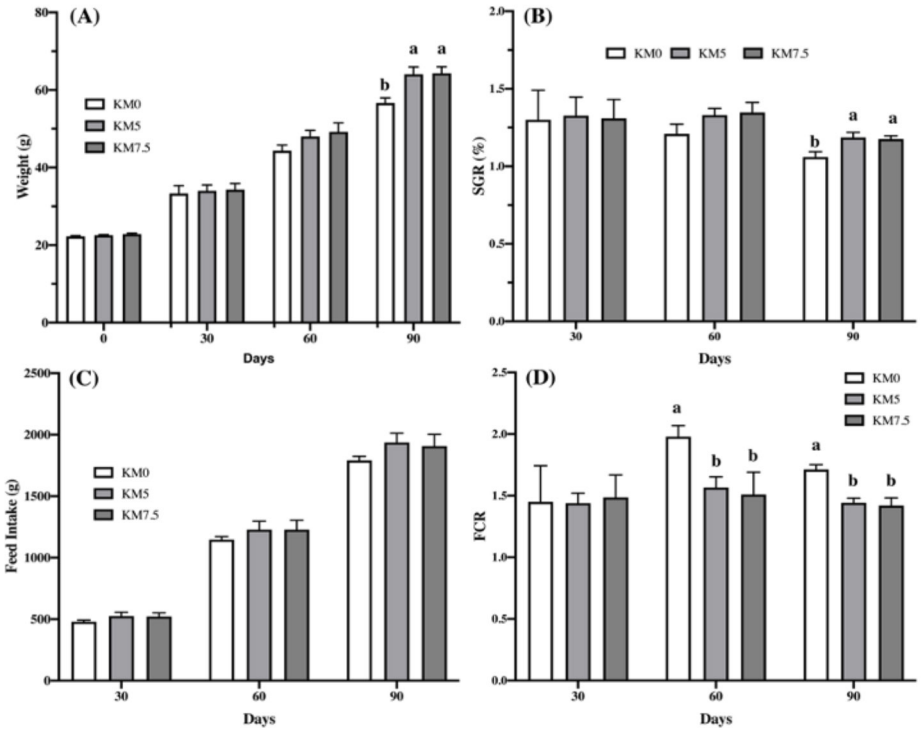


Figure 1. Weight, growth performance and feed utilization of European sea bass (*Dicentrarchus labrax)* juveniles fed diets with graded KM levels along the feeding trial. Values expressed in mean ± SD.

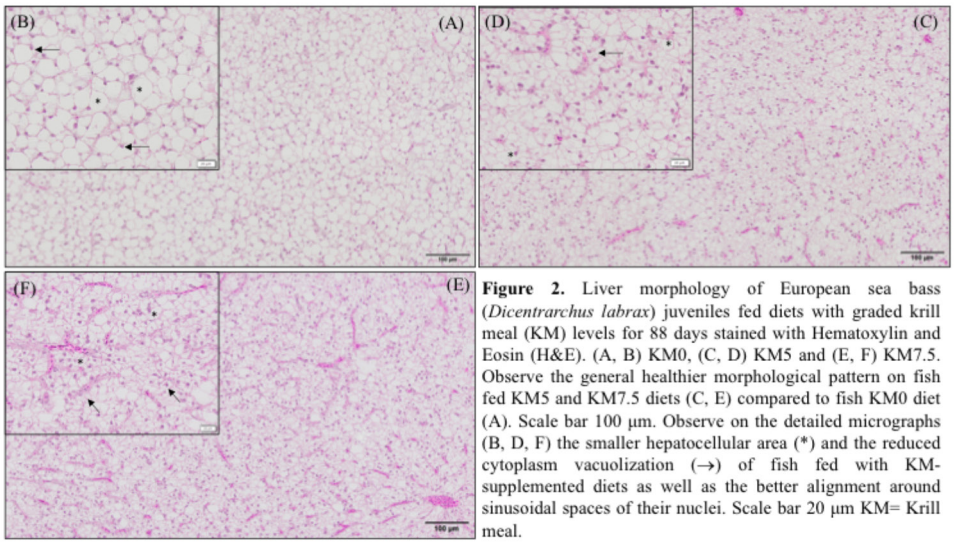


Figure 2. Liver morphology of European sea bass (*Dicentrarchus labrax*) juveniles fed diets with graded krill meal (KM) levels for 88 days stained with Hematoxylin and Eosin (H&E). (A, B) KM0, (C, D) KM5 and (E, F) KM7.5. Observe the general healthier morphological pattern on fish fed KM5 and KM7.5 diets (C, E) compared to fish KM0 diet (A). Scale bar 100 µm. Observe on the detailed micrographs (B, D, F) the smaller hepatocellular area (*) and the reduced cytoplasm vacuolization (→) of fish fed with KM-supplemented diets as well as the better alignment around sinusoidal spaces of their nuclei. Scale bar 20 µm KM= Krill meal.

EXPLORING EUTHANASIA METHODS FOR DEVELEPOMENT STAGES OF *Danio rerio*

J. Ramos; L. Tort

Department Cell Biology, Physiology and Immunology, Universitat Autonoma de Barcelona, Bellaterra, Spain
Email: juanramoblas@gmail.com

Introduction:

Zebrafish, *Danio rerio* is a small fish from the Hindukush valley and is one of the most used fish in research, special for biomedicine. During the early development stages, zebrafish are very sensitive to environmental conditions and also, they have to properly develop different organs like the gills, as they can breathe through the skin till day 14. These factors are really important for euthanasia; as euthanasic agents usually block muscular contraction. So, as relevant physiological features are different between adults and fries, the environmental conditions and protocols should be specially designed for the different development stages. Welfare supervision is mandatory in animal research including fish, but not for not larval feeding stages. So, protocols for husbandry, anesthesia or euthanasia have not been properly developed for these stages. As a consequence, this lack of standard protocols and no regulation for welfare supervision could end up with unreliable research and could negatively impact on fish welfare. In this work, we assessed euthanasic methods for developmental stages of zebrafish.

Material and methods:

Three different euthanasic agents, lidocaine, clove oil and tricaine(MS-222), were tested in three different stages of development, in order to see their efficacy and other implications. Lidocaine and clove oil were dissolved in ethanol, but tricaine was not. These anesthetics were used at high concentration in wild type *Danio rerio* gastrula stage, pharyngula and early larva. After exposing zebrafish to the anesthetics, efficacy, aversion, and specific gene expression was tested in order to verify their effects at different stages.

Efficacy was tested exposing the embryos for 1h to the euthanasic solution, removing them for the solution and watching heartbeat and embryo integrity after 24 hours of exposure. Aversion is the reaction of the embryos in order to escape from a noxious stimulus, anesthetics or euthanasic agents. In order to know how the different anesthetics, affect genetic expression, zebrafish samples were taken at 1, 3, 6, 10, 15 and 25 minutes for the 3 different development stages and for each anesthetic. In addition, embryos were recorded for 30 minutes in order to assess how anesthetic affects heart beat blocking, interference of cellular division or other processes related with the euthanasic procedure.

Results:

At the different development stages, zebrafish react in different ways and intensity when they are exposed to anesthetics. Their reaction depends not only on the product also the concentration and the water quality (specially the pH) affects to the aversion reaction. MS-222 and clove oil seems to be aversive products as zebrafish reacts the two last stages meanwhile no reaction was detected when exposed to Lidocaine. The reaction to the two aversive products tested was also different in time and intensity.

The embryos exposed to different anesthetics showed different functional processes in the different development stages. as observed under the microscope. For example, in the gastrula stage and earlier, an alteration in the cellular division or an embryo lysis can be detected, meanwhile in the last stage, raquis contraction, mucus hypersecretion and lysis can be observed. Heartbeat, that can be detected from the pharyngula stage, can be blocked, but after removing the embryos from anesthetics, recovered again.

Ten different genes (…….) were tested at different time points. Preliminary results showed differences in most of them.

Lidocaine	**Clove oil**	**Tricaine**
1g lidocaine	1ml clove oil	1g MS-222
2g bicarbonate		2g bicarbonate
50 ml ethanol	9 ml ethanol	
450 ml water	490 ml water	500ml water

(Continued on next page)

Conclusions:

Euthanasia is a regulated procedure by welfare law for adult fish. These procedures should be effective and reproducible, with no welfare implications for the animals and the technician.

Early development stages of zebrafish have different physiological traits than the adults, so especial protocols and studies should be developed. Euthanasia is commonly applied by an overdose of anesthetics, so fish lose consciousness and die by asphyxia, because muscular contraction is blocked. As during development stages, they are able to breathe through the skin, common anesthetics are not capable to euthanize, even when heart is not beating.

We find two different euthanasic protocols that are capable two kill at early development stages: Lidocaine and clove oil. But they affect zebrafish fry in a different way before and after losing the consciousness, as they express aversion to the anesthetic and different genetic pathways are expressed.

COMPARISON OF THE PREDATORY ACTIVITY OF DIFFERENT SPECIES ON THE SEA URCHIN *Paracentrotus lividus* (LAMARCK,1816)

N.Tourón*[1], E. Paredes[2], S. Campos[2], D. Costas[1] and J.M. García-Estévez[1]

[1]Centro de Investigación Mariña,Universidade de Vigo, ECIMAT, 36331, Vigo, Spain
noelia.touron.besada@uvigo.es
Tel.:722842627
*Corresponding author ORCID:0000-0001-6077-2801

[2] Centro de Investigación Mariña - Universidade de Vigo. Laboratorio de Ecoloxia Costeira (ECOCOST) Departamento de Ecoloxia e Bioloxia Animal

Abstract
In the existing bibliography to date, different species of crustaceans, fish and other invertebrates are mentioned as predators of the sea urchin *Paracentrotus lividus*, although no detailed information is found in this regard nor is it mentioned which is its main predator, due to which a Predation experiment of this species have been performed, introducing 3 different species as potential predators and observing both the capacity and the speed of predation of each one of them on individuals of the species *P.lividus* of different size ranges.

Within the framework of the OCIMER project (Optimization of the integral cultivation of the sea urchin *Paracentrotus lividus*) that is being developed in the facilities of the ECIMAT (Station of Marine Sciences of Toralla), two repopulations of coastal áreas of the Vigo Bay (Galicia,Spain) were carried out with juvenile specimens of the sea urchin species *P.lividus*); This information will be very useful for the subsequent monitoring of the individuals released to the natural environment, as well as to take into account the abundance of their largest predators in future repopulations with juvenile individuals of this species.

Material and Methods
Sea urchins (*P. lividus*) were introduced in 6 boxes of 150 l capacity with two size ranges: 10 mm in diameter (15 urchins) and approximately 20 mm in diameter (15 urchins) in each box; In 3 of them 5 adults were also introduced (boxes 1, 2 and 3), and a control box was placed where only sea urchins were kept without the predatory species; the urchins were fed with algae of the genus Laminaria sp. The 3 species that were tested as predators of *P. lividus* were the next: 3 starfish (*Marthasterias glacialis*), 3 velvet crab (*Necora puber*) and 2 spider crab (*Maja squinado*) were previously left without food for 5 days, and then were placed in the boxes containing urchins in 3 batches, leaving the experiment long enough for each predatory species to consume all the urchins at its disposal, with a maximum duration of the experiment of 28 days.

Highlights
- Only spider crabs were able to prey on adult urchins, while starfish and crabs only preyed on juvenile urchins of both size ranges, without a significative difference in predation rate between the two sizes.

- No significant protective effect of adults on juvenile individuals was observed against predators, probably due to the lack of interstices in the boxes that they do have in the rocks of the natural environment.

- All the urchins in the control box remained alive throughout the experiment.

Conclusion
Among the 3 predator species chosen, by far the largest predator of *P. lividus* is the spider crab, followed by the crab and lastly the starfish; Furthermore, the spider crab is the only one capable of predating adult specimens. It would be interesting to continue the experiment soon by introducing different species of fish. The starfish is not an active sea urchin predator.

Acknowledgements
We are grateful to the staff of the Toralla Marine Science Station (ECIMAT) belonging to the CIM (Centro de Investigación marina-Universitdade de Vigo) and to the San Xosé de Cangas do Morrazo Fishermen's Association. We are also grateful to GMA (Galician Marine Aquaculture) company, who collaborated selflessly with the OCIMER project. This work was developed within the framework of the OCIMER Project "Optimization of the integral cultivation of the sea urchin *Paracentrotus lividus*", financed by the PLEAMAR program of the Fundación Biodiversidad.

SEA URCHIN CRYOPRESERVATION AS A BIODIVERSITY CONSERVATION TOOL

S. Campos[2], N. Tourón[1] and E. Paredes*[1]

[1]Centro de Investigación Mariña,Universidade de Vigo, ECIMAT, 36331, Vigo, Spain
noelia.touron.besada@uvigo.es.
Tel.:722842627
*Corresponding autor ORCID:0000-0001-6077-2801

[2] Centro de Investigación Mariña - Universidade de Vigo. Laboratorio de Ecoloxia Costeira (ECOCOST)
Departamento de Ecoloxia e Bioloxia Animal

Introduction

Sea urchins, have been used as model systems in biology and ecotoxicology for more than a century now. The sea urchin is a gamete 'production powerhouse'. Given the transparency of the egg, the synchronicity of divisions, the ease of manipulation and the abundance of gametes, Sea urchins provide an excellent system to address general cell biological questions, including cryopreservation. Here we studied the cryopreservation of gametes, embryos and larvae of four species of sea urchins that could be located at the National Park "Illas Atlanticas de Galicia" in the North west coast of Spain. Three regular sea urchins, *Parancentrotus lividus, Echinus esculentus, Sphaerechinus granularis* and one irregular sea urchin *Echinocardium cordatum* were used for development of cryopreservation protocols.

Results

Results showed us that despite these species are close genetically, to the point that some can hybridize, and that their geographical distribution is overlapped, their response to cryopreservation is unique and individualized, and therefore it will require the development of individual protocols for their cryopreservation. In the case of sea urchins the main difference is the selection of the suitable cryoprotectant agent for each specific species and cell type. This might be due to the extraordinary sensitivity to chemicals present in the water that has made sea urchins perfect models for the study of ecotoxicology, as their early development stages could be used to identify negative effects of pollutants in the water in concentrations of parts per billion (ppb).

Conclusion

We have successfully cryopreserved sperm from two of the species with a 30-50% relative fertilization to fresh controls, egg cryopreservation was unsuccessful, in the case of embryos successful protocols could be developed for blastula embryos and no older larvae cryopreservation was achieved. This is the first time that three of this species were studied.

EUROPEAN PERCH (*Perca fluviatilis*) FED DIETARY INSECT MEAL (*Tenebrio molitor*): FROM A STABLE ISOTOPE PERSPECTIVE

H.Q. Tran[1]*, M. Kiljunen[2], V. Stejskal[1]

[1] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Na Sádkách, **České** Budějovice, Czech Republic
[2] University of Jyväskylä, Department of Biological and Environmental Science, Jyväskylä, Finland
Email: htranquang@frov.jcu.cz

Stable isotope analysis was conducted to investigate stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), diet-tissue discrimination factors of carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$). Bayesian mixing models were performed to assess relative contribution of insect meal and other ingredients to the development of tissues of European perch (*Perca fluviatilis*). Accordingly, four experimental formulations, characterized by the increasing inclusion levels of yellow mealworm (*Tenebrio molitor*) larvae meal (TM) at 0, 6.8, 13.5 and 20.3% as replacement for fishmeal at 0 (TM0), 25 (TM25), 50 (TM50) and 75% (TM75), respectively, were fed to juvenile perch (initial bodyweight, 20.81 ± 3.36 g) in a recirculated aquaculture system for 105 days.

$\delta^{13}C$ and $\delta^{15}N$ of TM were -16.75 and 3.53‰ and significantly distinguished from other terrestrial and marine feed components ($P < 0.05$). Inclusion of dietary TM did not affect $\Delta^{13}C$ value in blood and liver ($P > 0.05$) but did reduce in muscle ($P < 0.05$), whereas $\Delta^{15}N$ was significantly increased with the increasing inclusion level of TM in all tissues ($P < 0.05$). The growth of perch had a significant negative relationship with diet-muscle $\Delta^{15}N$. The contribution of TM to muscle (7.7 ± 3.8%) was comparable to its dietary inclusion (6.8%) in TM25 but double in the blood (13 ± 6%). TM appeared to be an essential ingredient incorporated into liver, as its contribution was consistent or higher than dietary inclusion (TM25: 25.4 ± 12.1 vs. 6.8%; TM50: 31.1 ± 14.9 vs. 13.5%; and TM75: 29.4 ± 14.4 vs. 20.3%). The higher inclusion levels of TM (more than 6.8%) did not elevate its contribution to muscle, blood, and liver (probability, $P_{BIC} < 0.95$) but significantly decreased that of fishmeal in all tissues ($P_{BIC} > 0.95$). Soy-derived ingredients, soybean meal and soy protein, were an important ingredient in the development of all tissues regardless of dietary TM.

The present study provided insightful information on the role of various diet components in perch tissues, which could underlie further development of aquafeed formulations for emerging perch farming in Europe.

Results

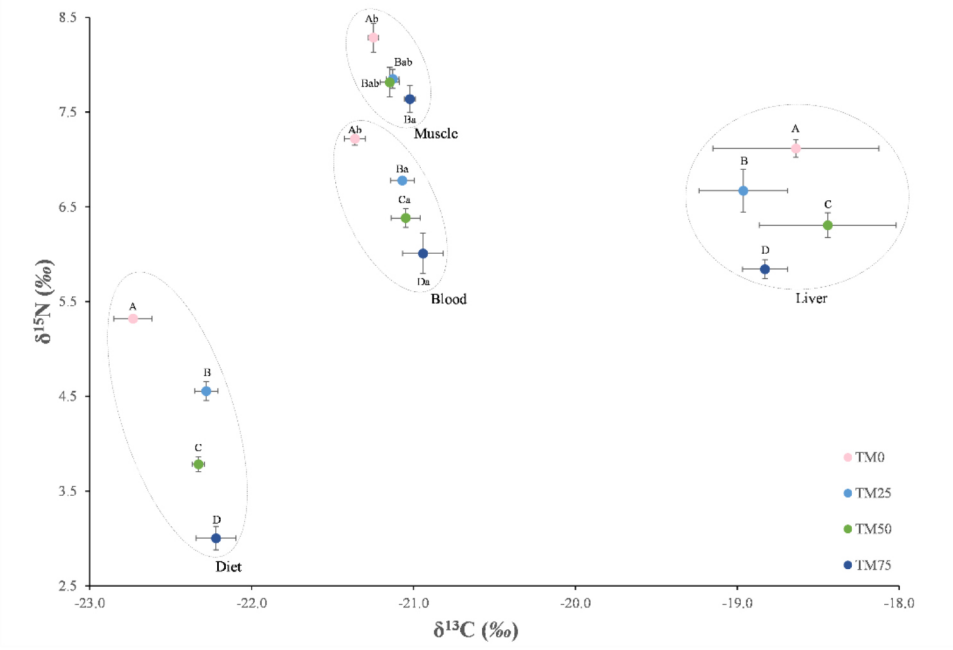


Figure 1. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of fillet, liver, and blood of European perch fed four experimental diets. Data were present as mean ± SD. Different lowercases and uppercases within the tissue group indicate significant differences in $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

(Continued on next page)

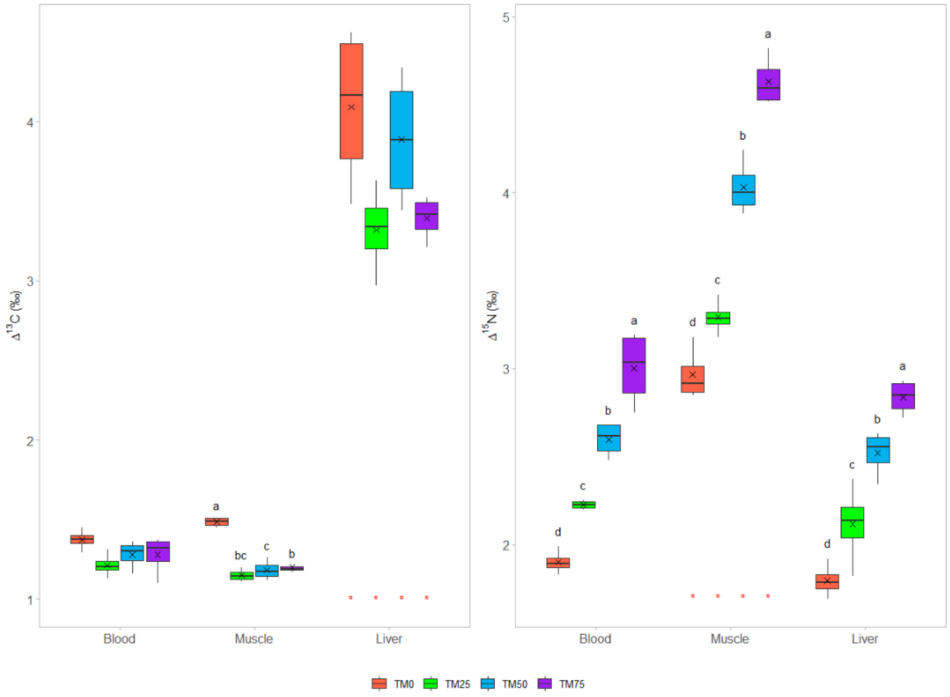


Figure 2. Discrimination factors, $\Delta^{13}C$ (left) and $\Delta^{15}N$ (right) of European perch's tissues and experimental diets. The black "x" represents mean value. The horizontal line inside each boxplot represents the median separating the interquartile range. Different lower cases within tissue group indicate significant difference across diet treatments ($P < 0.05$). The red asterisks within diet group indicate significant difference ($P < 0.05$) compared to the other tissues.

(Continued on next page)

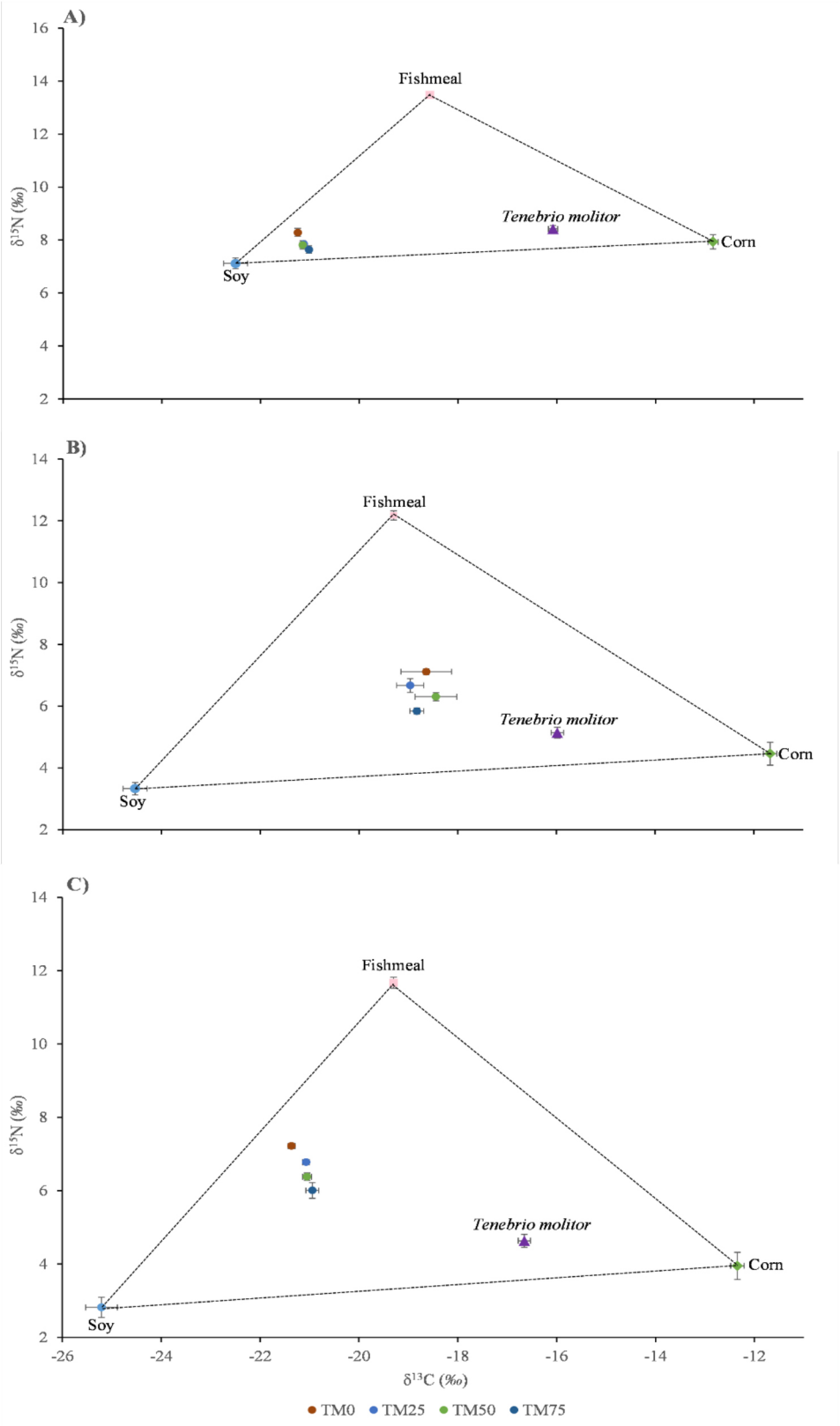


Figure 3. Iso-space plots of $\delta^{13}C$ and $\delta^{15}N$ signatures of four feed ingredients and tissues (muscle (A), liver (B) and blood (C)) of European perch fed for experimental diets.

(Continued on next page)

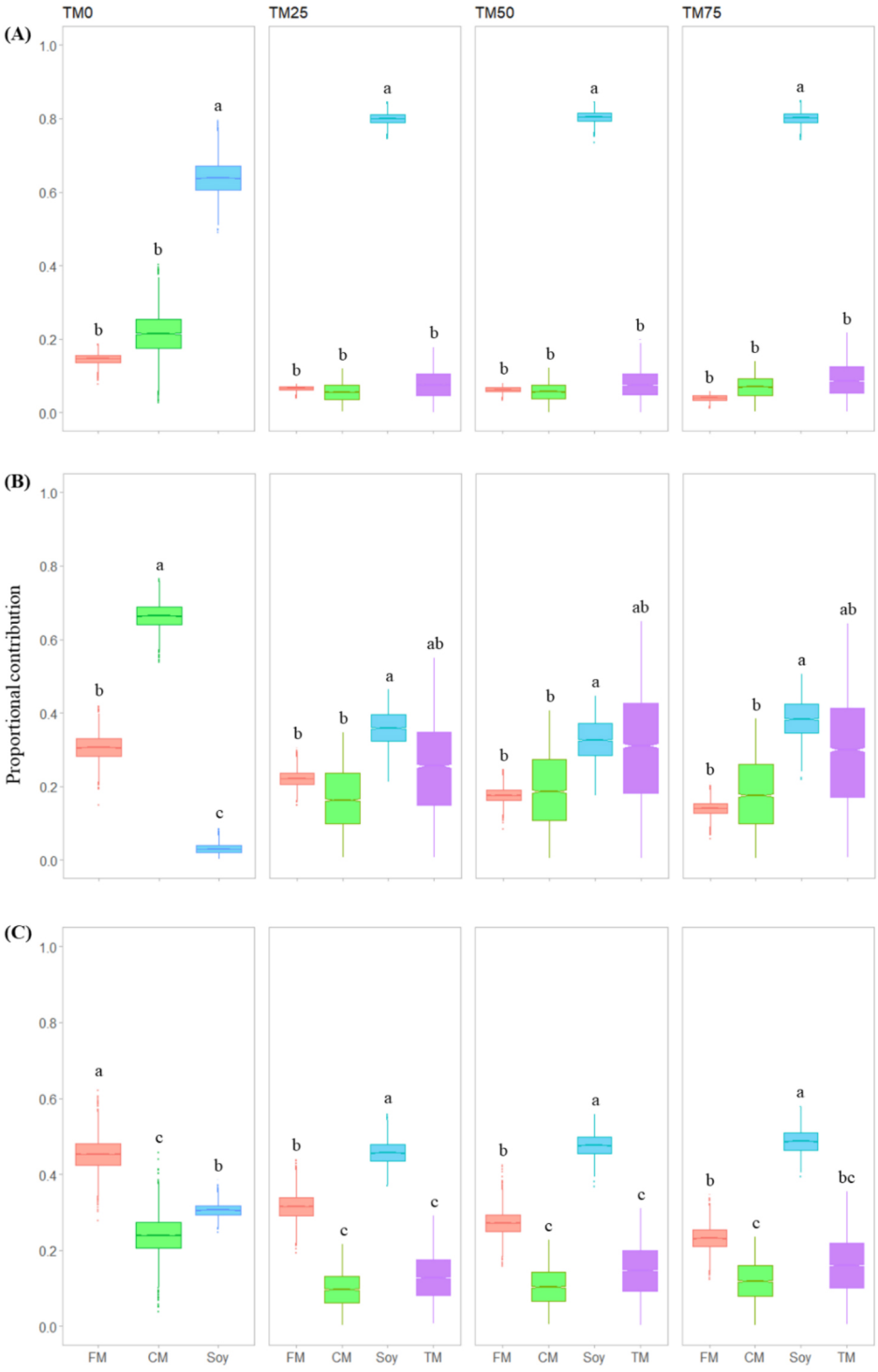


Figure 4. Boxplots from Bayesian isotopic mixing models representing proportional contribution (mean, interquartile range) of individual feed ingredient to muscle (A), liver (B) and blood (C) tissues of European perch fed experimental diets

ASSESSING AND OPTIMIZING GENOTYPE IMPUTATION STRATEGIES IN PACIFIC OYSTER (*Crassostrea gigas*)

S. Tsairidou[1]*, A. P. Gutierrez[2], C. Kriaridou[1], D. Robledo[1], and R. D. Houston[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK
[2]Institute of Aquaculture, University of Stirling, Stirling, UK

*E-mail:Smaragda.Tsairidou@roslin.ed.ac.uk

Introduction

Genomic selection uses genome-wide SNP genotypes to predict breeding values, and this methodology has been widely applied in terrestrial livestock and several aquaculture species, conferring cumulative improvements in a wide range of economically important production, health and welfare traits. The development of a medium-density SNP array for Pacific and European Oysters (Gutierrez *et al*., 2017), and the publication of a chromosome-level reference genome assembly (Peñaloza *et al*., 2021), have opened new promising avenues for the design of effective genomic selection strategies in these species. However, the capacity to routinely genotype at high density (HD) large numbers of individuals, as is required for genomic selection, remains prohibitively expensive for much of the sector. In previous studies in Atlantic salmon, genotype imputation has been proposed as a cost-effective method to implement genomic selection in breeding programmes (Tsairidou *et al*., 2020, Tsai *et al*., 2017). Imputation allows prediction of HD genotypes from low density (LD) SNP panels, by using information from haplotypes shared between related individuals (Li *et al*., 2009). Hence it requires dense genotyping only for a subset of the population, thereby substantially reducing the genotyping costs. The aim of this study was to test and assess genotype imputation for genomic prediction in Pacific oyster, using two different imputation software, and data for survival to the ostreid herpesvirus OsHV-1, a trait known to have a polygenic genetic architecture and hence being suitable for genomic selection (Gutierrez *et al*., 2020, Gutierrez *et al*., 2018).

Materials and Methods

Our study focused on a commercial breeding programme population challenged with the ostreid herpesvirus OsHV-1 (Cawthron Institute, New Zealand) (Gutierrez *et al*., 2020). Binary survival data recorded at the end of the trial and continuous time-to-death data were available for 718 individuals. All individuals, including their parents, were genotyped using the Affymetrix SNP array for oysters (Gutierrez *et al*., 2017) (25,600 SNPs). After mapping to the latest reference genome (Peñaloza *et al*., 2021) and performing quality control, 13,763 SNPs and 743 individuals (699 offspring and 44 parents) were retained for subsequent analyses. The analysis comprised three main parts: (a) heritability and genomic prediction analyses for the HD directly genotyped data; (b) generating a range of LD SNP panels from the HD data, and testing their genomic prediction accuracy; and, (c) imputing the LD SNP panels to HD to assess their imputation accuracy, and estimate genomic prediction accuracy using HD imputed data.

Genomic prediction was assessed via 20 repeats of 5-fold cross-validation, to estimate the mean prediction accuracy from the correlation between predicted breeding values (BVs) and phenotypes, corrected for the trait heritability. The BVs were estimated in ASReml/3.0 (Gilmour *et al*., 2009) using the following generalized linear mixed animal model which implements the logit link function for the binary survival data:

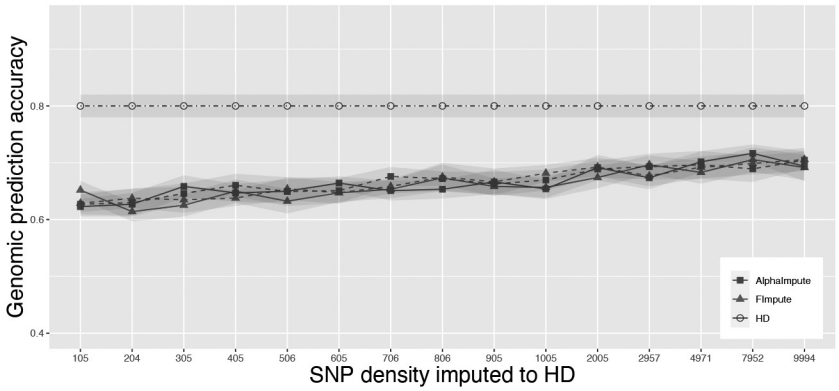


Figure 1. Genomic prediction accuracy calculated as means over 20 * 5-fold cross-validations, with LD genotypes imputed to HD using (a) FImpute (pink) and AlphaImpute (green)

(Continued on next page)

Tank was fitted as a fixed effect in $\boldsymbol{b}$, and genetic relationships between individuals were fitted as random effects through the genomic relationship matrix $\boldsymbol{G}$ calculated from SNP data with ~ N(0,). Heritability estimates were adjusted to the underlying liability scale. Time-to-death was analysed using a linear model. Low density *in silico* SNP panels were constructed via random sampling of SNPs within each chromosome but proportionally to chromosome length. Genotype imputation was performed using (a) AlphaImpute v2 (Whalen and Hickey, 2020, Hickey *et al*., 2011), and (b) FImpute v3.0 software (Sargolzaei et al., 2014). A range of imputation scenarios were tested where the majority of offspring were genotyped at LD for densities ranging between 100 and 10,000 SNPs, and parents were genotyped at HD.

Results and Discussion

The heritability estimated using the genomic relationship matrix calculated from HD SNP genotypes was 0.39 (s.e. 0.05) for survival and 0.64 (s.e. 0.05) for time-to-death. The mean genomic prediction accuracy over 20 replications of 5-fold cross validation was 0.80 (s.d. 0.02) for survival and 0.73 (s.d. 0.02) for time-to-death. Reducing the SNP panel density from HD to 100 SNPs resulted in ~ 31 % decrease of the genomic prediction accuracy for both survival and time-to-death. Imputation accuracy increased with increasing density of LD SNP panels imputed to HD, and overall, FImpute provided higher imputation accuracies than AlphaImpute in this data, although this difference was not statistically significant. Genomic prediction accuracies using imputed genotypes increased for higher densities imputed to HD, likely reflecting the better imputation accuracy for higher densities. Prediction accuracy was not found to differ significantly between genotypes obtained through using different imputation software (Figure 1). When using imputed genotypes, the prediction accuracies were found to be inferior to those obtained from directly genotyped HD data, regardless the software used for imputation.

While pedigree data can be used to predict breeding values only at the between-family level, in this study, genomic prediction was performed using the genomic relationship matrix calculated from imputed and directly genotyped data, which captures both between- and within-family variation. Further analyses are underway to test alternative scenarios of constructing the LD and HD groups, and to assess their impact on imputation and genomic prediction accuracy.

References

1. Gutierrez, A. P. et al. (2017) G3 (Bethesda) 7, 2209-2218.
2. Peñaloza, C. et al. (2021) GigaScience 10.
3. Tsairidou, S. et al. (2020) G3: Genes|Genomes|Genetics 10, 581-590.
4. Tsai, H.-Y. et al. (2017) G3: Genes|Genomes|Genetics 7, 1377-1383.
5. Li, Y. et al. (2009) Annu Rev Genomics Hum Genet 10:, 387-406.
6. Gutierrez, A. P. et al. (2020) Anim. Genet. 51, 249-257.
7. Gutierrez, A. P. et al. (2018) G3: Genes|Genomes|Genetics 8, 1273-1280.
8. Gilmour, A. et al. (2009) ASReml User Guide Release 3.0.
9. Whalen, A., J. M. Hickey (2020) bioRxiv, 2020.2009.2016.299677.
10. Hickey, J. M. et al. (2011) Genet. Sel. Evol. 43, 1297-9686. **11.** Sargolzaei, M. et al. (2014) BMC Genomics 15, 478.

DYNAMICS OF MOORED CLOSED CONTAINMENT SYSTEMS WITH COUPLED WAVE LOADS, SLOSHING AND ELASTIC DEFORMATIONS

A. Tsarau*, B. Su, Y. Shen, R. Firoozkoohi and P. C. Endresen

SINTEF Ocean, P.O. Box 4762 Torgarden, 7465 Trondheim (Norway)
E-mail: andrei.tsarau@sintef.no

Introduction

Net pens for sea-based fish farming are prone to parasite infestations and can be hard on the environment. To control the production environment, closed cages with impermeable walls have been designed in various shapes, commonly with a vertical symmetry axis and a circular shape in the waterplane measuring 10-35 m in radius (). A hydraulic system is used to exchange water and to create an internal current. Scaled models of closed and semi-closed (i.e., with a permeable bottom) cages are shown in Fig.1.

Being designed to withstand waves with significant height (*Hs*) up to 2 m and peak period (*Tp*) up to 4-5 s (as along the Norwegian coast), closed cages can experience significant wave-induced loads and may suffer from sloshing (i.e., liquid movement in a tank with a free surface) [1]. Both effects are challenging to predict due to the coupling between the mooring system, cage motions, its elastic deformations, sloshing and internal currents. We analysed these coupling mechanisms and their effects separately in [2-5] and present some findings here.

The effect of an internal current on sloshing in an axisymmetric cage with a rotating liquid is mainly due to the Coriolis effect. Experiments (Fig.1c) show that this effect is significant when where is the current angular velocity (rad/s) [2]. Neglecting this effect implies either large cages (>25 m) or small current velocities The latter is assumed in our further analyses due to the lack of appropriate models handling rotating flows.

The effect of mooring system and cage motion is seen in Fig.2 showing the surge, pitch and sloshing responses from model tests of the two cages in Figs. 1a,b (with = 20.25 m and draft in full scale) in waves with *Hs* =1 m and a white-noise spectrum truncated at T = 3-25 s. Commonly for both the closed and semi-closed cages and in all tested irregular wave conditions, the surge motions (and thus the mooring forces) were found to be dominated by the slowly-varying wave drift loads near T_{moring}= 116 s, corresponding to the natural period of the cage-mooring system [3]. Thus, slowly-varying and mean wave forces have great importance in the design of mooring system for closed and semi-closed cages. Further, the pitch motions were also significant at high periods, including events of parametric instabilities due to in-and-out-of-water motions of the floater [4].

The coupling of sloshing and cage motion caused the local minima and maxima of the cage responses at the wave frequencies, as seen in Fig. 2 near the vertical lines at T_{11} and T_{12}, which are the natural periods of sloshing in a rigid circular cylinder with modal shapes shown in Fig. 3. According to linear potential theory, the sum of the cage mass (M) and the added mass due to sloshing (A) can be infinitely great near T_{11} and T_{12}, and therefore the surge motion is minimal at these periods. At somewhat lower periods, when $A < 0$ and $M + A \approx 0$, the cage motions increase dramatically, and the sloshing waves heights exceed the exterior wave components at the same periods (Fig. 2). Coincidentally, this occurred near T_{21} – another natural period of sloshing that cannot be linearly excited in rigid cages but can be coupled to deformations in elastic cages.

The coupled sloshing and elastic deformations of the closed cage in Fig. 1a were observed mainly for the radial deformation modes shown in Fig.3, causing resonant amplification of interior waves near T_{21}. A numerical analysis of this effect was attempted by combining the structural analysis program LS-DYNA with WAMIT. The model correctly predicted the resonant frequencies, interior waves and cage deformations elsewhere (Fig. 4), but not in the resonant zone where the free-surface nonlinearities might matter [4]. Further studies of hydroelasticity, as in [5], may provide useful insights, but certainly more research is needed on this problem.

Fig. 1. Floating closed (a) and semi-closed (b) cages and a fixed closed cage with an internal current (c).

(Continued on next page)

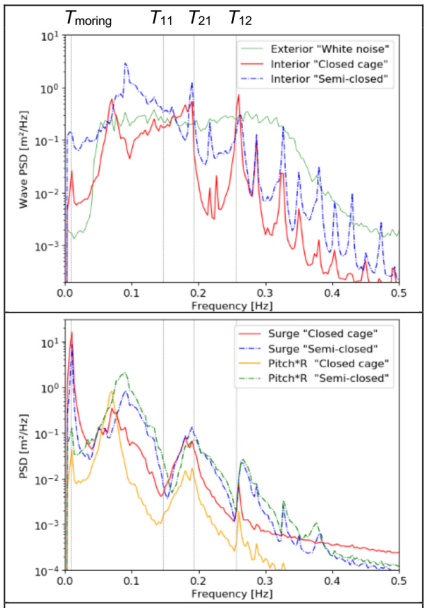


Fig. 2. Spectra of exterior and interior waves (upper) and cage motions (lower).

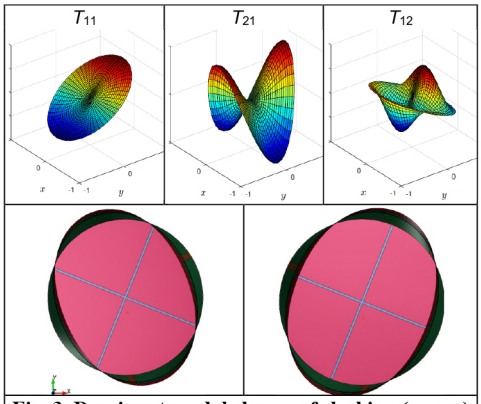


Fig. 3. Dominant modal shapes of sloshing (upper) and cage deformation (lower).

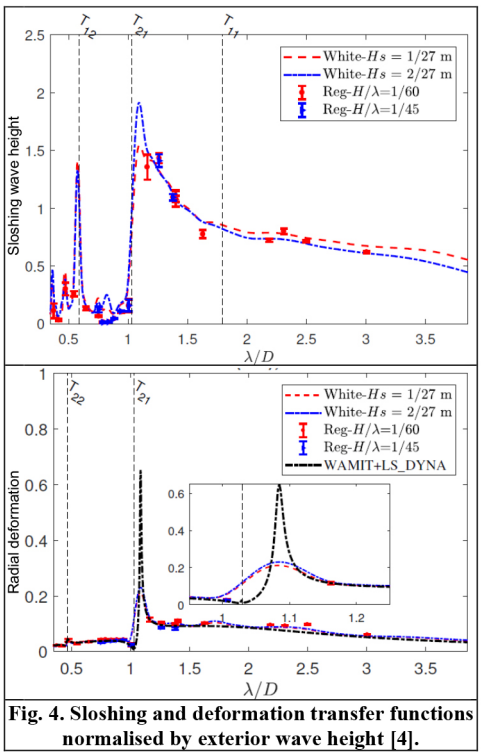


Fig. 4. Sloshing and deformation transfer functions normalised by exterior wave height [4].

Acknowledgement

This work was funded by the Norwegian Research Council's MAROFF program through grant no. 268402.

References

[1] Kristiansen D et al. Numerical and experimental study on the seakeeping behavior of floating closed rigid fish cages. OMAE; 2018.

[2] Tsarau A et al. Sloshing in a rotating liquid inside a closed sea cage for fish farming. Physics of Fluids; 2021.

[3] Su B et al. Numerical study of closed rigid fish cages in waves and comparison with experimental data. Under review in Ocean Engineering; 2021.

[4] Shen Y et al. Experimental investigation of a closed vertical cylinder-shaped fish cage in waves. Under review in Ocean Engineering; 2021.

[5] Strand IM & Faltinsen OM. Linear wave-induced dynamic structural stress analysis of a 2D semi-flexible closed fish cage. J. Fluids and Structures; 2020.

MYFISHCHECK: A MODEL TO ASSESS FISH WELFARE IN AQUACULTURE

Linda Tschirren[1,2,*], David Bachmann[1], Ali Cem Güler[1], Oliver Blaser[1], Nicola Rhyner[3], Andreas Seitz[1], Erich Zbinden[4], Thomas Wahli[2], Helmut Segner[2] and Dominik Refardt[1]

[1] Zurich University of Applied Sciences, Research Group for Aquaculture Systems, 8820 Wädenswil, Switzerland
[2] University of Berne, Centre for Fish and Wildlife Health, 3012 Bern, Switzerland
[3] Zurich University of Applied Sciences, Research Group for Environmental Genomics and Systems Biology, 8820 Wädenswil, Switzerland
[4] Zurich University of Applied Sciences, Research Group for Knowledge Engineering, 8820 Wädenswil, Switzerland

P.O. Box, Campus Grüental, 8820 Wädenswil (Switzerland)
E-Mail: linda.tschirren@zhaw.ch

Welfare in animal husbandry includes considerations of biology, ethics, ecology, law and economics. These diverse aspects must be translated into common quantifiable parameters and applicable methods to objectively assess welfare in animals. To assist this process in the field of aquaculture, where such methods are largely missing, we developed a model to assess fish welfare. A network of information was created to link needs, i.e. fundamental requirements for welfare, with parameters, i.e. quantifiable aspects of welfare. From this ontology 80 parameters that are relevant for welfare, have practicable assessment methods and deliver reliable results were selected and incorporated into a model. The model, named MyFishCheck, allows the evaluation of welfare in five distinct modules: farm management, water quality, fish group behaviour, fish external and fish internal appearance, thereby yielding five individual grades categorising welfare from critical, poor, acceptable to good. To facilitate the use of the model, a software application was written. With its adaptability to different fish species, farming systems, regulations and purposes as well as its user-friendly digital version, MyFishCheck is a next step towards improved fish welfare assessment and provides a basis for ongoing positive developments for the industry, the farmers and the fish.

Introduction

The aquaculture industry is in need of adequate methods for animal welfare assessment and the work presented is a next step towards this goal. The model described incorporates the specific advantages of existing welfare assessment attempts (Stien et al. 2013; Pettersen et al. 2014; Noble et al. 2018; Saraiva et al. 2019; Studer et al. 2020) in a single application. We focus on three key requirements:

Comprehensiveness: (I) We incorporate parameters from function-, nature- and feelings-based welfare concepts. This ensures an inclusive assessment that is unaffected by the potentially incomplete knowledge about welfare or bias of the assessor. (II) We assess the overall welfare in five modules (farm management, water quality, fish group behaviour, fish external and fish internal appearance). By not abstracting a high-resolution assessment into one overall index, the five distinct module grades facilitate the identification of potential causes of welfare problems. (III) With at least ten parameters per module we ensure sufficient coverage of signs of and prerequisites for welfare to allow an interpretation of the welfare state of the fish.

Applicability: (I) We ensure the applicability of the model by selecting the parameters based on three characteristics: science-based relevance for welfare, practicability of existing measuring methods and reliability of the results delivered. (II) The model can be used with only a subset of the modules or the parameters, enabling a flexible and purpose-oriented use. Scientists can benefit from a comprehensive model that allows a detailed assessment of fish welfare, while a simplified version of the same model has an increased practicability that assists fish farmers in their daily routines. (III) We provide a user-friendly version of the model by means of a software application. The users can profit from an efficient parameter evaluation and standardised documentation, which is important and should be as easy and intuitive as possible.

Developability: (I) Parameters that need to be adapted to specific fish species, production systems or local regulations in order to deliver meaningful results are highlighted. This facilitates future adaptation of the model to other species, systems or countries. (II) We provide access to the digital ontology the model is based on. This enables the inclusion of new knowledge by making it easy to adjust existing needs, parameters or relationships and to add new ones when pertinent.

Model development

The model development consisted of five phases (Fig. 1) where first a digital information network, an ontology, for fish welfare was created. On this basis welfare parameters were selected and grouped into five modules. In a third phase, a literature review and an expert survey were conducted to define the parameter intervals, scores and weights. These were incorporated into a mathematical equation delivering one grade per module. As a last step, two different applications were developed.

(Continued on next page)

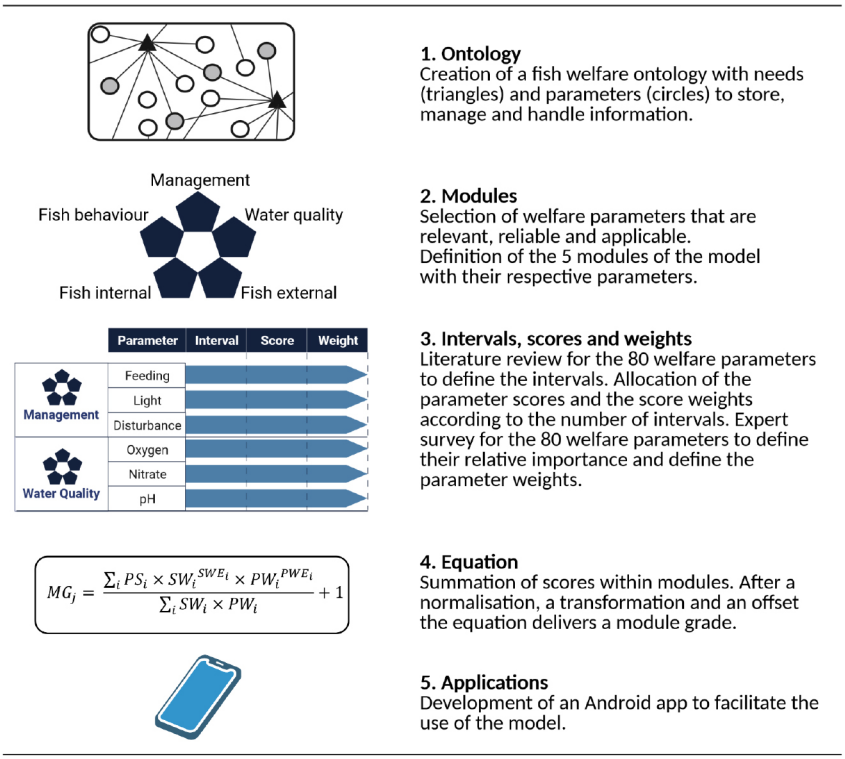


Figure 1: Graphical abstract of the model development of MyFishCheck

Conclusion

The MyFishCheck model developed here allows researchers to assess fish welfare based on the full model in a standardised and efficient way. This enables representative surveys of the whole industry, evaluations of measures across farms and validation of theoretical ideas or lab trials in practice. Initial tests on six different farms showed that the model is applicable on different fish species, different aquaculture systems and different locations. In addition, the available Microsoft Excel version of the model facilitates its use in science. Further, the model allows fish farmers to perform regular controls based on a customised version of the model as part of their quality control management. This enables the documentation of on-farm welfare standards, the tracking of improvements and the tracing of problems. During the testing, the model reliably produced lower module grades where parameters showed negative effects on welfare. Additionally, the app enables the user to perform these single-point evaluations more conveniently and to store, evaluate and compare past assessments. The model represents a next step towards a standardised evaluation of welfare, a digital documentation of assessments and a widespread application of welfare assessments. MyFishCheck will both in its current form as well as in future adaptations serve the field of aquaculture by assisting advancements for the common goal of better fish welfare.

References

Noble EC, Gismervik K, Iversen MH, Kolarevic J, Nilsson J, Stien LH, Turnbull JF (2018) Welfare indicators for farmed Atlantic salmon - tools for assessing fish welfare. Nofima, Tromsø, Norway.

Pettersen JM, Bracke MBM, Midtlyng PJ, Folkedal O, Stien LH, Steffenak H, Kristiansen TS (2014) Salmon welfare index model 2.0: An extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. Reviews in Aquaculture 6: 162–179.

Saraiva JL, Arechavala-López P, Castanheira MF, Volstorf J, Studer BH (2019a) A global assessment of welfare in farmed fishes: The FishEthoBase. Fishes 4: 30.

Stien LH, Bracke MBM, Folkedal O, Nilsson J, Oppedal F, Torgersen T, Kittilsen S, Midtlyng PJ, Vindas MA, Øverli Ø, Kristiansen TS (2013) Salmon Welfare Index Model (SWIM 1.0): A semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Reviews in Aquaculture 5: 33–57.

Studer BH, Castanheira M-F, Arechavala-López P, Volstorf J (2020) Development of practical fish welfare criteria for aquaculture. Fair-Fish International Association, Denens, Switzerland.

EFFECTS OF DIETARY SUPPLEMENTATION OF COPPER ON GROWTH, SURVIVAL, ANTIOXIDANT STATUS AND BONE DEVELOPMENT IN GILTHEAD SEABREAM (*Sparus aurata*) LARVAE

Y. Tseng[1]*, D. Domínguez[1], U. Sivagurunathan[1], K.M. Eryalçın[1,2], C.M. Hernández-Cruz[1], P. Antony Jesu Prabhu[3], P. Eckhard Witten[4] and M. Izquierdo[1]

[1]Grupo de Investigación en Acuicultura (GIA), University Institute Ecoaqua, University of Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[2]Istanbul University Faculty of Fisheries, Aquaculture Department, Phytoplankton and Zooplankton Culture Laboratory, Ordu Cad. No: 200, Laleli, 34470, Istanbul, Turkey
[3] Institute of Marine Research (IMR), Fish Nutrition Program, Bergen, Norway
[4]Department of Biology, Ghent University, Ghent, Belgium

Email: tyiyen@gmail.com

Introduction

Generally, rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) are commonly used as live prey for marine fish larvae in comparison with copepods due to the existence of standardized cost-effective protocols for their mass production (Conceição et al., 2010). However, their nutrient profile is deficient, particularly in many essential minerals (such as copper, zinc and selenium) in both rotifer and artemia in commercial hatcheries compared to copepods. The mineral profile of these live prey is not sufficient to cover the requirements of marine fish including gilthead seabream (*Sparus aurata*) (NRC, 2011; Hamre et al., 2008). Moreover, the nutrient composition of live prey is difficult to maintain and control during the long period in the larval rearing tanks. Therefore, it is necessary to establish balanced microdiets for early stage marine fish larvae.

Copper (Cu) is an essential trace element for survival, development, and normal growth in fish (NRC, 2011; Domínguez et al., 2019). Additionally, a Cu-dependent enzyme, lysyl oxidase (LOX) mediates the final step in the biosynthesis of collagen and normalizes the deposition of calcium and phosphorus in bones which impact the bone integrity (Tomaszewska et al., 2017). Cu deficiency caused low torsional strength of bone in chick tibia with the low lysyl oxidase activity (Opsahl et al., 1982). In fish, the copper deficiency resulted in reduced growth, high mortality and induced oxidative stress (Domínguez et al., 2019; NRC, 2011). Additionally, the negative results of copper excess caused reduced fish growth, increased oxidative risk, hepatic damage and cholestasis (Domínguez et al., 2019). Moreover, a high exposure of copper to larvae impact skeletal deformities (Barjhoux et al., 2012). Therefore, supplementation of optimal copper level in diet is necessary to fulfill the nutritional requirement of fish and to avoid the risk of toxicity. However, the information about mineral nutrition in marine fish larvae, and particularly gilthead seabream is still scarce. Hence the aim of this study was to investigate the effect of the dietary supplementation of Cu on growth, survival, antioxidant status and bone development in gilthead seabream larvae.

Materials and Methods

Diets. Copper sulfate pentahydrate ($CuSO_4.5H_2O$) was used as the copper source, supplemented in the basal diet composed of squid meal and casein at the levels of 0, 1.3, 2.3, 3.3 mg/kg in the isoenergetic and isonitrogenous diets. To contain a total analyzed levels of 17, 18, 19, 25 mg Cu/kg, respectively. ***Fish and experimental conditions.*** Larvae (initial dry weight 0.46 ± 0.09 mg, total length 7.9 ± 1.61 mm, 26 dph) were randomly distributed into twelve tanks (2100 individuals/ 200 L FRP tank) and hand fed every 45 minutes from 8:00 am to 8:00 pm until 46 dph. ***Sampling.*** Growth was monitored by measuring the weight and length of larvae at three different points: initial (26 dph), intermediate (33, 40 dph) and final (46 dph). At the final sampling, larvae were collected for histology, whole mount stain, gene expression, TBARS and remaining larvae for mineral analysis. Daily mortality was calculated for survival rate. ***Statistics.*** All data were tested for normality and homogeneity of variances and means compared by Ducan test and one-way ANOVA ($P < 0.05$). Quadratic regressions were used to establish a relation between dietary Cu level and their effect on the different indicators.

Results

Larvae fed the non-supplemented diet (Cu 17) showed the highest malondialdehyde (MDA) among the treatments (fig 1.a.). The antioxidant gene expression - cat was lowest in larvae fed the non- supplemented diet (Cu 17) (fig 1.b.). Severe skeletal anomalies and growth did not have significant differences when larvae were fed different dietary Cu levels ($p > 0.05$).

(Continued on next page)

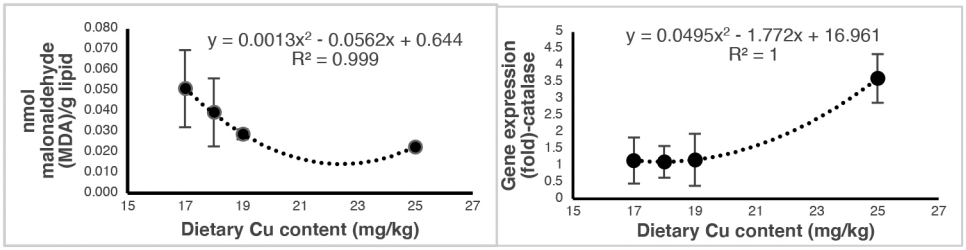


Figure 1 Thiobarbituric acid reactive substances (TBARS) - MDA (a) and antioxidant gene expression - *cat* (b) of gilthead seabream larvae (46 dph) fed different diets ($p < 0.05$)

Discussion and conclusion

Larvae fed diets with different levels of Cu (17-25 mg/kg) showed no significant differences in growth. However, in gilthead seabream fingerlings 5.5 mg Cu/kg were enough to cover their requirements for growth when fed a diet with only 10% fish meal and 75% terrestrial meals (Domínguez et al., 2019). This points out that small fish need higher nutrient requirements than larger fish. Despite dietary Cu did not have a significant effect on growth, there was a tendency to increase survival rate with increasing dietary Cu levels ($y = 0.0143x^2 + 0.1361x + 60.224$, $R^2 = 0.9975$). Additionally, a significant correlation between the DHA content in larvae and survival rate was found in the present study ($y=0.117x^2-4.3085+106.05$, $R^2=0{\cdot}9469$). Copper deficiency caused low torsional strength of bone in chick tibia with the low lysyl oxidase activity (Opsahl et al., 1982). However, no significant differences of severe skeletal anomalies were observed in the present study. Moreover, growth performance and skeletal anomalies are not the only indicator to quantify mineral requirements in fish. TBARS analysis is one of the most popular and commonly used methods to study tissue peroxidation. In contrast, to prevent oxidative damage, antioxidant enzymes such as *cat* (catalase) intercept reactive intermediates in the oxidant reactions. It has been demonstrated that either deficient or excess dietary Cu induced oxidative stress (Domínguez et al., 2019). In the present study, larvae fed the lowest Cu diet (17 mg/kg) showed the highest degree of the TBARS value and lowest *cat* gene expression. Overall, based on these results it is suggested that it is necessary to supplement Cu in the diet and the adequate dietary Cu concentration (25 mg/Cu kg) increased the antioxidant defense in gilthead seabream larvae (46 dph), while supplementing up to 25 mg/ Cu kg diet caused no negative effects on the growth.

Acknowledgment

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.

References

Barjhoux, I., Baudrimont, M., Morin, B., Landi, L., Gonzalez, P., Cachot, J., 2012. Effects of copper and cadmium spiked-sediments on embryonic development of Japanese medaka (*Oryzias latipes*). Ecotoxicol. Environ. Saf. 79, 272e282

Conceição LEC, Yúfera M, Makridis P, Morais S, Dinis MT (2010) Live feeds for early stages of fish rearing. Aquaculture Research 41: 613– 640.

Domínguez, D., Sarmiento, P., Sehnine, Z., Castro, P., Robaina, L., Fontanillas, R., Prabhu, P.A.J., Izquierdo, M. (2019). Effects of copper levels in diets high in plant ingredients on gilthead sea bream (*Sparus aurata*) fingerlings. Aquaculture, 507, 466–474. https ://doi.org/10.1016/j aquaculture.2019.04.044

Hamre K., Srivastava A., R_nnestad I., Mangor-Jensen A. & Stoss J. (2008) Several micronutrients in the rotifer *Brachionus* sp may not fulfill the nutritional requirements of marine fish larvae. Aquaculture Nutrition14,51^60.

NRC. (2011) Nutrient Requirements of Fish. National Research Council, National Academy Press, Washington, DC.

Opsahl W, Zeronian H, Ellison M, Lewis D, Rucker RB, Riggins RS. Role of copper in collagen cross-linking and its influence on selected mechanical properties of chick bone and tendon. J Nutr 1982;112:708–16

Tomaszewska, E., Dobrowolski, P., Kwiecień, M., Winiarska-Mieczan, A., Tomczyk, A., Muszyński, S., 2017. The influence of dietary Cu-glycine complex on histomorphology of cancellous bone,articular cartilage and growth plate, bone mechanical and geometric parameters is dose-dependent. Biol. Trace Elem. Res., 178: 54–63.

THE FIRST COMPLETE GENOME ASSEMBLY FOR THE MEAGRE *Argyrosomus regius*

V. Papadogiannis[1], T. Manousaki[1], J. Kristoffersen[1], A. Tsakogiannis[1], O. Nousias[1], C.C. Mylonas[1], D. Chatziplis[2], C. Batargias[3], C. S. Tsigenopoulos[1]

1. Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Crete, Greece
2. Department of Agriculture, International Hellenic University, Sindos, Thessaloniki, Greece
3. Department of Animal Production, Fisheries and Aquaculture, University of Patras
E-mail: v.papadog@hcmr.gr

Introduction

High-quality genome information is nowadays considered as a prerequisite of major significance in any animal and plant selection and breeding objectives. The meagre *Argyrosomus regius* is a fish species of elevated economic interest for the Mediterranean aquaculture in recent years, and there is ongoing effort to increase the efficiency of breeding performance. For this purpose, access to the full genomic sequence of the species would provide an important resource for exploring loci associated with quantitative traits and the genetic diversity of different wild populations and broodstocks. Here, we present the first complete nuclear genome for *A. regius*, which has been produced through a combination of long and short read technologies. This sequencing strategy, coupled with an efficient pipeline for assembling and polishing the genome, led to a contiguous, high-quality genome that provides an excellent base for future genomic studies in meagre.

Materials and Methods

Genome sequencing was carried out via a combination of third generation long read (Oxford Nanopore MinION) technology that provides contiguous long reads and a second generation (Illumina HiSeq4000) platform that produces short reads with high fidelity, which can be exploited to correct the former. Thus, a base assembly was first built using the Flye Assembler[1] and polished from the long-read data, followed by polishing and error correction using the low error short read data (Racon[2], Medaka (https://nanoporetech.github.io/medaka/), Pilon[3]), while all intermediate and final assemblies were assessed for the quality in terms of contiguity and completeness (Quast[4], Busco[5], Merqury[6]). This pipeline has been containerised in house, and is freely available (https://nellieangelova.github.io/De-Novo_Genome_Assembly_Pipelines/). Transcriptome sequencing was performed using total RNA extracted from 8 different tissues (listed in fig.1) on the Illumina HiSeq4000 platform. From these data, a final consensus transcriptome was constructed using Mikado[7], incorporating intron junction, ORF prediction and homology information. Augustus[8] and PASA[9] were then used to carry out gene prediction based on the genome and transcriptome assemblies.

Results and Discussion

The MinION platform produced a total of 38,119,965,327 bp, with more than 99.5% passing quality control, giving a final 54.5× genome coverage and 3,003,301 sequences with a mean length of 12,640 bp. Illumina sequencing produced a total of 166,912,732 sequences of 150 bp, with more than 85.8% passing quality control, giving a 30.9× genome coverage. Transcriptome sequencing produced more than 100,000,000 sequences of 150 bp per tissue sample, with more than 90% of them passing quality control. The genome assembly produced from the long and short read data consists of 1,012 contigs, totalling 696,249,749 bp in size, with an N50 of 2,798,312 bp and an L50 of 23. This high level of contiguity is coupled with high completeness scores from both Merqury (96.9%, based on Illumina data kmer counting) and BUSCO (98.7%, based on conserved gene presence/absence), while the final consensus transcriptome also shows comparably high completeness (BUSCO 96.3%). Based on these data, the gene prediction pipeline annotated a total of 24,589 genes with a mean size of 16,437bp and a final BUSCO score of 95.6%. The constructed reference genome will set the basis for gaining deeper understanding of meagre biology and will boost the efforts for selective breeding through genomic selection.

(Continued on next page)

Genome

Sequencing		
	MinION	Illumina
Number of reads	3,003,301	143,224,530
% Reads Pass	99.94	85.81
Mean read length	12,640.4	150
Read length N50	30,600	-
Total bases	37,962,807,176	21,483,679,500
Coverage	54.5	30.9

Genome Assembly			
Length	696,249,749bp (approx. 6.96 Mb)		
N50	7,813,463bp (approx.7.8 Mb)		
N75	2,798,312 bp		
Number of contigs	1,012		
L50	23		
L75	61		
Merqury Completeness Score	96.9147	Genome Repeat Content	25.90%

Transcriptome

Tissue	Trimmed reads	% reads pass
brain	126,387,852	90.20
gills	107,109,570	91.06
gonad	126,296,102	90.50
heart	113,954,698	91.09
liver	139,570,152	92.46
muscle	137,815,166	90.62
skin	127,358,490	91.18
spleen	131,482,808	90.74

BUSCO

	Genome	Transcriptome	Gene Prediction
BUSCO Score	98.70%	96.3	95.6
Complete BUSCOs	98.40%	95.30%	93.90%
Fragmented BUSCOs	0.40%	1.00%	1.70%
Missing BUSCOs	1.30%	3.70%	4.40%
total BUSCOs searched	3,640	3640	3640

Gene Prediction

Type	Number	Size total (kb)	Size mean (bp)
gene	24,589	404,183.91	16,437.59
transcript	49,553	982,213.56	19,821.48
cds	589,463	97742.68	165.82
exon	637,465	165,915.70	260.27
five_prime_utr	83,325	15,474.94	185.72
three_prime_utr	53,919	52,698.07	977.36

Fig.1 Sequencing and assembly metrics and statistics.

Acknowledgments

The study has received funding from the Greek Republic through the "MeagreGen" project under the call "Special Actions – AQUACULTURE" in the Operational Program "Competitiveness Entrepreneurship and Innovation 2014-2020".

References

1. Kolmogorov, M., Yuan, J., Lin, Y. & Pevzner, P. A. Assembly of long, error-prone reads using repeat graphs. *Nat. Biotechnol*. **37**, 540–546 (2019).
2. Vaser, R., Sović, I., Nagarajan, N. & Šikić, M. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res*. **27**, 737–746 (2017).
3. Walker, B. J. *et al*. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* **9**, (2014).
4. Gurevich, A., Saveliev, V., Vyahhi, N. & Tesler, G. QUAST: Quality assessment tool for genome assemblies. *Bioinformatics* **29**, 1072–1075 (2013).
5. Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. M. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**, 3210–3212 (2015).
6. Rhie, A., Walenz, B. P., Koren, S. & Phillippy, A. M. Merqury: Reference-free quality, completeness, and phasing assessment for genome assemblies. *Genome Biol*. **21**, 1–29 (2020).
7. Venturini, L., Caim, S., Kaithakottil, G. G., Mapleson, D. L. & Swarbreck, D. Leveraging multiple transcriptome assembly methods for improved gene structure annotation. *Gigascience* **7**, 1–15 (2018).
8. Stanke, M. & Morgenstern, B. AUGUSTUS: A web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Res*. **33**, 465–467 (2005).
9. Haas, B. J. *et al*. Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. *Nucleic Acids Res*. **31**, 5654–5666 (2003).

EFFECTS OF TWO DIETS WITH DIFFERENT PHYTOESTROGEN CONTENT ON BLOOD PARAMETERS AND GROWTH PERFORMANCE OF SEA BREAM *Sparus aurata*

A. Tsopelakos[1*], M. Athanasopoulou[1], C. Zantioti[1], D. Pavlopoulos[2], S.D. Koulocheri[2], E. Chatzoglou[1], E.D. Myrtsi[2], S.A. Haroutounian[2] and H. Miliou[1]

[1] Laboratory of Applied Hydrobiology, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, Votanikos 118 55, Athens, Greece
[2] Laboratory of Nutritional Physiology and Feeding, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, Votanikos 118 55, Athens, Greece
E-mail: aristsopelakos@aua.gr

Introduction

Sea bream (*Sparus aurata*) is one of the most commercially important aquaculture species. The increased incorporation of plant raw materials in the diets contributes to the increase of their phytoestrogen content. Major phytoestrogens found in fish feeds include genistein, daidzein, biochanin A and coumestrol (Matsumoto et al. 2004). These compounds have been associated with estrogenic potencies in fish (Pelissero and Sumpter 1992). Their metabolites may also possess estrogenic activity, with equol to be superior to all other isoflavones in its antioxidant activity (Rowland et al. 2000). The estrogenic effects are found to include alterations in lipid metabolism, plasma triglyceride and cholesterol levels, as well as overall growth rates (Kaushik et al. 1995; Chakraborty et al. 2014). The aim of this study was to estimate the effects of two diets with different phytoestrogen content on blood parameters and somatic indices 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 and fish fillets 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, consisting of six 147-L tanks (three replicates/treatment, 12 fish/tank). Fish were fed at satiation twice a day. At the end of the experiment, the following parameters were estimated: body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), viscerosomatic index (VSI), hepatosomatic index (HSI) and Fulton's condition factor (K). Blood samples were also collected from the caudal vein and stored in heparinized tubes. Blood samples were centrifuged and plasma was collected, for the assessment of cholesterol (CHOL), glucose (GLU), albumin (ALB), alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH) and triglycerides (TG) using commercial kits (Biosis).

Results

VSI and HSI were significantly higher in the low- (diet B) than in the high- (diet A) phytoestrogen treatment. In addition, daily feed consumption per fish was significantly higher in treatment B compared to A during the 3rd month of rearing, while FCR was lower during the 1st month. Condition factor, body weight and SGR were similar in both treatments. Plasma ALB, TG, CHOL, ALP and GPT levels were higher in fish fed the diet B compared to A, whereas no significant differences for GLU, LDH and GOT levels were observed. Equol was detected in certain fish of high-phytoestrogen treatment.

(Continued on next page)

Discussion

Fish fed the low-phytoestrogen diet demonstrated a trend for decreased FCR, indicating a better utilization of feed. It has been shown that the use of diets with high concentrations of phytoestrogens resulted in relatively low growth rates of fish (Chakraborty et al. 2014). According to previous studies, high inclusion of plant-derived raw materials, reduces liver lipids (Dias et al. 2005; Torno et al. 2019), leading to reduced VSI and HSI, similar to the present results. The low levels that were observed for ALB in treatment A, may be attributed to its role in the regulation of plant-deriving exogenous compounds to estrogen receptors (Baker 2002). Plasma CHOL and TG levels were decreased in high phytoestrogen dietary treatment, as has also been found in trout (Kaushik et al. 1995) and sea bass (Dias et al. 2005). The low ALP concentrations are in accordance with previous study on sturgeon, where decreased levels were observed in treatments with elevated isoflavonic phytoestrogens (Yousefi Jourdehi et al. 2014). The increased GPT and ALP levels in the low-phytoestrogen treatment may be related to the liver lipid accumulation (Michael et al. 1987). In conclusion, the differences in dietary phytoestrogen content, affect significantly blood parameters and somatic indices. However, these results could also be attributed to differences in dietary fat content and feed consumption. It has been suggested (Rowland et al. 2000) that the dietary fat intake decreases the capacity of gut microbiota to synthesize equol in humans, contributing to an interindividual variation in isoflavone metabolism, as in the present study. Further experimentation is required in order to clarify if the observed results were due to differences in dietary phytoestrogen concentrations, macronutrient content, gut microbiota, metabolites or interactions thereof.

References

Baker, M. (2002). Beyond carrier proteins. Albumin, steroid hormones and the origin of vertebrates. J. Endocrinol. 175: 121-127.

Chakraborty, S.B., Horn, P., Hancz, C. (2014). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Rev. Aquac. 6: 1-19.

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M., Kaushik, S.J. (2005). Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. A 142: 19-31.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M. (1995). Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133: 257-274.

Matsumoto, T., Kobayashi, M., Moriwaki, T., Kawai, S., Watabe, S. (2004). Survey of estrogenic activity in fish feed by yeast estrogen-screen assay. Comp. Biochem. Physiol. C 139: 147-152.

Michael, M., Hilmy, A., El-Domiaty, N., Wershana, K. (1987). Serum transaminases activity and histopathological changes in *Clarias lazera* chronically exposed to nitrite. Comp. Biochem. Physiol. C 86, 255-262.

Pelissero, C., Sumpter, J.P. (1992). Steroids and "steroid-like" substances in fish diets. Aquaculture 107: 283-301.

Rowland I.R, Wiseman H., Sanders T.A.B., Adlercreutz H., Elizabeth A. Bowey E.A. (2000). Interindividual variation in metabolism of soy isoflavones and lignans: Influence of habitual diet on equol production by the gut microflora. Nutr. Cancer, 36:1, 27-32.

Yousefi Jourdehi, A., Sudagar, M., Bahmani, M., Hosseini, S. A., Dehghani, A. A., Yazdani, M.A. (2014). Comparative study of dietary soy phytoestrogens genistein and equol effects on growth parameters and ovarian development in farmed female beluga sturgeon, *Huso huso*. Fish. Physiol. Biochem. 40: 117-128.

Acknowledgments

This work is part of EstroFish project (MIS: 5052097) that is co-financed by Greece and EU under the "Operational Programme Competitiveness, Entrepreneurship and Innovation - EPAnEK 2014-2020".

GROUP REGULATION AND ENVIRONMENTAL OUTCOME: EVIDENCE FROM ZONAL OUTPUT RESTRICTION IN NORWEGIAN AQUACULTURE

Ragnar Tveterås*, Tenaw G. Abate, Dagim G. Belay

University of Stavanger Business School, 4036 Stavanger, Norway
Email: ragnar.tveteras@uis.no

Introduction

Sea lice parasite has become a main environmental challenge for salmon farming. Sea lice creates concerns both for the welfare of farmed fish and the stocks of wild salmonids, as it can lead to increased mortality both for farmed salmon and wild salmon. In Norway sea lice are regulated both at the farm level and production area level, the latter through the so-called "traffic light system". A sea lice regulation at the farm site level was introduced in 2013. In 2017, the government of Norway in addition introduced the traffic light system (TLS), that divides the Norwegian coast into 13 aquaculture production zones and labels them 'Green', 'Yellow', or 'Red' based on the estimated sea-lice-induced mortality rates on the wild stock. If a production zone is labeled 'Green'/'Red', production capacity should increase/decrease by 6% whereas 'Yellow' means the current level of output should be maintained.

The research questions we ask are as follows: (1) Does output restriction improve environmental outcomes? Even though it seems that output restriction would automatically improve environmental outcomes, Helfand (1991) analytically outlined that the effect is not straightforward. (2) If output restriction improves environmental outcomes, does the improvement come at the cost of economic outcomes such as firms' efficiency loss and/or reduction in profits?

Empirical analysis

We use two data sources to empirically analyze the above research questions: (1) Publicly available weekly data on lice counts at farm level and other variables by The Norwegian Food Safety Authority (NFSA), the Norwegian Veterinary Institute and the Directorate of Fisheries. (2) Firm-level annual economic data (production data) from the Norwegian Directorate of Fisheries

For the first research question we have two complementary empirical strategies to establish a clear causal relationship between the TLS and environmental and economic outcomes, and obtain unbiased estimates. First, we have a regression discontinuity design in time (RDiT), where we explore the temporal aspect using 'week' as a running variable and the treatment date (week) as a threshold. When we estimate econometric RDiT models on the lice count

$$y_{it} = \gamma_0 + \gamma_1 1(TLS) + \gamma_2 x_{it} + \varepsilon_{it}$$

we find that TLS leads to a significant reduction in adult female sea lice count.

Second, we use a Difference in difference (DiD) estimation approach to explore the spatial aspect by exploiting variations in the intensity of output restriction. When we estimate econometric DID models on the lice count

$$y_{it} = \beta_0 + \beta_1 TLS + \boldsymbol{\beta_2} post_t \cdot TLS_{it} + \beta_3 x_{it} + \eta_t + \alpha_i + \vartheta_z + \varepsilon_{it}$$

we find that the introduction of TLS leads to a significant reduction in adult female sea lice count.

To address the second research question on the economic effect of sea lice restriction we undertake an econometric analysis of production costs and profits. We estimate cost functions and profit functions which enable us to separate the effects of input prices, scale economies, technical change and external effects on production costs in salmon farming. The results are mixed. Overall, an increase in sea lice prevalence is associated with an increase in production costs. However, the effect is only statistically significant when full production costs is the dependent variable. For profits the effect of an increase in sea lice prevalence is significantly negative when we estimate a pooled model, i.e. lower sea lice prevalence is associated with higher profits, but not significantly different from zero when we include firm-specific effects in the profit function.

(Continued on next page)

Table. Estimated mean elasticity with respect to average adult female sea lice per salmon from translog cost and profit functions

Model	Estimate	Std.error	t-value	p-value
Pooled cost	0.019	0.013	1.53	0.127
Firm effects cost	0.015	0.012	1.20	0.229
Pooled full cost	0.044	0.016	2.72	0.006
Firm effect full cost	0.030	0.017	1.73	0.084
Pooled profit	-0.152	0.072	-2.12	0.034
Firm effect profit	0.027	0.030	0.92	0.358

Conclusions

Using unique data from this policy experiment, we investigate whether sea lice regulation improves environmental outcomes and/or affects firms' cost efficiency and profit. We take advantage of spatial and temporal variation in the regulation's implementation to show that a threat of zonal output restriction did reduce the prevalence of sea lice in all production zones across different specifications. Difference-in-difference estimations show that a greater reduction in the prevalence of sea lice is observed in 'Red' labeled zones compared to 'Green' and 'Yellow' zones. Evidence from estimation of cost functions and profit functions indicate that the introduction of the TLS resulted in cost increases and a reduction in profit.

POTENTIAL INTERACTION BETWEEN VITAMIN D AND VITAMIN K IN RELATION TO GROWTH, CALCIUM METABOLISM AND BONE MAINTENANCE IN GILTHEAD SEABREAM JUVENILE (*Sparus aurata*)

U. Sivagurunathan[1]*, Yiyen Tseng[1], D. Dominguez[1], L. Robaina [1], C. Boglione[2] and M. Izquierdo[1]

[1]Grupo de Investigación en Acuicultura (GIA), University Institute Ecoaqua, University of Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[2]Biology Department, Laboratory of Experimental Ecology and Aquaculture, Biology Department, University of Rome Tor Vergata, Rome, Italy
Email: siva05.guru@gmail.com

Introduction

Studies on plant-based ingredients have been increasing for aquafeed production due to the diminished availability and increasing price of high-quality fish meal and fish oils (FAO, 2020). This alternative source of plant-based ingredients may alter the nutritional profile of the produced aquafeed, which directly affects the nutritional requirement of the fish (Francis et al, 2001) including vitamins, which play a major role in growth, health, and reproduction in fish. Vitamin requirements for various fish species have been established in NRC, 2011, but the requirement level for gilthead seabream (*Sparus aurata*) was still unknown.

Gilthead seabream is one of the most cultured fish species in the European Union (EU) aquaculture system and contributes around 6.77% of all European aquaculture production (APROMAR, 2020). Even though the production rate is high, the gilthead seabream is often detected with skeletal anomalies during the culture period which affects the production cost, growth, and fish survival rate (Andrades *et al*., 1996). There are several studies on skeletal anomalies in gilthead seabream based on developmental stage, temperature, genetic background and nutrition (Boglione and Costa, 2011, Izquierdo *et al*., 2016). Therefore, the present study focused on the micronutrients, especially vitamin D and vitamin K, due to the lack of information about these vitamins on gilthead seabream juveniles.

Vitamin D (VD) and Vitamin K (VK) are fat soluble vitamins, which deliver synergistic effects on calcium (Ca) metabolism, skeletal development, and mineralization of bones. In fish, the source of vitamin D depends on the dietary intake, and it helps in calcium deposition and skeletal development (Lock *et al*., 2010), while vitamin K helps in posttranslational modification and activation of the vitamin K-dependent proteins, that eventually help in skeletal development of fishes (Krossøy *et al*., 2011). This suggests a pleiotropic effect in vitamin D and K, that helps in understanding the interaction of these vitamins in growth and skeletal development of fish. Thus, the aim of the study was to evaluate the potential interaction between vitamin D and K in skeletal development and to identify the optimum requirement of these vitamins in gilthead seabream juveniles.

Materials and methods

Seven different isoenergetic and isonitrogenous plant-based diets (FM – 10%, FO – 6%) were formulated with increasing levels of VD_3 and VK_3 (Table 1,2). Gilthead seabream juveniles with initial weight of 72.63 ± 0.33 g were randomly distributed into 21 tanks in triplicate groups and manually fed 2% body weight until apparent satiation for 105 days. Every two weeks, fish were sampled for growth parameters such as length and weight. Water quality and feed intake were monitored throughout the experiment. At the end of the trial, fish were analysed for growth performance, protein utilization and body indices. Samples were taken for X-ray, histology, gene expression and vitamin analyses.

Results

After a period of 105 days feeding, fish doubled the weight, in which the final body weight (FBW) showed no significant difference (P – 0.19) among the groups by one-way ANOVA. But with respect to two-way ANOVA the VD*VK interaction showed a significant difference (P – 0.002) between treatments. Other parameters such as Feed conversion ratio (FCR), Specific growth rate (SGR), Feed intake (FI), showed no significant difference in one-way ANOVA as well as no interaction effect ($P \leq 0.05$) by two-way ANOVA among the groups, except for Protein efficiency ratio (PER) which showed significant differences among the groups in both statistical tests (Table 2).

(Continued on next page)

Table-1: 3x4 factorial design of supplemented dietary VD_3 and VK_3 mg/kg of diet.

		Vitamin K_3 (mg/kg)		
		0	**6**	**12**
Vitamin D_3 (mg/kg)	**0**	0,0	-	-
	0.04	-	0.04,6	0.04, 12
	0.08	-	0.08, 6	0.08, 12
	0.50	-	0.50, 6	0.50, 12

Table-2: Supplemented dietary VD_3 and VK_3 mg/kg of diet

DIET	VDK1	VDK2	VDK3	VDK4	VDK5	VDK6	VDK7
Supplemented dietary VD_3 (mg/kg)	0	0.04	0.04	0.08	0.08	0.50	0.50
Supplemented dietary VK_3 (mg/kg)	0	6	12	6	12	6	12

Table-3: Growth performance and feed utilisation of *Sparus aurata* fed experimental diets.

Growth Indices	VDK1	VDK2	VDK3	VDK4	VDK5	VDK6	VDK7	*ONE-way* ANOVA (*P* value)	*TWO-way* ANOVA (*P* value)
FBW (g/fish)	148.36 ± 6.34	147.99 ± 2.26	150.54 ± 3.45	148.11 ± 6.09	139.75 ± 5.16	141.64 ± 9.35	151.61 ± 5.78	0.19	0.002
WG (%)	103.32 ± 8.23	103.64 ± 3.72	108.28 ± 4.71	103.83 ± 7.75	92.38 ± 6.92	95.06 ± 13.17	109.13 ± 9.28	0.18	0.051
SGR (%)	0.68 ± 0.04	0.68 ± 0.02	0.70 ± 0.04	0.68 ± 0.04	0.62 ± 0.03	0.64 ± 0.07	0.70 ± 0.04	0.18	0.051
FCR	1.55 ± 0.08	1.56 ± 0.06	1.51 ± 0.05	1.53 ± 0.07	1.70 ± 0.14	1.65 ± 0.18	1.49 ± 0.09	0.24	0.055
PER	1.05 ± 0.05^{ab}	1.05 ± 0.04^{ab}	1.07 ± 0.04^{ab}	1.07 ± 0.05^{ab}	0.91 ± 0.07^{a}	0.99 ± 0.11^{ab}	1.10 ± 0.06^{b}	0.04	0.009
FE	0.47 ± 0.02	0.47 ± 0.02	0.48 ± 0.02	0.47 ± 0.02	0.43 ± 0.03	0.44 ± 0.05	0.49 ± 0.03	0.23	0.05
FI	159.97 ± 5.29	161.48 ± 2.21	163.55 ± 3.72	159.04 ± 6.51	156.61 ± 7.80	154.86 ± 6.14	161.92 ± 4.55	0.49	0.349

*Values represent means ± standard deviation of triplicates.

Discussion and Conclusion

The results from the present study suggest no significant difference in growth performance by one-way ANOVA. However, two-way ANOVA suggests an interaction effect among the groups with respect to FBW. Moreover, there were no significant differences observed in FI by both statistical tests, indicating there might be a combination effect of dietary VD and VK in FBW of the gilthead seabream juveniles. Abawi and Sullivan (1987) also suggest that higher supplemental levels of vitamins D and K would improve growth performance of poultry. On the other hand, in Atlantic salmon (*Salmo salar*) VD, VK, Ca, and dissolved CO2 exposure did not cause any deleterious effects on bone mineralization or fish health (Graff *et al*.,2002). Therefore, further analyses are being conducted to understand the interaction effects of these vitamins in skeletal development and calcium metabolism in gilthead seabream juveniles.

Acknowledgment

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

References

Abawi, F.G. and T.W. Sullivan. (1989) Interaction of vitamins A, D_3, E and K in the diet of broiler chicks. Poultry Science, 68: 1490-1498.

Andrades, J.A., Becerra, J., Fernandez-Llebrez, P. (1996) Skeletal deformities in larval, juvenile, and adult stages of cultured gilthead sea bream (*Sparus aurata* L.), Aquaculture 141, 1 – 11.

APROMAR. (2020) Informe realizado por la Asociación Empresarial de Acuicultura de España. www.apromar.es

Boglione C, Costa C. (2011) Skeletal deformities and juvenile quality. In: Pavlidies MA, Mylonas CC, editors. Sparidae. Biology and Aquaculture of Gilthead Seabream and other Species. Wiley-Blackwell Chichester. pp. 233– 294.

FAO. (2020) The State of World Fisheries and Aquaculture (SOFIA) Departamento de Pesca. Roma.

Francis, G., Makkar, H.P.S., Becker, K. (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199(3-4):197-227.

Izquierdo, M.S., Ghrab, W., Roo, J., Hamre, K., Hernández-Cruz, C.M., Bernardini, G., Terova, G., Saleh, R. (2016) Organic, inorganic and nanoparticles of Se, Zn and Mn in early weaning diets for gilthead seabream (*Sparus aurata;* Linnaeus, 1758). Aquaculture Research 1–16.

Krossøy, C., Waagbø, R., Ørnsrud, R. (2011). Vitamin K in fish nutrition. Aquaculture Nutrition 17; 585–594.

Lock, E-J., Waagbø, R., Bonga, S.W., Flick, G. (2010) The Significance of vitamin D for fish: A review. Aquaculture nutrition 16; 100–116.

NRC. (2011). Nutrient Requirements of Fish and Shrimp. The National Academic Press, Washington D.C.

Graff, I.E., Waagbø, R., Vermeer, C., Lie, Ø. & Lundebye, A.K. (2002) A multivariate study on the effects of dietary vitamin K, vitamin D_3 and calcium, and water carbon dioxide on growth, bone minerals, vitamin status and health performance in smelting Atlantic salmon (*Salmo salar* L.). Journal of Fish diseases 25, 599–614.

METHODS AND APPLICATION FOR PARAMETERIZATION OF FISH SKIN COLOUR EXPERIMENTS

J. Urban*, O. Mashchenko, P. Urbanová

Laboratory of Signal and Image Processing, Institute of Complex Systems, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Zámek 136, Nove Hrady, 37333 (Czech republic)
email: urbanj@frov.jcu.cz

Introduction

Using methods of obtaining information by a non-invasive method in the aquatic environment is in demand and is constantly used in all sectors of aquaculture. In biology, we often estimate the state of the object by its color. The classical spectrophotometric methods have a disadvantage of the point measurement, where only small area of the fish skin could be evaluated. Such method is invasive, since there is a direct contact. The correctly set camera could give a valuable color characteristic across the whole fish body, if image processing methods are applied. Proper light and camera calibration are two related, but separated tasks. To improve the measurement acquisition, we developed a simple device, a box or chamber, with standardized light conditions, automatic camera calibration, environmental sensors, weighting scale, and software applications for color analysis and evaluation.

Materials and Methods

The existing methods for the white balance were mostly developed for the corrections of the image taken with the incorrect settings. However, they are general enough to work directly on camera setting. Since the amount of light from the LEDs illumination is suffcient, two simplest methods of white balance algorithms could be used, the Retinex method, and GrayWorld method.

The captured images could be easily analysed with the software application for the segmentation (fish to background), color transformations and characteristics (sRGB, cRGB, LAB, HSV), image and set characteristics. The segmentation and color analysis is tuning automatically the parameters and thresholds.

Results and Discusion

The camera is calibrated automatically, using described methods. The standard color checker chart was used for the comparison of white balance calibration.

The device is standardizing the condition for the color measurements, automatically setup the camera parameters as white balance, brightness, and contrast to allows accurate color analysis from the captured images. The devise is easy to use, without advance user operation. It is connected with the analysing software for the segmentation, color transformation, and statistic. The device should help with the data aquisition as well as the analysis, comparison, and color evaluation.

Acknowledgment

The study was _supported by the Ministry of Education, Youth and Sports of the Czech Republic - project CENAKVA (LM2018099), the CENAKVA Centre Development (No:CZ:1:05/2:1:00/19:0380) and project Biodiversity CZ:02:1:01:/0:0/0:0/16/025/0007370), as well as TACR GAMA (TG03010027 PoC02_23) and by the GAJU 017/2016/Z. This project also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein

References

Urban, J. 2020. Automatic Calibration, Acquisition, and Analysis for Color Experiments. In International Work-Conference on Bioinformatics and Biomedical Engineering (pp. 298-309). Springer, Cham.
Zapryanov, G., Ivanova, D., Nikolova, I.2012, Automatic white balance algorithms fordigital stillcameras{a comparative study. Information technologies and control (2012) 16-31

ENVIRONMENTAL PREFERENCE OF NILE TILAPIA ACCORDING TO BEHAVIOURAL TRAITS

A. V. Montalt*, P. Arechavala-Lopez, C.M. Maia, M. Cabrera-Alvarez, J.L. Saraiva

Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal
*Corresponding author: avmontalt@gmail.com

Introduction

Along their life-history, animals develop behavioural strategies to ensure their survival. Nonetheless, inside fish schools, completely opposite conducts among individuals are revealed. Some display a bolder role while others are shyer; in between both extremes, a continuum of behavioural profiles takes place. When those behaviours are stable across a wide range of social and environmental contexts, individual personality is defined. This proactive-reactive pattern has been identified in nature but also in aquaculture, where the interest on animal welfare is growing exponentially and, alongside it, the will to improve rearing methods. Yet, little is known about how to adapt artificial habitats to fish behavioural patterns. Arechavala-Lopez et al. (2021) suggested environmental enrichment (EE) as a potential way of reducing stress and enhance fish welfare; while Maia et al. (2017) presented preference tests as a tool to accurately determine fish preferred choices. Merging both, EE and preference concepts, this project aims to assess whether proactive and reactive individuals of Nile tilapia *Oreochromis niloticus* have different preference responses for any of two types of EE.

Material and Methods

We studied 50 Nile tilapia (Silver Natural Male Tilapia™ , Til-Aqua International B.V., Netherlands) weighing 44.2 ±9.7g. Every week we anaesthetised and tagged 10 individuals with t-Tag100 (BTS-ID ™), transferred them to a 300L holding tank (100x55x55cm; density: 1.5kg/m^3) and left them undisturbed for a 64h recovery period.

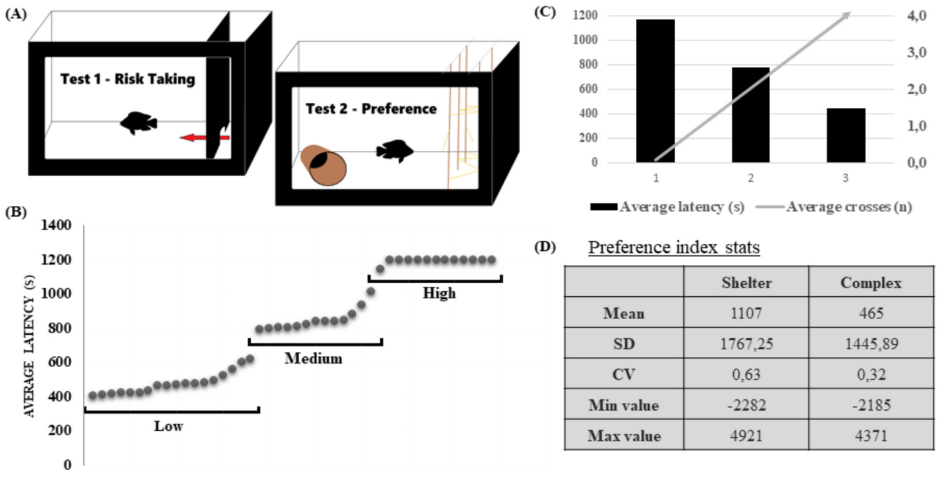
We first ran a risk-taking test (RT), placing one subject fish in a testing tank (100x55x55cm) divided into a $^1/_5$ and a $^4/_5$ areas by a vertical acrylic wall (Fig.1A). The wall had a bottom opening (10x9cm), initially covered by a lid. After 5min in the $^1/_5$ area, we opened the hole and recorded subject's activity for 20min. Subsequently, we returned the fish to its holding tank. We tested each fish of the 10-fish group once a day in 3 consecutive days before 64h of recovery. We calculated the average latency to cross the opening, rating the individuals as high or low risk-takers. After the recovery, we ran a preference test (PT). The testing tank (100x55x55cm) had 3 vertical areas of the same size. The central area was empty while the right and left areas displayed one of two kinds of EE: a shelter structure (pot), and a framework of hanging ropes and sticks (Fig.1); their position was counterbalanced after each trial. We released one fish into the central area and recorded its behaviour for 20min. After 3 repetitions in consecutive days, we euthanised them. We used The Observer XT ™ (Noldus, the Netherlands) to analyse the videos, tracking subjects' position and quantifying behavioural traits. We calculated a preference index (Maia et al., 2017) for each subject based on its EE choices (duration and intensity). We are currently studying links between choice patterns and RT profiles.

Results

Risk-taking test: We identified three main categories of latency (Low, Medium and High) by ordering individuals by their average latency among trials (Fig.1B). We also evaluated the Coefficient of variation of the latency (CV), discriminating a similar 3-category pattern. The average latency to cross the hole decreased over the trials while the average number of crosses through the hole increased (Fig.1C).

Preference test: We calculated the preference index for each individual, categorizing them into three groups: Pref-Shelter (44%), Pref-Complex (30%) and Undecided (26%). Intensity of response for EE heavily fluctuated among individuals (Fig.1D).

(Continued on next page)



	Shelter	Complex
Mean	1107	465
SD	1767,25	1445,89
CV	0,63	0,32
Min value	-2282	-2185
Max value	4921	4371

Fig. 1. A: Experimental tanks schemes for RT and PT tests on Nile Tilapia; B: Distribution of individual average latency to cross the hole on RT test; C: Average latency and number of crosses in RT test; D) Preference index stats for both EE, (+)values mean preference while (-)values mean non-preference.

Discussion

Until now, we have evaluated risk-taking behaviour and preference tendencies, which provide an overview of the main types of responses. Decreasing latency and increasing crossing events over trials suggest a habituation process to the RT arena. We are pondering different proxies of fish personality to develop a compound RT index. First analyses of the CV of latency already gave us an insight on the consistency of fish performance in consecutive RT trials, showing that some fish continuously behave in the same way while others vary their behaviour on each trial. Also, the addition of variables linked to activity levels (e.g. zone changes per minute, total number of zone changes) and exploratory behaviour (e.g. max. distance from hole, surface/bottom ratio) will supplement the mentioned index providing a complete fish behavioural profile. Regarding the PT, further than our qualitative categorization, an exploration of the quantity, intensity, and nature of interactions with the two EE types will give us a deep perspective on fish preferences. Further development of behaviour-preference profiles will yield the need to design environments adapted to fish requirements, favouring their welfare and thus, increasing their added value as product.

References

Arechavala-Lopez et al. (2021). Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Reviews in Aquaculture*, in press.

Maia, C. M. & Volpato, G. L. (2017). Preference index supported by motivation tests in Nile tilapia. *PloS one,* 12(4), e0175821.

MICROBIOLOGICAL BIODIVERSITY AND COMPOUNDS COMPOSITION OF PELOID MUD IN SERVICE OF HEALTH

D. Vadlja[a,b,*], M. Bujak[a,b], I. Tartaro Bujak[a], S. Kazazić[a], N. Topić Popović[a,b], R. Čož-Rakovac[a,b]

[a]Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
E-mail: denis.vadlja@irb.hr
[b]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute, Bijenička cesta 54, Croatia

Introduction

Peloids are naturally formed mud suspensions with variable content of clay, inorganic and organic compounds, water, and gases throughout the seasons. Consequently, their microbiological composition is in a continuous change and little is known about their prokaryotic distribution and community structures (Carretero et al., 2020).

Since Roman times, a peloid micro-location on the Croatian Adriatic coast near the small-town Nin is used for pelotherapy (use of peloids for medicinal purposes) (Gomes et al., 2013). Nowadays, the health resort is under constant supervision of the Zadar Health Institute and part of the tourist attraction and health tourism of Croatia. Healing of arthritis, spinal deformities, muscular and skeletal system problems, skin diseases, and rheumatic disorders are just some of the applications of Nin peloid mud. However, insufficient knowledge and limited scientific reports concerning the abiotic and biotic content of peloid mud is still a barrier for understanding its contribution to the healing effects. To that end, investigation of the microbiological diversity and compound composition of Nin peloid mud is highly warranted. Therefore, the main objective of this work was to identify the most common bacterial strains in the Nin peloid mud, their biochemical profile, and their fatty acid composition.

Materials and methods

Sample collection was conducted in August 2020 from several locations across the Nin peloid mud micro-location (44°14'59.2'' N, 15°10'24.5'' E). Peloid mud samples were collected into sterile Falcon tubes, stored at 4° C, and analysed the next day for chemical composition and presence of yeasts and bacteria. Samples were inoculated onto Marine agar plates and incubated for 48 h at 20 °C. A total of 39 primary isolates were analysed using matrix-assisted laser-induced desorption/ionization (MALDI) connected to the time of flight (TOF) mass spectrometry (MS) channel (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) (Topić Popović et al., 2017). A total of 33 bacterial isolates were cryo-preserved and selected for further testing. DNA extraction (Qiagen, Hilden, Germany) and 16S sequencing (Macrogen, Amsterdam, Netherlands) were utilized to identify bacterial strains unidentifiable by MALDI-TOF MS. The API 20E tests (BioMerieux, Marcy l'Etoile, France) (Topić Popović et al., 2014) and fatty acid (FA) analyses (Varian Inc., Palo Alto, USA) (Bujak et al., 2018) were conducted on overnight bacterial cultures to provide insights into their biochemical profile and cellular fatty acid composition.

Results

Analytical and microbiological methods were conducted on seawater and sediment samples of peloid mud. Consumption of $KMnO_4$ was between 32.6 and 56.3 mg O_2 L^{-1} seawater. Ammonia concentration was under 0.01 mg N L^{-1}. Obtained nitrate concentration was between 0.2 and 0.7 mg N L^{-1}. Microbiological analysis showed that 100 g of sediment contained 108 colonies of coliform bacteria, 41 colonies of *E. coli* (limit 500), 71 colonies of faecal enterococci (limit 200), 7349 colonies of sulphite-reducing clostridia, zero colonies of *P. aeruginosa* and yeasts per 100 g of sediment.

The API 20E results revealed that none of the isolated bacterial strains synthesized arginine dihydrolase and urease. Half of the strains were positive for β-galactosidase, tryptophane deaminase, gelatinase, and indole production. A fifth of the isolated bacterial strains fermented sucrose, mannose, glucose, produced indole and acetoin, and decarboxylate lysine and ornithine. A quarter of the isolated bacterial strains fermented amygdalin, while almost all strains produced oxidase. 16S sequencing and MALDI-TOF MS analysis showed that peloid harbours a richness of bacterial species, with at least three distinct community types. The predominant fatty acids in Gram-positive bacteria were i-C15:0 and ai-C15:0, whereas in Gram-negative bacteria the main fatty acids were C16:0, C16:1, C18:0 and C18:1.

(Continued on next page)

Discussion and conclusion

Analytical and microbiological results revealed no anthropological impact on the microbiological composition of peloid mud. Overall results have proven remarkable quality of water and sediment for pelotherapy purposes.

The primary screening of bacterial isolates was conducted using MALDI-TOF MS. It is a powerful diagnostic method for the fast screening of isolated bacteria that helped us in the isolation process. However, we could not identify most of the bacteria isolates because the reference database spectra lack the spectra of environmental bacteria. Results obtained using the API 20E method provided additional information on the biochemical profile and metabolic pathways of isolated bacterial strains. The results differ depending on the bacterial strains and are in correspondence with literature data. The FA composition of bacterial cells varies depending on the species and has thus been used as a biomarker in taxonomy. It was an excellent tool to overcome the shortcomings of the MALDI-TOF MS regarding its lower level of taxonomic resolution for environmental samples. Despite their drawbacks, together with the 16S sequencing, which is the golden standard for the identification of bacteria, we identified all isolated bacterial strains from the peloid.

The acquired data can provide a better understanding of peloid muds, their microbiological and chemical properties. It is of great importance to identify metabolic pathways and compounds produced by the microorganisms, which could benefit human health and healing processes. Further studies will involve detailed tests on isolated bacterial strains and their properties, including several parameters, such as antimicrobial and antibiotic testing and bioinformatics models of the relationship between microbiological communities in the peloid mud.

References

C. Gomes, M.I. Carretero, M. Pozo, F. Maraver, P. Cantista, F. Armijo, J.L. Legido, F. Teixeira, M. Rautureau, and R. Delgado. 2013. Peloids and pelotherapy: Historical evolution, classification and glossary. Applied Clay Science 75-76: 28-38.

M. Bujak, I. Tartaro Bujak, S. Sobočanec, M. Mihalj, S. Novak, A. Ćosić, M. Tolušić Levak, V. Kopačin, B. Mihaljević, T. Balog, I. Drenjančević, M. Baus Lončar. 2018. Cellular Physiology and Biochemistry 47: 827-841.

M.I. Carretero. 2020. Clays in pelotherapy. A review. Part II: Organic compounds, microbiology and medical applications. Applied Clay Science 189: 105531.

N. Topić Popović, R. Sauerborn Klobučar, I. Maguire, I. Strunjak-Perović, S. Kazazić, J. Barišić, M. Jadan, G. Klobučar, R. Čož-Rakovac. 2014. High-throughput discrimination of bacteria isolated from *Astacus astacus* and *A. leptodactylus*. Knowledge and Management of Aquatic Ecosystems 413: 04.

N. Topić Popović, S.P. Kazazić, I. Strunjak-Perović, R. Čož-Rakovac. 2017. Differentiation of environmental aquatic bacterial isolates by MALDI-TOF MS. Environmental Research 152: 7-16.

REDUCTION IN HUMAN HANDLING-DERIVED STRESS INCREASES SENEGALESE SOLE GROWTH

D. G. Valcarce*†, J. M. Martínez-Vázquez[1†], M. F. Riesco[3], J. L. Rodríguez Villanueva[2] and V. Robles[3]

† Equal contribution
[1] Spanish Institute of Oceanography (IEO-CSIC). Monte-Corbanera, 39012, Santander, Spain
[2] Instituto Galego de Formación en Acuicultura, Xunta de Galicia, Illa de Arousa, 36626, Pontevedra, Spain
[3] Cell Biology Area, Department of Molecular Biology, Universidad de León, 24071, León, Spain
* E-mail: david.garcia@ieo.es

Introduction

The zootechnics in aquaculture is in constant evolution, in order to reach the maximum production rates, making the initial economical investment the more profitable as possible in the shortest time. Biomass control and precise feed estimation for finest fish growth are key and routinary activities in culture facilities. These periodical protocols usually imply animal persecution, tank withdrawn, and human handling, which may lead to stress induction on the individuals.

Over recent years, different mechanisms based on image analysis for quantifying the tank biomass have been studied in some commercial species, showing different levels of accuracy in their estimations (Saberioon et al., 2017). In addition, in the particular case of *Solea senegalensis* some specific constraints, such as overlapping of flatfish individuals in the bottom of the tank, should be considered in the development of these artificial recognition systems.

The initial hypothesis of this work was that the implementation of a precise image recognition system via artificial neural networks for biomass prediction in *Solea senegalensis* might provide an accurate biomass estimation, reducing human handling, potential stress induction and, eventually, an improvement on animal growth.

Material and methods

In order to validate this hypothesis, 30 adult F1 Senegalese soles born and reared in our facilities were divided into two homogeneous cohorts in terms of weight. One of them was established as control group (CTRL), which was standard manually sampled and the other one, the experimental group (EXP), was maintained under a lower human-animal interaction culture relying on a new image analysis predictor for biomass calculation with a lowest estimation of 0.8 centimetres, and around 95% of accuracy detection (Marco et al., 2021). Thus, animals belonging to CTRL group were exposed to a monthly manual sampling which included tactile, visual, olfactory, gustatory and auditory stimuli contrary to EXP counterparts. This sampling was performed following standard protocols in animal production centers for biometrical measurements. Each group was split into three replicates (n =5; biomass density: 1.6700 ± 0.009 kg/m^2 per tank). All animals included in the experiment were fed daily with a commercial diet of Europa 5® pellets from Skretting Spain S.A (Burgos, Spain) to an adjusted quantity of the 0.5% biomass of each tank.

Visible implant elastomers (VIE) tagging was used to monitor individual growth. Before the beginning of the experiment, each fish was tagged with a unique code based on the number and position of marks on the dorsal area. This approach allowed fish recognition using a UV lamp, avoiding withdrawing them from the tank in the EXP group. Top view photographs from each EXP tank were taken to calculate the precise biomass within them using the developed algorithm. Biomass data provided by the image analysis system were used to adjust food ration in the experimental tanks.

The experiment lasted 10 months. Two key points were established at: 5 months (halfway of the experiment) and 10 months (end of experimentation).

Specific Growth Rate (SGR) at 5 and 10 months were represented as the mean ± standard error of mean (S.E.M.). The comparison of biometrical data between groups was analyzed using a Mann Whitney test. Statistical analyses were performed with Prism8 (GraphPad Software, San Diego, CA, USA). p-values < 0.0500 were considered statistically significant.

The experimental design and all procedures including animals in the present study were approved by the institutional Animal Care and Use Committee (authorization number PI-10-16) at the Marine Culture Plant El Bocal of the Spanish Institute of Oceanography in Santander, Spain. All animals were standard manipulated according to the Guidelines of the European Union Council (2010/63/EU), following Spanish regulations (RD/2013) for the use of laboratory animals.

(Continued on next page)

Results and discussion

Animals included in the EXP group (lower human-animal interaction) reported a statistically significant higher SGR at both evaluation points: 5 months ($p=0.0038$) and 10 months ($p=0.0040$) when compared to CTRL animals. The reduction of the human-animal interaction by the application of this artificial neural network system based on image analysis would benefit flatfish aquaculture, reducing the cost associated with manual labour. Furthermore, the validation of this system opens up new opportunities for the development of easy-to-use tools for flatfish aquaculture industry since the algorithm can be implemented by any device such as a mobile phone. Our results indicate that a lower human-animal interaction, implying potentially lower stress induction, improves biomass gain in Senegalese sole individuals in a short period of time of its life cycle. Further studies will be focus on the study of stress related mechanisms that could trigger this handling response in order to provide specific treatments to counteract this effect.

References

Marco V. S., Valcarce D. G., Riesco M.F., Robles V. 2021. Low-cost automatic fish measuring estimation. *Proceedings of the ACM Symposium on Applied Computing*. SAC 2021, 168525. 90-97.

Saberioon, M., Gholizadeh, A., Cisar, P., Pautsina, A., Urban, J. 2017. Application of machine vision systems in aquaculture with emphasis on fish: State-of-the-art and key issues. *Reiews in Aquaculture*. 2017, 9, 369–387. 5.

Acknowledgments

Authors would like to acknowledge PID2019-108509RB-I00 project (Ministerio de Ciencia e Innovación), FCJ2018-037566-I grant and Stolt Sea Farm S.A.

LONG TERM EFFECTS OF EARLY PROBIOTIC INTAKE ON *Solea senegalensis* CULTURE

J. M. Martínez-Vázquez[1], I. Martín[1], E. Chaves-Pozo[2], V. Robles[3], I. Rasines[1], D. G. Valcarce[1*]

[1] Spanish Institute of Oceanography (IEO-CSIC) Monte-Corbanera, 39012, Santander, Spain
[2] Spanish Institute of Oceanography (IEO-CSIC). Puerto de Mazarrón, 30860, Murcia, Spain
[3] University of León (ULe). Department of Molecular Biology, 24071, León, Spain

*E-mail: david.garcia@ieo.es

Introduction

Lactic acid bacteria (LAB) are one of the most studied probiotic bacteria in terms of their beneficial effects on aquatic species health. *Pediococcus acidilactici* CNCM MA18/5M, is a LAB strain which is registered in the European Union for use in feed for aquaculture. Previous studies have focused on the positive effects of this probiotic on a variety of species with commercial interest such rainbow trout (Ramos et al., 2013), tilapia (Standen et al., 2013) or blue shrimp (Castex et al., 2010) among others.

The current experiment aims to explore the potential beneficial effects of a prolonged probiotic supplementation (up to 150 days post hatching (dph)) with this strain on growth performance in *Solea senegalensis* culture.

Material and methods

This experiment was conducted in accordance with the Spanish and European law on animal experimentation and was specifically approved by the Bioethics Committee of the University of Cantabria (authorization number 2021-02).

Embryos were obtained by *in vitro* fertilization (IVF) (Rasines et al., 2013) using F1 *Solea senegalensis* gametes from only one couple. General culture protocol and feeding regime were based on Cañavate and Fernández-Díaz (1999) with slight modifications. One dph, the batch of siblings was homogenously split into 6 tanks (Volume: 200 L; density: 50 larvae L^{-1}). Two experimental groups (3 tanks/group) were created differing in the feeding program: 1) the control group (CTRL) in which live food (rotifer and artemia metanauplii) was enriched with a commercial product (Easy Dry SELCO®, Inve Aquaculture) and 2) the experimental group (PROBIO) in which live food was enriched (10^{11} CFU mL^{-1}) with *Pediococcus acidilactici* MA 15/5M (Bactocell®, Lallemand). Once weaning was over (90 dph), the PROBIO group maintained the probiotic supplementation in the pellets. The probiotic was included in the inert food of this experimental group using the oil coating technique (20 % w/w vegetal oil).

Growth was evaluated taking into account three temporal frames: 1) Live-inert cofeeding (3 dph; beginning of feeding-90 dph; end of weaning), 2) Inert feeding (90 dph-150 dph), and 3) Complete trial (3 dph -150 dph). Specific Growth Rate, SGR $((\ln W_f - \ln W_0)*100/T_f-T_o)$ was used as variable to evaluate fish growth. Statistical analysis was performed using SPSS 21.0. Normally distributed variables were analyzed using the student's t-test. Values with $p < 0.05$ were considered to be statistically significant. Data are expressed as mean ± SEM.

Results and Discussion

The prolonged dietary supplementation with *P. acidilactici* CNCM MA18/5M from the beginning of larval feeding (PROBIO) equaled CTRL group in terms of growth ($p = 0.372$) after the first temporal frame (live-inert cofeeding) (Table 1). These results report evidence on the capacity of the bioencapsulated probiotics in live food to provide similar growth rates upon the end of weaning when compared to tanks in which live feeding was enriched using a commercial enrichment product (SELCO®) in *S. senegalensis* specimens. In the second temporal frame involving inert feeding exclusively (90 dph-150 dph) the statistical analysis did not report significant differences ($p = 0.0720$) between CTRL and PROBIO (maintaining probiotic supplementation with probiotic-coated pellets) although a higher mean SGR value was registered in this experimental group (Table 1). Overall, taking into account the complete trial, up to the last evaluated sampling (150 dph), a significant increase ($p = 0.037$) was reported in the growth values in the PROBIO tanks (Table 1). It is our purpose to maintain constant probiotic supplementation during more months to monitor the evolution of growth, as well as other indicators such as gonad development. So far, our data provide clues indicating the potential positive effect of constant probiotic supplementation in the culture of Senegalese sole, which may be the starting point for the development of new specific feeding programs based on this approach.

(Continued on next page)

Table 1. Growth performance of *S. senegalensis* cultured with the two experimental feeding programs: control (CTRL) and long-term supplemented with *P. acidilactici* CNCM MA18/5M (PROBIO) during the three studied temporal frames in the culture. Data are expressed as mean ± SEM. Mean values with different superscript letters in the same column were significantly different among treatments ($p< 0.05$)

	SGR (%)		
Experimental Group	**Live-inert cofeeding (up to 90 dph)**	**Inert feeding (90-150 dph)**	**Complete trial (up to 150 dph)**
CTRL	10,839 ±0,057[a]	2,925 ±0,147[a]	7,495 ±0,089[a]
PROBIO	10,929 ±0,068[a]	3,541 ±0,207[a]	7,807 ±0,048[b]

References

Cañavate J.P. and Fernández-Díaz C. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. *Aquaculture*. 174 (3-4), 255-263.

Castex, M.; Lemaire, P.; Wabete, N.; Chim, L., 2010: Effect of probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress of *Litopenaeus stylirostris* under *Vibrio nigripulchritudo* challenge. Fish Shellfish Immunol. 28, 622– 631.

Ramos, M. A.; Weber, B.; Gonçalves, J. F.; Santos, G. A.; Rema, P.; Ozório, R. O., 2013: Dietary probiotic supplementation modulated gut microbiota and improved growth of juvenile rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol*. 166, 302– 307.

Rasines I., Gómez M., Martín I., Rodríguez C., Mañanós E., Chereguini O. 2013. Artificial fertilisation of cultured Senegalese sole (*Solea senegalensis*): Effects of the time of day of hormonal treatment on inducing ovulation. *Aquaculture* 392–395, 94-97

Standen, B. T.; Rawling, M. D.; Davies, S. J.; Castex, M.; Foey, A.; Gioacchini, G.; Carnevali, O.; Merrifield, D. L., 2013: Probiotic *Pediococcus acidilactici* modulates both localised intestinal and peripheral-immunity in tilapia (*Oreochromis niloticus*). Fish *Shellfish Immunol*. 35, 1097– 1104.

Acknowledgments

Authors would like to acknowledge FCJ2018-037566-I grant, PROBISOLE project (Fundación Biodiversidad; PLEAMAR2020-FEMP), Lallemand Animal Nutrition S.A. and STOLT Sea Farm S.A.

MARINE FINFISH AQUACULTURE, ANTIBIOTICS AND ESCAPED FISH: THE ELEPHANT IN THE ROOM

J.M. Valero-Rodriguez*; P. Sanchez-Jerez; K. Toledo-Guedes.

Department of Marine Sciences and Applied Biology, University of Alicante. Alicante, Spain
* Email: Juanma.valero@ua.es

Introduction:
Climate change is profoundly affecting worldwide aquaculture. For example, heat waves could increase the incidence of diseases that could be typical of warmer environments. The use of antibiotics to treat individuals in the culture may be associated with a risk (Dang *et al*. 2021). Extreme meteorological events can also occur as a result of this global change, leading to damage in facilities, breakage of networks and, consequently, escapes of individuals from aquaculture farms (Callaway *et al*. 2012). These escapees can be caught and misclassified as wild product by local and recreational fishermen and depending on the timing of the event, considerable amounts of antibiotics could reach consumers as a result (Figure 1). This study focuses on identifying critical points and suggesting management measures than may be used to face possible harmful consequences derived from these events. We try to convey the need of a modern prevention and mitigation approaches to ensure safety for all consumers.

Materials and methods:
An initial set of searches using ISI Web of Science and Google Scholar catalogues were done between March & June 2021 using search strings aimed at different objectives. The first search string was aimed at identifying the current state of influence that climate change has on the aquaculture industry, as well as the relation between warmer temperatures, illnesses emergence and escape events. The second and third search strings were aimed at pre- and post-escape management measures and their current trends. Some publications missed by the initial search were provided by experts in the field or discovered by reading the full reference lists of all articles that provided relevant data, as well as review articles on related topics.

Results:
A total of 500 papers and reports were thoroughly examined. More than 50% of the selected papers were based on experiments located in Asia, 25% from Europe, 6% from Oceania, 9% from South America and 10% from North America. More than 40% of the papers delved on the detection of antibiotics resulting from aquaculture activities and 40% of them looked for methodologies for the bioremediation and/or reduction of the amount of said substances. While 40% of the papers collected explored the methodologies involved in good practices to avoid aquaculture escapes, only 20% of the papers mentioned the effect of climate change on antibiotic presence in the environment.

On the one hand, we compiled information and suggested improvement measures regarding current trends aimed at the decision-making process for the aquaculture facilities location. We also delved on the rationale of antibiotics use in cultured species and suggested alternatives with less associated risk, such as vaccination.

On the other hand, we identified knowledge gaps within: the development of management plans focused on the contingency of escaped specimens; the refinement of good practice manuals on culture management; early detection measures; preparation of recapture plans; communication networks that notify local authorities about the escapees and traceability of the amounts of antibiotics that reach local markets in the form of poorly labelled fish.

Guidelines and conclusions:
This work showed the need for improvement in different areas aimed at avoiding unwanted quantities of antibiotics from reaching consumers. Regarding prevention work, a series of measures were suggested based on the data found. E.G.: while countries such as Norway are very experienced in implementing preventive vaccines, others are still using large amounts of antibiotics with their cultures, showing a time lag relevant in our globalized world. Likewise, the fact of restructuring the data used in the site selection systems is necessary to incorporate useful information related to climate change that could lead to a full prediction model. Data conveying frequency of thermal anomalies could be an applicable example. Likewise, post-escape measures such as mitigation and management plans or the traceability of escaped fish likely to contain significant amounts of antibiotic must be improved to ensure the sale of a safe consumable product. Ultimately, only the combination of improved pre- and post- escape measures can minimally ensure a risk- free quality product.

(Continued on next page)

References

-Callaway, R., A. P. Shinn, S. E. Grenfell, J. E. Bron, G. Burnell, E. J. Cook, M. Crumlish, S. Culloty, K. Davidson, R. P. J. A. C. M. Ellis and F. Ecosystems (2012). "Review of climate change impacts on marine aquaculture in the UK and Ireland." **22**(3): 389-421.

-Dang, L. T., L.-H. T. Nguyen, V. T. Pham and H. T. T. Bui (2021). "Usage and knowledge of antibiotics of fish farmers in small-scale freshwater aquaculture in the Red River Delta, Vietnam." Aquaculture Research.

Funding:

This work is part of project GLORiA. GLORiA is supported by the Biodiversity
Foundation of the Spanish Ministry for the Ecological Transition and Demographic Challenge, through the pleamar programme and co-financed by the European Maritime and Fisheries Fund (EMFF). It is also part of the LIFE IP INTEMARES project "Integrated, innovative and participatory management of the Natura 2000 Network in the Spanish marine environment".

THE ROLE OF SEAWEEDS IN FUTURE CIRCULAR CLIMATE SMART FOOD SYSTEMS

Sander van den Burg, Sophie Koch, Trond Selnes, Maggie Skirtun

Wageningen Economic and Research
Droevendaalsesteeg 4
6708 PB Wageningen, the Netherlands
E-mail: sander.vandenburg@wur.nl

Introduction
The increased global demand for food and feed combined with a changing climate confronts societies with the challenge to develop Climate Smart Food Systems. Increased food production has to be achieved in the context of a growing set of climate change-related risks, competition for space, scarcity of resources and the need to preserve the world's ecosystems. In recent years, there has been significant interest in the potential contribution of marine lower trophic species, including seaweeds, in such Climate Smart Food systems, for multiple reasons.

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 CO2 and mineral fixation, nutrient recycling, and long term carbon storage (Froehlich et al. 2019; Duarte et al. 2017).

The valuation of such ecosystem services can support the seaweed farmers in setting up their business case for seaweed cultivation and can also be an additional source of income in low-income countries (van den Burg, Dagevos, and Helmes 2019; Hasselström et al. 2020). In this line of reasoning, various initiatives such as Seaforester and Oceans2050 emphasise the value of restoring global kelp ecosystems.

Objective
This study provides a realistic perspective on the role of seaweeds in a future Climate Smart Food system. It identifies different pathways for climate mitigation, opened up by seaweed cultivation and use. For each of the identified pathways, we review the evidence base and identify challenges and risks. Furthermore, the pathways are discussed in the light of the organisation of current seaweed value chains and economic feasibility, currently and in the foreseen future. The study concludes with recommendations for business and government to advance the role seaweeds.

Methodology
The study pulls together information from two multidisciplinary research programmes: "Towards a circular and climate positive society: Marine Resources" and "Safe Seaweed by Design". The first phase involves a review of literature on the role of seaweed in climate mitigation, including Life-Cycle Analysis and Blue Carbon literature. In the second phase, identified pathways are evaluated, using economic models and literature.

Intended output
The results from the study will support development of seaweed application possibilities in future Climate Smart Food systems. It will also be used to inform policy decisions in meeting goals set out under EU strategy to Sustainable development for climate smart food systems and circular economy.

(Continued on next page)

References

Abbott, D W, Inga M Aasen, Karen A Beauchemin, Fredrik Grondahl, Robert Gruninger, Maria Hayes, Sharon Huws, et al. 2020. “Seaweed and Seaweed Bioactives for Mitigation of Enteric Methane: Challenges and Opportunities.” *Animals* . https://doi.org/10.3390/ani10122432.

Burg, S W K van den, H Dagevos, and R J K Helmes. 2019. “Towards Sustainable European Seaweed Value Chains: A Triple P Perspective.” *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsz183.

Buschmann, Alejandro H, Carolina Camus, Javier Infante, Amir Neori, Álvaro Israel, María C Hernández-González, Sandra V Pereda, et al. 2017. “Seaweed Production: Overview of the Global State of Exploitation, Farming and Emerging Research Activity.” *European Journal of Phycology* 52 (4): 391–406. https://doi.org/10.1080/09670262.2017.1365175.

Duarte, Carlos M., Jiaping Wu, Xi Xiao, Annette Bruhn, and Dorte Krause-Jensen. 2017. “Can Seaweed Farming Play a Role in Climate Change Mitigation and Adaptation?” *Frontiers in Marine Science* 4 (April). https://doi.org/10.3389/fmars.2017.00100.

Froehlich, Halley E, Jamie C Afflerbach, Melanie Frazier, and Benjamin S Halpern. 2019. “Blue Growth Potential to Mitigate Climate Change through Seaweed Offsetting.” *Current Biology : CB* 29 (18): 3087-3093.e3. https://doi.org/10.1016/j.cub.2019.07.041.

Hasselström, Linus, Jean-Baptiste Thomas, Jonas Nordström, Gunnar Cervin, Göran M Nylund, Henrik Pavia, and Fredrik Gröndahl. 2020. “Socioeconomic Prospects of a Seaweed Bioeconomy in Sweden.” *Scientific Reports* 10 (1): 1610. https://doi.org/10.1038/s41598-020-58389-6.

Seghetta, Michele, Daina Romeo, Martina D’Este, Merlin Alvarado-Morales, Irini Angelidaki, Simone Bastianoni, and Marianne Thomsen. 2017. “Seaweed as Innovative Feedstock for Energy and Feed – Evaluating the Impacts through a Life Cycle Assessment.” *Journal of Cleaner Production* 150: 1–15. https://doi.org/https://doi.org/10.1016/j.jclepro.2017.02.022.

THE TRANSCRIPTOMIC RESPONSE OF ATLANTIC SALMON *Salmo salar* TO MICROALGAE DIETS AND ENVIRONMENTAL STRESS

Doret van Muilekom[1]*& Jonas Müller[2,3], Michael Schlachter[3], Ronald M. Brunner[1], Henrike Seibel[3], Svenja Starke[4], Alexander Rebl[1] and Tom Goldammer[1,5]

[1]Institute for Farm Animal Biology (FBN), Institute of Genome Biology, Fish Genetics Unit Dummerstorf, Germany
[2]Institute of Animal Breeding and Husbandry, Department for Marine Aquaculture, Christian-Albrechts University Kiel, Germany
[3]Gesellschaft für Marine Aquakultur mbH (GMA), Büsum, Germany
[4]Microganic GmbH, Melle, Germany
[5] Molecular Biology and Fish Genetics, Faculty of Agricultural and Environmental Sciences University of Rostock, Rostock, Germany
E-mail: muilekom@fbn-dummerstorf.de

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 to be promising functional feed ingredients, as they have beneficial effects on immune status. Knowledge on the immune-promoting and stress-compensatory effects of microalgae diets in Atlantic salmon remains however limited.

This study aims to investigate the transfer into marine farming systems and the influence of microalgae diets as probiotic feed on the expression of genes functioning as biomarkers for fish health status. The Biomark HD/Fluidigm test system is used for multigene expression analysis. The results from this study will identify the most promising microalgae candidates and help the development of a BioChip indicating fish health status. Since NF-kB inhibitors emerged to be important immune regulators, these molecules will be analyzed in more detail.

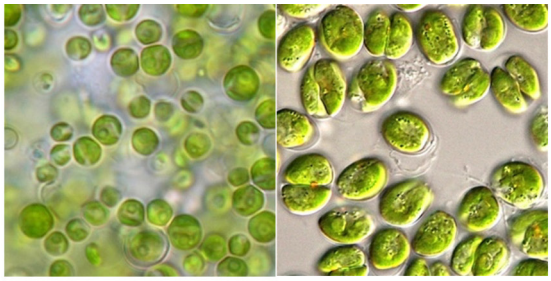


Figure 1: Microalgae species used as supplements to the diet.

Blaue Bioökonomie BioFiA

PREDICTING FEED CONVERSION RATIO AT COMMERCIAL SIZE FROM JUVENILE PERFORMANCE IN INDIVIDUALLY REARED *Oreochromis niloticus*

C. Rodde[1,2,3], M. Vandeputte*[3,4], T. Q. Trinh[2], J.A.H. Benzie[2,5] and H. de Verdal[1]

[1] CIRAD, UMR 116 ISEM, Montpellier Cedex 5, France
[2] Worldfish, Penang, Malaysia
[3] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France
[4] GABI, INRAE, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France
[5] School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland
Contact: marc.vandeputte@inrae.fr

Introduction

Using breeding programs to reduce feed conversion ratio (FCR), namely the ratio between feed intake (FI) and body weight gain (BWG), could highly strengthen aquaculture sustainability. However, selective breeding programs require phenotyping individual FCR, and thus FI, on a large number of fish, which is particularly challenging. A solution is to rear fish in individual aquaria and collect uneaten pellets (Besson et al., 2019). In literature, this method is exclusively used on juvenile fish for practical reasons. Estimating individual FCR beyond juvenile stage is, however, critical since much more feed is consumed during the later stages of growth than during the younger stages. Determining whether FCR estimated at a young age accurately predicts FCR at commercial size has major implications for the design of selective breeding program. In particular, when individual rearing is used, labour costs and space needed would be much lower to individually phenotype juvenile fish rather than fish at commercial size. Selecting male Nile tilapia on their FCR estimated over only two weeks might permit to improve the whole production cycle FCR by about 1% per generation with a 50% selection intensity (Rodde et al, 2020). However, these results were established on only 30 male tilapia and needed to be confirmed on more individuals, including females. In the present study, 60 fish (30 females and 30 males) were reared over 30 weeks and we assessed whether phenotyping individual FCR over only two weeks could accurately predict individual FCR over the 30 weeks.

Material and methods

Animals were originating from the 18th generation of GIFT (Genetically Improved Farmed Tilapia) produced from the 4th March to the 4th April 2019 at the WorldFish Research Station in Jitra (Kedah State, Malaysia). On the 22nd of July 2019, fish were isolated in 10 L aquaria part of a same water recirulating system. Over the whole experiment, fish were fed a commercial diet (Cargill®) to 90% of the optimal feeding rate to reduce feed wastage, as explained in Rodde et al (2020). After two weeks of acclimation, the experiment started on the 5th of August 2019 (35.3 g and 35.5 g for females and males BW, respectively). On the 14th of October (around 90 g), fish were transferred to individual 60 L aquaria to provide them extra space to grow. The experiment ended after 30 weeks, on the 2nd of March 2020, once fish at commercial size (342.7 g and 288.4 g for females and males BW, respectively). Each fish was anaesthetized with clove oil once a week and weighed to adjust feed ration. Every day, uneaten pellets were removed from the aquaria at least two hours after the last meal and counted to estimate the total amount of feed wasted by each fish. Body weight gain, FI and FCR were calculated over 15 two-week periods. The FCR was also estimated over the 30 weeks of the experiment (global FCR named "FCRg"). Individual FCR measured over the two-week time steps and FCRg were normalized using log-transformation (lnFCR and lnFCRg) and Pearson's correlation between each two-week period lnFCR and lnFCRg was estimated. Then, the potential genetic gain on FCRg was compared when fish were selected for i) FCRg directly and ii) FCR estimated over a two-week period as an indirect selection criterion. To perform this simulation, selection intensity was set to 50% (the 15 best females and males were selected) and heritability was set to 0.32 as estimated for juvenile FCR in GIFT Nile tilapia by de Verdal et al. (2018).

(Continued on next page)

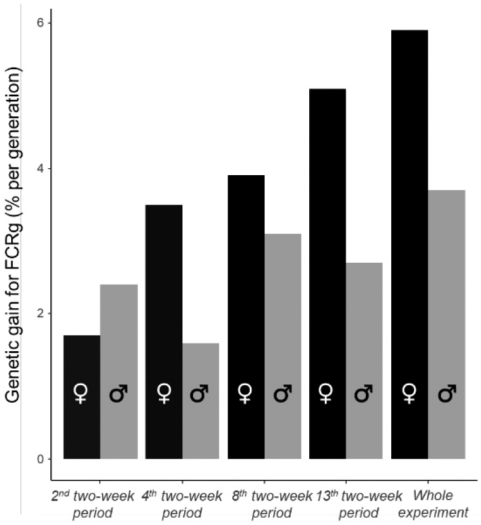


Figure 1 Genetic gain for FCRg depending on various selection periods

Results

In total, 30 females and 29 males were successfully phenotyped. FCRg was 1.87 ± 0.44 (CV = 23.4%) and 1.94 ± 0.33 (CV = 16.7%) for females and males, respectively. When including both females and males in the analyses (n = 59), lnFCR appeared to be significantly correlated with lnFCRg over 14 two-week periods out of 15 (r = 0.26-0.75 for significant correlations). For females and males separately, lnFCR and lnFCRg were significantly correlated over 11 and seven periods, respectively (r = 0.48-0.88 and r = 0.39-0.82, respectively). Projections revealed that direct selection on FCRg would improve FCRg by 5.9 and 3.7% per generation for females and males, respectively (Fig. 1). In comparison, selection on FCR on the 4th two-week period (worst correlation, r = 0.26) would improve FCRg by 3.5 and 1.6% for females and males, respectively, whereas selection on FCR on the 13th period (best correlation, r = 0.75) would improve FCRg by 5.1 and 2.7% for females and males, respectively (Fig. 1). Two other simulations were made selecting on the 2nd and the 8th periods to balance correlations of respectively r = 0.40 and 0.65 with time to wait before performing selection. An improvement of FCRg by 1.7 and 2.4% was projected for females and males, respectively, for the 2nd period, and by 3.9 and 3.1% for females and males, respectively, for the 8th period (Fig. 1).

Discussion and conclusions

These results suggest that estimating the FCR of juvenile tilapia over two-week periods could be relevant to perform a selective breeding program for FCRg at lower costs in both female and male tilapia. Genetic gains projected here were higher than the 1% per generation estimated in Rodde et al. (2020), this being explained by both stronger correlations between lnFCR and lnFCRg and higher variability of FCRg (the CV of FCRg was only 10.8% in Rodde et al., 2020). The impact of individual rearing on fish performance remains, however, debatable. Moreover, the heritability of 0.32 used here and published by de Verdal et al. (2018) was estimated over only one week at juvenile stage with fish reared in small groups, which differs greatly from experimental conditions presented here.

References

Besson, M., Allal, F., Chatain, B., Vergnet, A., Clota, F., Vandeputte, M., 2019. Combining individual phenotypes of feed intake with genomic data to improve feed efficiency in sea bass. Frontiers in Genetics 10, 219.

de Verdal, H., Vandeputte, M., Mekkawy, W., Chatain, B., Benzie, J.A.H., 2018. Quantifying the genetic parameters of feed efficiency in juvenile Nile tilapia *Oreochromis niloticus*. BMC Genetics 19, 105.

Rodde, C., Chatain, B., Vandeputte, M., Quoc Trinh, T., Benzie J.A.H., de Verdal, H., 2020. Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*? Aquaculture Reports 17, 100349.

EVALUATION OF SEMI-MOIST FEED IN GROWTH PERFORMANCE AND FEED UTILIZATION OF JUVENILE GREATER AMBERJACK, *Seriola dumerili (RISSO,1810)*. VALIDATION IN LARGE SCALE EXPERIMENT

A. Vasilaki*, G. Rigos, C. Nikoloudaki, G. Pyrenis, D. Kogiannou, S. Adamidou, I. Nengas

Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Anavyssos, Attika, Greece
Email: avasilaki@hcmr.gr

Introduction

Greater amberjack (*Seriola dumerili*) is considered an excellent candidate for the diversification of Mediterranean aquaculture species due to its fast growth rate, adaptability to intensive culture conditions, its excellent flesh quality, its global market recognizability and high market price. Aquaculture of greater amberjack was established in Japan and later adopted in European countries (Sicuro & Luzzana 2016). Although significant scientific progress has been made globally concerning the rearing and reproduction of greater amberjack, important steps of the production process, such as nutrition, need further development. Innovative semi-moist feeds are very promising showing improved KPIs in commercial scale (moisture 20-30% compared to conventional extruded feeds with <10%). Semi-moist feeds result in enhanced digestion process and better utilization of feed nutrients due to the reduced time required for intestinal hydration compared to the standard dry pellets (Ruohonen et al,1997). This type of feed is also used in Japan for Japanese amberjack (*S.quinqueradiata*) (Sicuro & Luzzana 2016).

Materials and methods

Two experimental trials were conducted with the first one carried out in the facilities of the Hellenic Centre for Marine Research, Institute of Marine Biology, Biotechnology and Aquaculture in Athens. Greater amberjack of an initial body weight of 83.9±11.6g were obtained from Argosaronikos Fishfarms S.A. and distributed in groups of 20 fish in 9 cages (three replicates of each treatment) of $1m^3$ capacity. Conventional feed, specially designed for greater amberjack, was modified to three different levels of moisture content (Table 1). Fish were fed the experimental diets *at libitum* three times per day. Temperature ranged between 24-26 °C.

The second trial took place in Argosaronikos Fishfarms S.A., in Salamina. Eight thousand fish with an initial body weight of 125±10g were used for the validation of the selected lab scale diet. Four cages (two replicates) were used with approximately two thousand fish per cage. Both experimental diets were produced with extrusion, a commercial feed with 8% moisture content and a semi-moist feed with 20% moisture (Table 1).

Results and Discussion

The results of the first trial showed a positive effect of moisture content in growth performance and feed utilization (Table 2). Specifically, feed consumption had a positive correlation with the increase of moisture content, this was observed for other species too (Ruohonen et al.1997). Correlation was equal to R=0.912. Higher feed consumption (in dry matter) had as result the increase of final body weight for fish fed semi-moist diets (13.9 & 23.8%). Specific growth rate (SGR) showed significant differences between the groups fed the experimental diets compared to those fed the conventional moisture feed. A trend was observed for better feed conversion ratio (FCR) for fish fed the experimental diets with P=0.055. The field trial validated the aforementioned results (Table 2). Fish fed the semi-moist feed gained more weight compared to those fed the conventional moisture feed. Showing significant higher SGR for experimental population fed the semi-moist diet compared to those fed the conventional feed. No significant differences were observed in FCR between the experimental groups. Although feed consumption was lower for groups fed the semimoist feed, those populations performed higher final weight. Similar results with both trials were also observed by Papadakis et al. 2008 for the same species.

Papadakis IE, Chatzifotis S, Divanach P, Kentouri M (2008) Weaning of greater amberjack (Seriola dumerilii Risso 1810) juveniles from moist to dry pellet Aquaculture international, 16(1), 13-25

Ruohonen K, Grove DJ, McIlroy JT (1997) The amount of food ingested in a single meal by rainbow trout offered chopped herring, dry and wet diets Journal of Fish Biology, 51(1), 93-105

Sicuro B, Luzzana U, 2016 The State of Seriola spp. Other Than Yellowtail (S. quinqueradiata) Farming in the World. Reviews in Fisheries Science & Aquaculture 24, 314-325

(Continued on next page)

Table 1: Feeds composition Trial 1 & 2

Trial 1	Moisture	Ash	Protein	Fat
Diet 1	9.4	9.56	49.83	15.4
Diet 2	13.9	9.02	47.38	14.64
Diet 3	23.8	8.04	41.93	12.96
Trial 2	**Moisture**	**Ash**	**Protein**	**Fat**
Conventional	8	10	46.92	15.76
Semi-moist	20.36	9.36	41.02	13.64

Table 2: Growth performance: weight increase, Feed conversion ratio (FCR), Specific growth rate (SGR), % feed consumption

Trial 1	Diet 1	Diet 2	Diet 3
Weight increase (g)	$101.96^{a} \pm 10.77$	$121.33^{b} \pm 1.58$	$119.87^{b} \pm 3.81$
FCR	0.85 ± 0.04	0.79 ± 0.02	0.81 ± 0.02
SGR	$2.66^{a} \pm 0.16$	$2.98^{b} \pm 0.08$	$2.95^{b} \pm 0.07$
% consumption	$2.68^{a} \pm 0.04$	$2.75^{ab} \pm 0.01$	$2.79^{b} \pm 0.05$
Trial 2	**Semi-moist**	**Conventional**	
Weight increase (g)	$355.5^{a} \pm 7.78$	$278.5^{b} \pm 2.12$	
FCR	1.88 ± 0.02	2.58 ± 0.41	
SGR	$0.59^{a} \pm 0.01$	$0.50^{b} \pm 0.02$	
% consumption	$1.78^{a} \pm 0.08$	$2.31^{b} \pm 0.13$	

SEABASS AND SEABREAM CONSUMPTION: A CASE STUDY IN SPAIN

Y. Vecchio[1], M. Masi[1], J. Di Pasquale[2], E. Tribilustova[3], and F. Adinolfi[1]

[1] Department of Department of Veterinary Medical Sciences - Alma Mater Studiorum University of Bologna (Italy)
yari.vecchio@unibo.it, margherita.masi4@unibo.it, felice.adinolfi@unibo.it
[1] Faculty of Veterinary Medicine - University of Teramo (Italy)
jdipasquale@unite.it
[3] Eurofish International Organisation
ekaterina.tribilustova@eurofish.dk

Introduction

Several scholars have deepened the analysis of consumer behaviour towards fish products and many determinants of choice have emerged (Carlucci et al., 2015). Many studies have focused on the role of demographic variables (Tomic et al., 2016), highlighting in particular the relevance of age and household dimension (Birch & Lawley, 2012). Among the most considered aspects in empirical surveys, the perception of health benefits is considered as one of the promoting factors that could explain fish consumption patterns (Neale et al., 2012). As well, the issue of perceived convenience or discomfort of fish has often emerged as influential in consumer choice (Cosmina et al., 2012). Although there is a high propensity for the purchase of fresh products by most consumers (Neale et al., 2012), numerous empirical evidences have observed among the barriers also the perceived difficulty in preparing fresh fish has an influence negative on purchasing behavior (Altintzoglou et al., 2010). Origin and production methods are considered among the most important attributes in food preferences, also in the case of fish products (Mauracher et al., 2013), and as well, great attention is given to nutrition claims and sustainability labels (Menozzi et al., 2020). The agroecological context exert at the same time an influence on consumer habits, showing for example how consumers who traditionally live in areas fish-oriented people prefer to eat unprocessed seafood products (Tomic et al., 2016). The role played by the main factors contributing to consumer awareness complete the general framework, which include trust in information sources (Pieniak et al., 2007), self-efficacy in quality assessment (Sveinsdóttir et al., 2009) and the ability of quality signals to provide accurate information (Adinolfi et al., 2011). Price is still little explored in the study of fish consumption behaviour, since the quantity of different species and the wide range of their prices make it difficult to perform any assessment (Neale et al., 2012). However, empirical evidences show that fish is widely perceived as more expensive than other sources of protein and particularly meat (McManus et al., 2012) although there is little certainty about how this perception affects purchasing behaviour towards fish and fish products (Khan et al., 2018).

Aim

The analysis of this consumer survey collected the main factors influencing the consumption of fish products from the literature. The empirical review is aimed to determine how these factors impact on the consumption of seabass and seabream (BB) determining homogeneous groups of consumers.

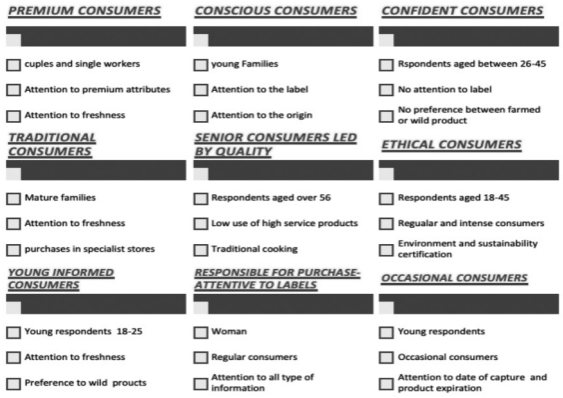
Method

Cluster analysis techniques have been commonly used by economists to investigate the characteristics and motivations of food consumers and it's one of the most widely used methods in marketing research. The segmentation of consumers into different homogeneous groups allows consumers' behaviour to be summarized in a limited number of distinct consumption profiles. Vanhonacker et al. (2013) investigated consumer behaviour towards fish products using this method. A survey was conducted as part of the Horizon PerformFISH project, which gathered relevant information on consumer decision making, in order to explore the behaviour of Italian consumers towards BB products. The application of the MCA-CA (multiple correspondence analysis-cluster analysis) algorithm allows to define typological groups based on previously selected dimensions corresponding to the survey section.

Results

The analysis showed 9 different consumer groups shown in the following image.

(Continued on next page)



Bibliography

Adinolfi F., De Rosa M., Trabalzi F., (2011) Dedicated and generic marketing strategies: The disconnection between geographical indications and consumer behavior in Italy, British Food Journal 113 (3), 419-435

Altintzoglou T., Verbeke W., Vanhonacker F., Luten J. ,(2010) The Image of Fish from Aquaculture Among Europeans: Impact of Exposure to Balanced Information, Journal of Aquatic Food Product Technology Vol. 19, Iss. 2,

Birch, D., Lawley, M., (2012), Buying seafood: understanding barriers to purchase across consumption segments, Food Quality and Preference Volume 26, Issue 1, October 2012

Carlucci D., Nocella G., De Devitiis B., Viscecchia R., Bimbo F., Nardone G., (2015), Consumer purchasing behaviour towards fish and seafood products. Patterns and insights from a sample of international studies, Appetite,Volume 84,

Cosmina M., Demartini E., Gaviglio A., Mauracher C., Prestamburgo S., Trevisan G., (2012), Italian consumers' attitudes towards small pelagic fish, 11 (1) (2012), pp. 52-57

FAO (2014), The state of world fisheries and aquaculture. Opportunities and challenges, Food and Agriculture Organization of the United Nations, Rome.

Khan AQ, F. Aldosari and S.M. Hussain, Fish consumption behavior and fish farming attitude in Kingdom of Saudi Arabia (KSA), Journal of the Saudi Society of Agricultural Sciences 17(2), 195-199 (2018)

Ismea, 2019, I consumi domestici delle famiglie italiane

Mauracher C., Tempesta T., Vecchiato D., (2013), Consumer preferences regarding the introduction of new organic products. The case of the Mediterranean Sea bass (Dicentrarchus labrax) in Italy, Appetite, 63, pp. 84-91

McManus A., Hunt W., Howieson J., Cuesta-Briand B., McManus J., Storey J., (2012), Attitudes towards seafood and patterns of consumption in an Australian coastal town, Nutrition Bulletin, 37 (3), pp. 224-231

Menozzi, D., Nguyen, T. T., Sogari, G., Taskov, D., Lucas, S., Castro-Rial, J. L. S., & Mora, C. (2020). Consumers' preferences and willingness to pay for fish products with health and environmental labels: Evidence from five European countries. Nutrients, 12(9)

Neale E.P., Nolan-Clark D., Probst Y.C., Batterham M.J., Tapsell L.C., (2012), Comparing attitudes to fish consumption between clinical trial participants and non-trial individuals Nutrition and Dietetics, 69 (2), pp. 124-129

Pieniak Z., Verbeke W., Scholderer J., Brunsø K., Olsen S.O., (2007), European consumers' use of and trust in information sources about fish, Food Quality and Preference, 18 (8), pp. 1050-1063 SveinsdóttirK.,MartinsdóttirE.,Green-PetersenD.,HyldigG.,SchelvisR.,DelahuntyC.,(2009),Sensorycharacteristicsof differentcodproductsrelatedtoconsumerpreferencesandattitudes,FoodQualityandPreference,Volume20,Issue2,March M. Tomic´, D. Matulic´ and M. Jelic´, What determines fresh fish consumption in Croatia? Appetite **1(106)**:13-22 (2016). Vanhonacker F., Van Loo E.J., Gellynck X., Verbeke W., (2013), Flemish consumer attitudes towards more sustainable food choices, Appetite, Volume 62

INFLUENCE OF DIETARY INCLUSION OF BLOOD HYDROLYSATES ON EUROPEAN SEABASS MUSCLE GROWTH

Cristina Velasco*[1], Daniela Resende[1,2,3], Beatriz Oliveira[1,2], Miguel Pereira[3], Carlos Pereira[4], Bianca Marques[5], Cristina Rocha[5], Manuela Pintado[3], Luísa M.P. Valente[1,2]

[1]CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal
[2]ICBAS, UP, Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal
[3]CBQF, Laboratório Associado, ESB-UCP, R. Arquiteto Lobão Vital 172, 4200-374 Porto, Portugal
[4]Politécnico de Coimbra/ESAC, Bencanta, 3045-601 Coimbra, Portugal
[5]CEB, UM, Campus de Gualtar, 4710-057 Braga, Portugal
*Presenting author. Email: cvelasco@ciimar.up.pt

Introduction

The success of aquaculture not only depends on the use of sustainable protein sources, but also on the ability to produce high quality products. From a commercial viewpoint, flesh firmness is one of the most appreciated characteristics, and it is strongly influenced by a set of intrinsic traits such as the number and size distribution of muscle fibres [1]. In addition, both growth performance and muscle protein synthesis are regulated by extrinsic factors including diet characteristics, environmental conditions or stressors [2]. Several studies have demonstrated that plant and animal protein hydrolysates provide bioactive peptides able to confer physiological benefits, through the regulation of gene expression and signalling pathways, making them suitable ingredients for inclusion in aquafeeds with potential advantages on economically important traits such as growth performance, body composition and flesh quality. Although some pathways related to muscle fibre proliferation and hypertrophic growth have been studied in fish and associated to their nutritional status, there is no knowledge on the effects of swine blood hydrolysates (BH) in the regulation of these genes in aquaculture fish species, which were investigated in this work.

Methods

Three fractions of swine BH obtained by autohydrolysis (AH) or enzymatically were selected. The enzymatically obtained BH were further submitted to a micro- (MF) and nanofiltration (NF). Dried hydrolysates were then included in five isolipidic and isoproteic diets for European seabass: a fishmeal (FM) based diet (positive control, PC), a commercial-based diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g), and fed to apparent satiation in a recirculating saltwater system (RAS). After 12 weeks, 9 fish per treatment were sampled: fish were softly scaled before taking a cross-sectional slab, that was photographed for determination of the cross-sectional area (CSA). A white muscle sample was collected for RNA extraction, and a small portion of the fillet was frozen in isopentane cooled by dry ice, for histological analysis.

Results

Fish fed NF had similar final weight and length as NC but differed significantly from the PC that had the best performance. Fish fed MF diet had the lowest values for both parameters (MF<AH≤NF≤NC<PC). Regarding muscle cellularity results, MF diet showed significantly smaller white muscle CSA compared to all other diets, and the lowest number of fibres. However, fibre density did not vary significantly among experimental diets. Fibre's diameter mostly varied between 60 and 65 μm, with only 5-7% of them being lower than 20 μm and 3-4% higher than 120 μm. The MF group had the highest percentage of intermediate size fibres (40-60 μm) and lowest percentage of small-sized fibres (< 20 μm) and highest percentage of large sized ones (>120 μm).

MF diet down-regulated gene expression of *myod1*, *fgf4* and *capn1* compared with the PC, and up-regulated *mafbx* and *mymk*; NF diet down-regulated *fgf4, fgf6 and capn1*; while fish fed the AH diet showed no significant differences in the expression of these muscle growth markers compared to the PC group.

(Continued on next page)

Conclusions

Both *myod1* and *fgf4* play a pivotal role in myoblast proliferation and differentiation, and its downregulated expression in fish fed MF suggests an impairment of muscle hyperplastic growth that was further reflected in the lowest number of muscle fibres. This was further supported by the upregulation of *mafbx,* responsible for protein breakdown. However, a possible compensatory growth mechanism was observed through the concomitant downregulation of *capn1* and upregulation of *mymk* suggesting a reduction in muscle proteolysis but increased myoblast fusion. Nevertheless, these compensatory mechanisms were not sufficient as the MF group presented the worst growth performance. Fish fed the AH diet, despite showing a reduced growth compared to both controls, did not show any change in the expression of growth markers in muscle. But fish fed NF showed a significant downregulation of *capn1* compared to both the PC and the NC diets, which seems to have a pivotal role in supporting muscle accretion and final muscle cross sectional area.

In conclusion, different swine blood hydrolysates (BH) regulate muscle growth through the expression of genes either involved in myoblast proliferation, differentiation and fusion or muscle atrophy.

References

1. Valente, L.M., et al., *What determines growth potential and juvenile quality of farmed fish species?* Rev. Aquac. 2013, **5**, S168–S193.
2. Johnston, I. A. *Environment and plasticity of myogenesis in teleost fish*. J. Exp. Biol. **209**, 2249-2264

Acknowledgments

Work supported by Project MOBFOOD, POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT, SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019).

HIGH QUALITY *Paracentrotus lividus* GONADS CAN BE PRODUCED UNDER A RECIRCULATION PILOT-SCALE SYSTEM

Cristina Velasco[1*], Inês Garrido[1,2], Rui J. M. Rocha[3], Luísa M. P. Valente[1,2]

[1]CIIMAR, Interdisciplinary Centre of Marine and Environment Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[2]ICBAS, Abel Salazar Biomedical Sciences Institute, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[3]Riasearch Unipessoal Lda, Cais da Ribeira de Pardelhas 21, 3870-168 Murtosa, Portugal *Presenting author: cvelasco@ciimar.up.pt

Introduction
Sea urchin gonads have been increasingly demanded, for their unique organoleptic properties. Therefore, urgent action is needed to protect this species and its natural habitat, being echinoculture a potential solution to this problem. In fact, in the past years, feasible ways of producing sea urchins with marketable gonads, namely *Paracentrotus lividus*, have been studied. The market price and consumers' acceptability of cultured sea urchins' gonads are influenced by sensory traits. Colour is a highly important feature, since it is the first stimuli presented to the consumer, therefore, a major influencing factor in marketability. Echinenone is the most abundant carotenoid in the gonads and its deposition depends on availability, uptake and bioconversion of β-carotene from dietary sources (Symonds et al. 2007). In this study, the effectiveness of an extruded diet supplemented with a natural source of β-carotene was evaluated after 4 and 8 weeks of feeding.

Materials and methods
A control diet (CTRL) was formulated and compared to an isonitrogeneous and isoenergetic diet (DUN), containing *Dunaliella salina* (1.5% inclusion) as a natural rich source of β-carotene. The diets were cold extruded (<30 °C) and softly dried (<45 ºC). The diets were distributed every 48h, during 8 weeks, to triplicate groups of sea urchins, placed in plastic mesh cages in a saltwater recirculation aquaculture system (RAS) with a stocking density of 3.0 $kg.m^{-2}$; temperature 15.4 ± 0.7 ºC, salinity 33‰, and a 10h L/14h D photoperiod. After 4 and 8 weeks of feeding, 4-5 sea urchins per tank were individually weighted, measured, and gonads sampled for texture and colour evaluation (lightness, redness, yellowness, chroma and hue angle, *i.e*. L*, a*, b*, C* and h* respectively) using CIElab colour space.

Results and Discussion
The tested diets were well accepted by the sea urchins. Feeding the sea urchins for 4 weeks did not produce a significant increment in GSI. However, GSI increased significantly from 8.1 to 16.1 in females and from 9.4 to 16.9 in males, after 8-week of feeding the experimental diets. No differences could be observed among diets for GSI values. After 8 weeks of feeding, female from DUN group showed significantly higher a*, but lower L*, b* and h*values than those from the CTRL. In males, a significantly higher b* value could be observed in gonads from DUN group compared to those from the CTRL. Gonads resilience and firmness did not vary among dietary treatments. To ascertain whether the gonads from DUN group would be accepted by consumers, the results were compared with previous studies performed with *P. lividus* (Baião et al. (2021) which gonads were scored 8 (from 1-9) in acceptability by the consumer (ACC8)). The C* value of females fed DUN diet was similar to that of ACC8, showing a high similarity. Moreover, L* value was lower whilst the a* value was higher than those observed in ACC8 gonads, which are reported as favourable features. Resilience was also improved in DUN fed urchins. In males, gonads were very similar to those highly scored by consumers (similar CIElab values).

Conclusion
The tested diets were able to successfully enhance gonadal growth in both sexes during the 8-week growth trial under a recirculation pilot-scale system. When considering consumers' demands reported by Baião et al. (2021), lower L*, but higher a*, b* and resilience values were related to more appealing gonads. The present results clearly showed that DUN diet is effective in producing gonads corresponding to such quality traits that will be highly accepted by consumers.

(Continued on next page)

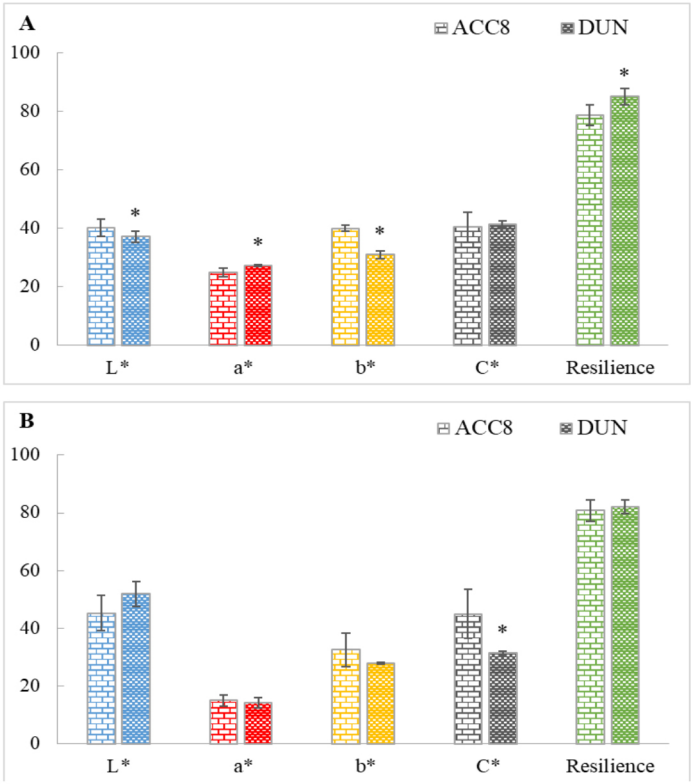


Figure 1 – Colour and texture parameters of (**A**) female and (**B**) male sea urchins with acceptability score of 8 (ACC8) and sea urchins fed DUN for 8 weeks. Asterisk marks significant differences between ACC8 and DUN groups.

Acknowledgments

Work supported by Project CAVIAR – Market valorisation of sea urchin gonads through dietary modulation (FA05_2017_015), financed through programme Fundo Azul.

References

Baião, L.F., Rocha, C., Lima, R.C., Marques, A., Valente, L.M.P., Cunha, L.M., 2021. Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications. Food Research International. 140, 109873.

Symonds, R.C., Kelly, M.S., Caris-Veyrat, C., Young, A.J., 2007. Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9'-cis-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 148, 432-444.

MICROALGAE BY-PRODUCTS FOR AQUACULTURE FEEDS AND ITS PROSPECTIVE APPLICATIONS IN BIOTECHNOLOGY

A. Ventura-Castellano[1], S. Ramírez-Bolaños[1], R. Quirós-Pozo[1], P. [2]E. Portillo-Handfield[2], L. Robaina [1]

[1]Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[2]Technological Institute of the Canary Islands (ITC). Pozo Izquierdo 0 S.N. 35119, Vecindario, Canary Island, Spain
E-mail: anais.venturacastellano@gmail.com

Introduction

Algae have become almost 30% of the 120 million tons of global aquaculture production in 2019. The microalgae stand out in the use as functional foods or dietary supplements and extraction of bioactive compounds (Barkia *et al*., 2019). In fish feed, these theoretically provide the necessary amino acids, beneficial polysaccharides, fatty acids, antioxidants, vitamins, and minerals, becoming promising candidates for the reduction or replacement of fish meals and also fish oils. However, their incorporation either as direct feed ingredient or in its defatting forms as a co-product for the human industries has not met the expectations. Nowadays, their incorporation seems limited as additives with moderate levels of inclusion (2.5 to 10 mg/kg) (Milad *et al*., 2016; Abdulrahman *et al*., 2018). Different microalgae factors (cultivation conditions, processing, physiological state, bioavailability, digestibility, etc) have been directly associated with poorer and different results in fish trials, part of which stems from fish species and culture conditions. In addition, their high cost of production together with the above mentioned has slowed down their incorporation in aquaculture with respect to what was initially expected and desired. Therefore, research into new processing techniques to improve the use of microalgae in fish nutrition is mandatory and can also help the necessary sustainability of this industry. Nowadays, *Isochrysis* sp. (ISO) is one of the most experienced and important microalgae due to its physiological characteristics and nutritional value that confers antioxidant, antibacterial, and immunomodulatory effects. ISO fat is widely used in cosmetics and as a feed supplement, being the remaining by-product usually discarded as residue. In aquaculture, some investigations for the substitution of fish oil and fish meal as additives with non-moderate amounts of ISO reported adverse effects that compromise fish health and welfare. Due to the observed effect of the microalgae as is in fish nutrition, and the globally increased amount of its defatted by-products derived from other industries, it is becoming interesting to consider them as novel aquafeed materials. The aim of present study is to theoretically compare and discuss biochemical properties and benefits for the ISO by-products against the raw ISO, and biotechnological needs to improve their use in the near future.

Material and Methods

Processed: ISO and CO-ISO were obtained from the Biotechnology Department (ITC, Canary Islands), the latter obtained after fatty acid extraction process by supercritical CO_2 extraction from the raw correspondent ISO.

Biochemical analysis: The proximate composition of the ISO and CO-ISO (moisture, ash and crude protein) was determined according to AOAC protocol (2000). Lipids were determined according to Folch *et al*. (1957). Amino acid profile was analysed by HPLC according to the EU rules (ISO 13903:2005; EU 152/2009).

Results

CO-ISO has a lower percentage of lipids, proteins (8.22 and 36.71% respectively) and amino acids (table 1) but an increase in ash (14.02% table 1) compared to ISO. A priori this may be a disadvantage, however, it has been seen that percentages of 5 and 10% of ISO inclusion in feed for *Seriola dumerili* can cause negative effects on animal growth and health (Ventura-Castellano *et al*., 2019) and its substitution by CO-ISO can allow good growth performance and animal welfare (Ventura-Castellano *et al*., 2021). Due to the negative effect on the parameters analysed in the diets with ISO, which could be associated with an excess of fucoxanthin and the fatty part of it, an optimized approach would be the use of CO-ISO. Even so, the accessibility, bioavailability, digestibility, and utilization of aa, lipids, carbohydrates, and other bioactive of this by-product and microalgae could be improved by means of pretreatments based on the combination of different biotechnological techniques for their degradation, once again without losing sight of the use of waste from other industries to mitigate climate change and achieve a circular economy.

(Continued on next page)

Amino acid	g 100 g^{-1}	g 100 g^{-1}
	ISO	**CO-ISO**
(a) Cysteine + Cystine	0.33 ± 0.05	0.199 ± 0.03
(a) Methionine	0.80 ± 0.11	0.410 ± 0.06
(a) Alanine	2.33 ± 0.33	2.56 ± 0.36
(a) Arginine	4.70 ± 0.66	1.45 ± 0.20
(a) Aspartic acid	11.2 ± 1.6	2.29 ± 0.32
(a) Glutamic acid	12.3 ± 1.7	2.96 ± 0.41
(a) Glycine	1.88 ± 0.26	1.33 ± 0.19
(a) Histidine	0.70 ± 0.1	0.458 ± 0.06
(a) Hydroxyproline	<0.05 (LOQ)	<0.05 (LOQ)
(a) Isoleucine	1.47 ± 0.21	1.11 ± 0.16
(a) Leucine	2.83± 0.40	1.99 ± 0.28
(a) Lisin	1.90 ± 0.27	1.33 ± 0.19
(a) Ornithine	<0.05 (LOQ)	<0.05 (LOQ)
(a) Phenylalanine	1.85 ± 0.26	1.30 ± 0.18
(a) Proline	1.77 ± 0.25	1.70 ± 0.24
(a) Serine	1.63 ± 0.23	1.22 ± 0.17
(a) Threonine	1.76 ± 0.25	1.22 ± 0.17
(a) Tyrosine	1.20 ± 0.17	0.956 ± 0.13
(a) Valine	1.94 ± 0.27	1.35 ± 0.19
Proximal composition (DW%)		
Total Lipid	16.89 ± 0.33	8.22 ± 0.65
Total Protein	67.24 ± 0.052	36.71± 0.66
Ash	8.75 ± 0.01	14.02 ± 0.17
Moisture	9.90 ± 0.03	7.69 ± 0.013

Table 1*: Isochrysis sp*. cysteine and methionine content determined by oxidative method (ISO 13903:2005; EU 152/2009 (F)) and amino acid profile with acid hydrolysis (ISO 13903:2005; EU 152/2009 (F)). Proximal composition of raw material from the Canarian Institute of Technology (ITC)

References

Barkia, I., Saari, N. & Manning, S.R. 2019. Microalgae for high-value products towards human health and nutrition. Marine Drugs, 17(5): 304.

Ventura-Castellano A., Hernández-Rivero L., Ramírez-Bolaños S., Quirós-Pozo R. & Robaina L. (October 7-10, 2019). Microalgae *(Isochrysis galbana)* in diets for juvenile amberjack (*Seriola dumerili,* risso 1810); effects of on growth, color performance, body composition and liver morphology [Poster]. Aquaculture Europe, International Conference and Exposition, EA.

Ventura-Castellano A., Monzón-Rivero[1] C., Ramírez-Bolaño S., Quirós-Pozo R. & Robaina L. (April 12-15, 2021). Microalgae by-product in diets for amberjack (*Seriola dumerili,* risso 1810); effects of on growth, body composition, and liver morphology. [Poster]. Aquaculture Europe, International Conference and Exposition, EA

NUMERICAL MODELLING OF THE DYNAMIC BEHAVIOUR OF A SIMPLIFIED SEAWEED (*Laminaria saccharina*) CULTIVATION NET SYSTEM SUBJECTED TO WAVES AND CURRENT-INDUCED LOADS USING A LUMPED MASS APPROACH

Gael, Verao Fernandez[1]*, Ajie Pribadi[1], Julian Pforth[2], Eva Strothotte[2], and Evert Lataire[1]

[1]Maritime Technology Division, Ghent University, Ghent, Belgium.
[2]R & D Centre, University of Applied Sciences Kiel GmbH, Kiel, Germany
*E-mail: Gael.VeraoFernandez@UGent.be

Introduction

The multi-use concept for the North Sea, encouraged by the European Union, have brought up new commercial opportunities for the offshore and aquaculture sectors. The European Horizon 2020 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 (UNITED 2021) appears as a joint effort between research and industrial partners to enhance aquaculture in the vicinity of offshore wind turbine parks. One of the activities within the UNITED project was the design of a seaweed cultivation net system for *Laminaria saccharina* (the design is owned by Offshore Seaweed Systems; Offshore Seaweed Systems 2021). The system will be installed at the FINO3 research platform (FuE-Zentrum FH Kiel GmbH 2021), located 45 nautical miles offshore in the German part of the North Sea. As part of the design process, a numerical study of a simplified seaweed cultivation net system was performed using a lumped-mass approach numerical model to evaluate the dynamic behaviour of the system subjected to waves and currents induced loads.

Theoretical Background

The two key parameters when modelling seaweed net systems is to correctly capture the hydrodynamic interactions of the net and the seaweed with the incident flow, respectively. The former has been studied mainly for fish nets by means of experimental testing (DeCew et al. 2010) and numerical models based on dynamic 3D models (Lader and Enerhaug 2005) and the lumped-mass approach (Cifuentes and Kim 2017). Cifuentes and Kim (Cifuentes and Kim 2017), adapted the experimental drag coefficient formula estimated by DeCew and Tsukrov (DeCew et al. 2010) validating it with experimental results improving the dynamic 3D models by addressing the influence of net solidity, , and Reynolds number, .

The latter has focused on experimentally obtaining the drag coefficients of a single blade of seaweed subjected to an unidirectional flow on a wave flume (Vettori and Nikora 2019). However, these experimental studies do not represent the behaviour of a net full of seaweed. To solve this limitation, the authors of this research propose to consider the growth of the seaweed in the net as biofouling on the net structure. Therefore, the drag coefficients of the net and seaweed combination will be adjusted based on the thickness of the seaweed following the recommendations of the DNV OS 301 (DNV GL 2018) for marine growth.

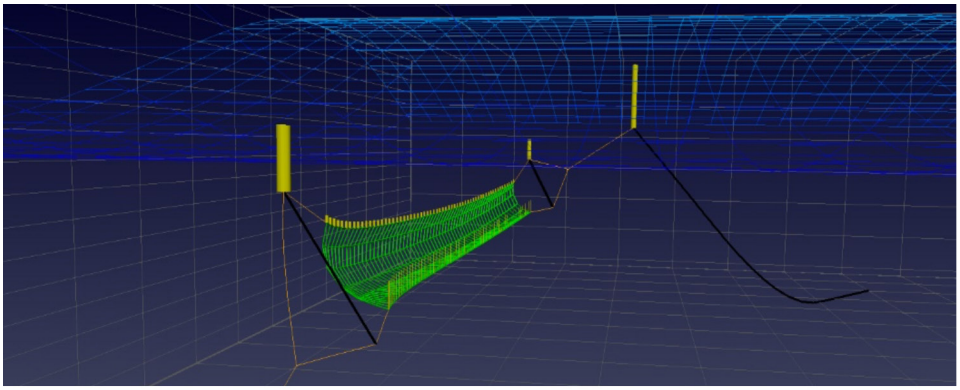


Figure 1 Snapshot of hydrodynamic calculation for $H = 14.5\ m$ and $T = 12.1\ s$.

(Continued on next page)

Numerical model

The numerical calculations have been performed using the in-house developed mooring dynamic solver MoorDyn-UGent, based upon the open source code MoorDyn (Hall 2017) and adapted by Pribadi et. al (Pribadi, Donatini, and Lataire 2019). A numerical net of 30 m length and 3 m width (for the cultivation of *Laminaria saccharina*) installed vertically supported by different buoys and clump weights has been modelled and subjected to different wave and current combinations. The net has been discretized as a combination of cylindrical elements with two mesh sizes: i) equivalent to the real net (0.5 m x 0.5 m) and ii) coarser than the real net (1.0 m x 1.0 m). The drag coefficient of the net and seaweed combination has been obtained using the adapted formula of Cifuentes and Kim (Cifuentes and Kim 2017), corrected with the DNV OS 301 (DNV GL 2018) standards.

Results

Figure 1 shows a snapshot of the hydrodynamic simulation for a wave height, , and a wave period, , with a net discretization of 0.5 m x 0.5 m. In the numerical study performed it was observed that the numerical net discretization does not affect the tensions calculated on the anchor lines of the simplified seaweed cultivation net system. Furthermore, it was noticed that at the test site location, the major part of the load suffered by the system origins from the wave-induced hydrodynamic loads with a small contribution of the current-induced load.

Discussion and conclusion

A new numerical approach for calculating the mooring line tensions of a seaweed cultivation net system has been presented. This numerical approach is based on considering the growth of seaweed as fouling over a net following the DNV-OS 301 recommendations for cylindrical elements. The numerical results presented in this abstract will be validated against experimental data gathered during the UNITED project to assess the applicability of the numerical approach.

References

Cifuentes, Cristian and M. H. Kim. 2017. "Numerical Simulation of Fish Nets in Currents Using a Morison Force Model." *Ocean Systems Engineering* 7(2):143–55.

DeCew, J., I. Tsukrov, A. Risso, M. R. Swift, and B. Celikkol. 2010. "Modeling of Dynamic Behavior of a Single-Point Moored Submersible Fish Cage under Currents." *Aquacultural Engineering* 43(2):38–45.

DNV GL. 2018. *DNVGL-OS-E301 Position Mooring*.

FuE-Zentrum FH Kiel GmbH. 2021. "FINO3 - Research Platform." Retrieved April 30, 2021 (https://www.fino3.de/en/).

Hall, Matthew. 2017. *MoorDyn User's Guide*. Charlottetown, PEI, Canada.

Lader, P. F. and B. Enerhaug. 2005. "Experimental Investigation of Forces and Geometry of a Net Cage in Uniform Flow." *IEEE Journal of Oceanic Engineering* 30(1):79–84.

Offshore Seaweed Systems. 2021. *Cultivator Specifications 17th January 2021*.

Pribadi, Ajie Brama Krishna, Luca Donatini, and Evert Lataire. 2019. "Numerical Modelling of a Mussel Line System by Means of Lumped-Mass Approach." *Journal of Marine Science and Engineering* 7(9).

UNITED. 2021. "UNITED Project." Retrieved April 30, 2021 (https://www.h2020united.eu/).

Vettori, Davide and Vladimir Nikora. 2019. "Flow-Seaweed Interactions of Saccharina Latissima at a Blade Scale: Turbulence, Drag Force, and Blade Dynamics." *Aquatic Sciences* 81(4):61.

OYSTER PRODUCTION IN A MAN-MADE SALTWATER CREEK SYSTEM IN THE NETHERLANDS

G.P. Verbeeke*, E.P.A. Merks, L. Sanders, A.C. Lataes, J. van Houcke

HZ University of Applied Sciences, P.O. Box 364, 4380 AJ Vlissingen (The Netherlands)
Email:gabrielle.verbeeke@hz.nl

Introduction

In the Netherlands traditionally two main sites are used for oyster production: Lake Grevelingen and the Eastern Scheldt. Production in these systems have their challenges as food availability is low and competition high in the Eastern Scheldt, while in Lake Grevelingen water quality issues arise occasionally. The sector is therefore looking for additional cultivation sites. Recently a man-made saltwater creek system was constructed as a new nature reservation area in the Southwestern part of the Netherlands. Within the 300 ha area approx. 30 ha is designated for aquaculture activities. In 2020 first experiments focusing on the oyster production potential within the area started. As growth of oyster is dependent on the environmental circumstances (Barillé et al., 1997; Bernard et al., 2011; Cognie et al., 2003; Palmer et al., 2021) the system characteristics were also investigated.

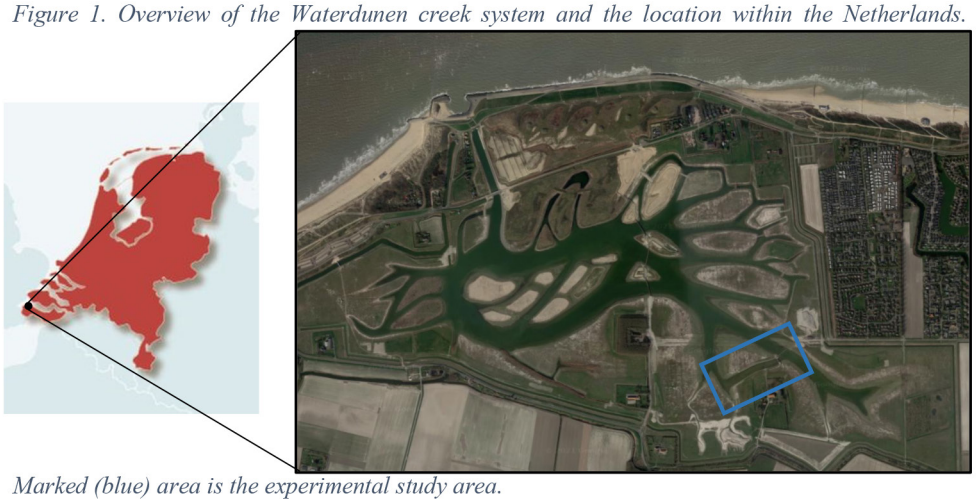
Methodology

The Waterdunen area, a man made saltwater creek, is located near Groede, The Netherlands. The creek system is fed by North sea water through culverts that dampen the tide. The system is exposed to a tidal curve of approx. 1.2 m. At the end of the 3.2 km creek system a 7.500 m^2 experimental location has been developed. At the inlet of this area three paddlewheels are able to increase the flow through of oxygen rich water.

System characteristics were investigated using continuous data loggers (water temperature, dissolved oxygen, chlorophyll, turbidity and flow velocity). Furthermore every three weeks water samples were analyzed for microalgae abundancy during the period December 2020-August 2021.

Growth of the Pacific cupped oyster (*Crassostrea gigas*) at different live stages (oyster spat of 14mm, half-grown oysters of 32.5g and commercial size oysters of 60.3g) was assessed using three oyster cultivation methodologies (off bottom and bags with an inundation time of 16 h. and bags with an inundation time of 12 h.). The growth of oyster spat was assessed every three weeks using measurements of the length of 300 individuals per treatment. Growth of the half-grown and commercial size oyster was assessed using total weight, tissue weight and condition index (AFNOR) every six weeks for 90 individuals per treatment. In addition the growth of on bottom European flat oysters (*Ostrea edulis,* 52.5g) were also assessed using the same measurements and sample size. Mortality of all oyster groups was evaluated over time by counting dead individuals.

Figure 1. Overview of the Waterdunen creek system and the location within the Netherlands.



Marked (blue) area is the experimental study area.

(Continued on next page)

Results

Preliminary results show relatively high chlorophyll content within the water system (3.3 – 16.7 μg L^{-1} December – March) as compared with the main cultivation sites in the Netherlands (0.51 – 4.1 μg L^{-1} and 0.48 – 11.6 μg L^{-1} December – March for Lake Grevelingen and Eastern Scheldt respectively (Rijkswaterstaat, 2021)). Due to the low water column and tidal curve temperature fluctuations can be fast (water temperature of 6.5˚C at 6 February 2021 and 0.5˚C at 12 February 2021).

A clear effect of inundation time on the growth and mortality of all oyster groups was found. Highest growth occurred in the oysters that had the longest inundation time (while these groups showed lowest mortality. Pacific cupped oyster spat started growing within the winter months while half-grown and commercial size Pacific cupped oysters started showing growth in spring. In all cases growth was mainly related to the chlorophyll content in the water. The European flat oysters did not shown any growth and high mortality was found in this group, most likely due to high turbidity in the water column.

References

Barillé, L., Prou, J., Héral, M., & Razet, D. (1997). Effects of high natural seston concentrations on the feeding, selection, and absorption of the oyster Crassostrea gigas (Thunberg). *Journal of Experimental Marine Biology and Ecology*, *212*(2), 149–172.

Bernard, I., de Kermoysan, G., & Pouvreau, S. (2011). Effect of phytoplankton and temperature on the reproduction of the Pacific oyster Crassostrea gigas: Investigation through DEB theory. *Journal of Sea Research*, *66*(4), 349–360. https://doi.org/10.1016/j.seares.2011.07.009

Cognie, B., Barillé, L., Massé, G., & Beninger, P. G. (2003). Selection and processing of large suspended algae in the oyster Crassostrea gigas. *Marine Ecology Progress Series*, *250*, 145–152.

Palmer, S. C. J., Barillé, L., Kay, S., Ciavatta, S., Buck, B., & Gernez, P. (2021). Pacific oyster (Crassostrea gigas) growth modelling and indicators for offshore aquaculture in Europe under climate change uncertainty. *Aquaculture*, *532*, 736116. https://doi.org/10.1016/j.aquaculture.2020.736116

Rijkswaterstaat. (2021). *Veldparameters-OW (Expert)—Rijkswaterstaat Waterinfo*. Waterinfo. https://waterinfo.rws.nl/#!/kaart/Veldparameters-OW/(massa)Concentratie___20chlorofyl-a___20in___20Oppervlaktewater___20ug___2Fl

PHENOTYPIC CHARACTERIZATION OF THE THREE MAIN NATURAL POPULATIONS OF EUROPEAN SEA BASS (*Dicentrarchus labrax)* ON GROWTH AND QUALITY TRAITS PERFORMANCES

A. Vergnet*[a], F. Clota[a,b], D. Edoh[a,b] , F. Ruelle[a], M.O. Blanc[a], S. Lallement[a], M. Leitwein[a], M. Vandeputte[a,b], F. Allal[a]

[a] MARBEC, University of Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France
[b] Université Paris-Saclay, INRAE, AgroParisTech,, GABI, Jouy-en-Josas, France
E-mail: alain.vergnet@ifremer.fr

Introduction

The European sea bass is one of the most important species of Mediterranean aquaculture with 191,000 tons produced in 2016 (FAO). With a repartition area ranging from the Atlantic Ocean (southern Norway to Senegal) to the Mediterranean Sea, the European sea bass is genetically structured in three populations: Atlantic, West Mediterranean and East Mediterranean (Bahri-Sfar et al. 2000)with the noticeable exception of one Egyptian sample which grouped within the western clade, a fact attributable to the introduction of aquaculture broodstock. No heterogeneity was observed within the western basin ($\theta = 0.0014$ and n.s.. Characterizing these three populations can enable hatcheries to select the breeders with the best genetic background for specific breeding goals and rearing conditions, and thus to speed up genetic improvement. A previous study has investigated phenotypic differences between these populations using a partial diallel design with five natural populations and sub-populations (Vandeputte et al. 2014). In this study we focused on the main three natural populations in a "pure strain" design, to characterize their growth and quality traits with repeated measurements along two years.

Material and Methods

Three groups were produced by artificial fertilization in February 2018. Atlantic (AT) and West Mediterranean (WM) groups were produced by mating wild sires and wild dams from each of the populations in a full-factorial mating design, thus producing pure AT and WM offspring. Equal numbers of eggs from 22 WM dams, fertilized with sperm from 40 WM sires, and eggs from 9 AT dams, fertilized by 26 AT sires, were used. The East Mediterranean (EM) group was produced by mating 39 wild EM sires with 13 F1 EM x WM dams, thus producing 75% EM-25% WM backcross progenies. Each group was reared in triplicate tanks until 244 dph (30g), where 2106 fish were PIT-tagged, mixed and split into three replicate common garden groups. The fish stayed in 1.5 m^3 tanks until 432 dph at an average temperature of 20.9°C, and were then transferred to three 5 m^3 tanks. They were fed *ad libitum* with commercial feed (Le Gouessant Neo Grower) using self-feeders. The temperature was kept at an average of 20.5°C until 704 dph, and then lowered to 15.6°C until 858 dph where all fish were measured for the last time. Body length was measured at 0, 11, 40, 53, 80, 108, 136, 157, 188, 213, 244, 300, 341, 390, 432, 472, 522, 579, 634, 704 and 858 dph, as well as body weight starting at 108 dph, to calculate DGC (Daily Growth Coefficient) following this formula:

$$DGC_i = \frac{\omega_f^{\frac{1}{3}} - \omega_i^{\frac{1}{3}}}{t} \times 100,$$

where ω_f is the final body weight and ω_i is the initial body weight of the fish during a period of t days. Muscle fat content was estimated using a Distell FishFatmeter in the last 8 biometrics. At 864 dph, fish were sacrificed and dissected, their sex was identified and liver, digestive tract and gonads parts were weighed to the nearest 0.1 g. Then, the fish were de-headed, and manually filleted on the left side. Head, left fillet and half-carcass were weighed to the nearest 0.1 g. Liver, viscera, gonads, head, fillet, vertebral axis, carcass and gills yields were calculated. Different types of Anova models were built in order to test the effect of the strains and larval tanks for each biometry. Individual growth and yields were modeled as follows:

$$Y_{ijklm} = \mu + S_i + P_j + t_{k(j)} + T_l + \varepsilon_{ijklm}$$

With the phenotype of fish m, μ the intercept, S_i the fixed effect of sex i with three levels (male, female, unknown), P_j the fixed effect of population j; $t_{k(j)}$ the random effect of initial tank k, nested within population j, with three levels per population, the fixed effect of common garden tank l, with three levels, and ε_{ijklm} the random residual.

(Continued on next page)

Results and Discussion

During the first period of growth after tagging (244-463 dph), there was a highly significant effect of populations on DGC ($P<0.001$) with the highest DGC (1.28± 0.01) recorded in the EM population and the lowest DGC (1.07± 0.01) obtained in the WM population. During the second period of growth (463-704 dph), generally characterized by low growth performance, a significant effect of the populations on DGC was also detected ($P<0.05$) with the same ranking, although differences between populations were much smaller. During the third period (704-858 dph), characterized by a low temperature, the AT population has the highest DGC ($P<0.001$).

On quality traits, Table 1 shows significantly ($P<0.001$) higher head and vertebral axis for the Mediterranean populations compared to the Atlantic population, with the EM population having the highest values. There was a significantly ($P<0.001$) higher viscera yield for Atlantic population compared to Mediterranean populations. Filet yield showed significant differences ($P<0.001$) with means ranging from 55.6 ± 0.7 % (AT) to 57.0 ± 0.7 % (WM).

Conclusion

Most of the traits studied were significantly affected by the origin of the fish (AT, WM or EM). The EM population was found very promising for hatchery production, with fast growth, high carcass and fillet yield, and low fat in the fillet. The AT population was less efficient (poor processing yields and high fillet fat), however this may be due to the high rearing temperatures conditions (>20°C) potentially favouring the Mediterranean populations. Consequently, it would be extremely interesting to test these populations using different temperature profiles, along with robustness in those conditions, as it seems more and more clear that AT fish have a specific adaptation to low temperatures (and maybe a maladaptation to high temperatures).

Acknowledgement

The data presented here were obtained in the AQUAEXCEL^{2020} project which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 652831. This study was also supported by the French Ministry of Environment under grant CRECHE2020

References

Bahri-Sfar, Lilia, Christophe Lemaire, Oum Kalthoum Ben Hassine, and François Bonhomme. 2000. "Fragmentation of Sea Bass Populations in the Western and Eastern Mediterranean as Revealed by Microsatellite Polymorphism." *Proceedings of the Royal Society B: Biological Sciences* 267(1446): 929–35.

Vandeputte, Marc et al. 2014. "Multi-Site Evaluation of the Rearing Performances of 5 Wild Populations of European Sea Bass (Dicentrarchus Labrax)." *Aquaculture* 424–425: 239–48.

AUTOMATED DOSING OF FREEZE-DRIED MICROALGAE FOR BIVALVES AND SHRIMP IN RAS

V. Vermeylen[1,*], M. Wille[1], L. Roef[2], M. Muys[2], J. Dantas Lima[3], N. Nevejan[1] and P. Bossier[1]

[1] Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Coupure Links 653, 9000, Gent, Belgium
[2] Proviron Holding NV, George Gilliotstraat 60, 2620 Hemiksem, Belgium
[3] Imaqua BVBA, Ambachtenlaan 27A, 9080 Lochristi, Belgium
*E-mail: Vincent.vermeylen@ugent.be

Introduction

Many aquaculture species, for example penaeid shrimp and several fish species, require microalgae as feed during certain life stages. For bivalves it even constitutes the main feed during their entire life. Growing microalgae can be a burden for individual hatcheries and farms as it requires considerable investment and space, is relatively labour-intensive and not always reliable, resulting in variable quality and sometimes even crashes.

To date several companies worldwide have specialized in producing a variety of microalgae species in systems ranging from open (raceway) ponds to highly advanced photobioreactors. Together with advances in processing techniques they offer (increasingly cost-effective) alternatives to the use of live microalgae.

Despite these advances, the use of processed algae-products in aquaculture to date still seems limited and mostly directed towards production of live feed (*e.g.* rotifers) or inclusion as ingredient in formulated feeds. Only a limited number of publications exist on the use of these processed algae, especially for shrimp. Moreover, algae concentrates and pastes seem to be preferred over dried forms. The latter however have the advantage of being more stable, resulting in easier transportation and longer shelf life making them a convenient product.

In the current presentation we report on trials conducted with different species of commercially available freeze-dried microalgae (Proviron, Belgium), as feed for penaeid shrimp (*Litopennaeus vannamei*) larvae and cupped oyster (*Crassostrea gigas*) spat as compared to live algae. In addition, we adopted RAS technology and an automated feeding unit. We believe these introductions can improve the currently used culture technology, making it more (cost-)effective and reliable, especially in a European context.

Materials & Methods

L. vannamei nauplii were obtained from Imaqua BVBA (Lochristi, Belgium) and stocked in 100-L cylindroconical poly-ethylene tanks at a density of 100-150 larvae. L^{-1} in a temperature- and light-controlled room. The larval tanks were connected to a filter unit consisting of a biological filter and a protein skimmer, allowing to operate the tanks in batch or in RAS. A standard feeding protocol was applied using microalgae at around 100 cells μL^{-1} for zoea stages and introducing Artemia instar I nauplii from the late zoea 3 stage onwards. Suspensions of freeze-dried algae were cooled and automatically fed to the larval tanks at regular intervals using a newly developed dosing device.

Factors tested for shrimp larvae were algae species (*Chaetoceros muelleri* and *Thalassiosira pseudonana*), algae processing (fresh or freeze-dried), water management (batch or RAS) and algae feeding system (manual or automated). Trials were terminated when all treatments reached postlarval stage (10-12 DPH). Evaluation criteria were water quality, larval development, survival to postlarval stage and visual observation (fouling, fecal strings).

For the oyster experiments, *C. gigas* spat (size T2) were obtained from De Oesterput bvba (Ostend, Belgium), and stocked at a density equal to 3 kg per m² in sieves which were hanging in100L cylindroconical poly-ethylene tanks. Temperature was set at 20°C, and a light-dark cycle at 12:12 hours. Three independent recirculating systems (each with drum filter, protein skimmer and automated feeding system) and one batch system (control) operated each with three rearing tanks. Three feeding regimes were tested in the recirculating systems, all consisting of the same three algae species (*Tetraselmis chuii*, *Chaetoceros muelleri* and *Isochrysis galbana*): 100% live algae, 100% freeze-dried algae and a 50:50 mixture. The batch system received live algae only. All oysters were fed 1.5% of the spat wet weight in dry weight microalgae, in a 1:1:1 ratio in algae dry weight. The trial ran for 5 weeks. Oyster growth (shell and meat weight, and shell length), water quality and water mineral levels were monitored and evaluated.

(Continued on next page)

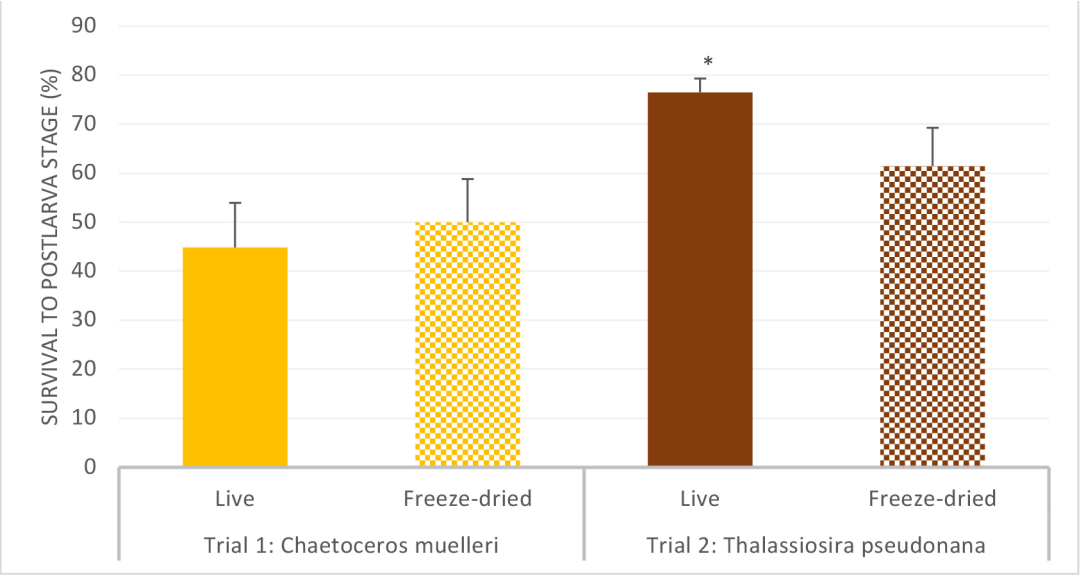


Fig. 1. Survival of shrimp larvae to the postlarval stage, fed either live or freeze-dried *Chaetoceros* or *Thalassiosira* algae as part of their diet. * denotes statistically significant differences between treatments with the same alga species.

In all trials, all microalgae were supplied by Proviron (Belgium), including live *C. muelleri*, *T. pseudonana*, *Isochrysis* sp. Tahitian strain and *T. chuii* and their freeze-dried form, known under the product names ChaetoPrime, ThalaPrime P, IsoPrime and TetraPrime C, respectively. The fatty acid profiles of the different algae were also determined.

Results

High survival (50-61%) to the postlarval stage was obtained for shrimp larvae fed freeze-dried *C. muelleri* and *T. pseudonana* , similar or only slightly lower compared to larvae fed the live form (figure 1). Effects on water quality, larval development rate and external appearance will be discussed as well.

Similarly, results of the trials on (partial) replacement of live algae with freeze-dried algae for *C. gigas* oyster spat will be presented.

Lastly, results on automated dosing of freeze-dried algae in combination with adopting RAS technology, instead of batch systems with manual water exchange (common for shrimp) or flow through systems (common for oysters), will be discussed.

Conclusions

Our study demonstrates that freeze-dried algae can be a good alternative for live algae resulting in almost similar growth and survival for some species. Adopting RAS and automated feeding systems could improve water quality, reduce inputs and overall make aquaculture systems function more autonomously, which is considered especially advantageous for application in Europe.

Acknowledgements

This study is part of the BlueMarine³.Com project funded by the Flemish government through Flanders Innovation and Entrepreneurship (VLAIO) and is facilitated by the Blue Cluster program.

ANAESTHETIC PROFILE AND BIOCHEMICAL EFFECTS OF MENTHOL AND THYMOL IN ZEBRAFISH MODEL

R. Vieira[1*], D. Sousa[2], C. Venâncio[1,3], L. Félix[1,4]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[2] School of Life and Environmental Sciences, UTAD, Vila Real, Portugal
[3] Departement of Animal Science, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[4] Institute of Investigation and Innovation in Health (i3S), Laboratory Animal Science (LAS), Institute of Molecular Cellular Biology (IBMC), University of Porto (UP), Porto, Portugal.
Email: raquelvieira1920@gmail.com

Introduction

In intensive aquaculture, practical procedures as weighing, measuring, spawning or transport are frequent, which can induce stress in fish and influence their welfare. To minimize these negative effects which can have significant economic impacts, the use of anaesthetics is increasingly recommended. Tricaine methanesulphonate (MS-222) is a synthetic anaesthetic often used in aquaculture and research, however some studies support that this compound may induces further changes on different parameters of the fish welfare. In this sense, it is necessary to find compounds that have anaesthetic properties and are harmless to fish, the environment and the consumer with the purpose of enable safe and sustainable use. Thymol and menthol are natural antioxidant compounds with proven anaesthetic properties in different species. The zebrafish is a teleost fish which has been used as a model fish for aquaculture research. Its larval stages are highly suitable for addressing welfare mechanisms by anaesthetics as most receptors are found after 72 hours post-fertilization (hpf). Therefore, the objective of this work was to evaluate the anaesthetic profile of menthol and thymol and biochemical changes in anaesthetized and recovered animals.

Material and methods

Zebrafish larvae (72 hpf) were anaesthetized with different concentrations of thymol (12.5 – 400 mg L^{-1}) and menthol (25 – 600 mg L^{-1}) prepared in E3 medium (negative control). MS-222 at 200 mg L^{-1} was used as a positive control group. The time it took to lose the ability to swim and the response to a touch stimulus on the tail was registered. Then, the animals were placed to recover in E3 medium and the time until the animals returned to swimming normally noted. After finding the concentrations best suited for aquaculture procedures (induction time be less than 180s and of recovery less than 300s), a new set of animals was anesthetized and collected while others were allowed to recover to evaluate biochemical changes. After 24 and 48h of recovery, the animal's heartbeat was evaluated.

Results

The induction and recovery time of MS-222 was 35.60 (17.00 – 60.45), 86.40 (60.90 – 159.60) seconds, respectively. The natural compounds tested had an induction time very similar with that of MS-222: for thymol 200 mg L^{-1} it was 33.92 (30.00 – 48.83) seconds and for menthol 400 mg L^{-1} it was 21.00 (17.00 – 60.45) seconds. However, the recovery time was higher in all concentrations tested in comparison to MS-222. In the heartbeat evaluation, all animals had a normal heartbeat after 48 hours of recovery. At the biochemical level, larvae under the effect of thymol showed an increase in SOD activity and a decrease in LDH and ATPase activity. After the recovery, there was an increase in SOD activity and a decrease in GSH and GSSG levels and ATPase activity. In the case of menthol, there was an increase in SOD, GST and GPx activities and a decrease in GSH, GSSG, LPO and LDH after induction. After the recovery, a decrease in LPO level and ATPase activity and an increase in ROS, AChE, CarE and GST activities were observed.

(Continued on next page)

Discussion and conclusion

Natural compounds tested showed a good anaesthetic profile similar to MS-222 with a low induction while increased recovery times. No mortality rates were obtained, and no alterations in heartbeat rate were observed. Regarding the biochemical changes observed, the induction of SOD and other antioxidant defences activity by thymol or menthol during and after anaesthesia could be an adaptive response to the stress which neutralizes the impact of ROS generated induced, as described in other studies. LPO reduction with menthol anaesthesia may indicate that the compound used prevents the oxidation process of the lipids present in cell membranes. The decrease in LDH activity in relation to control might represent a reduction of cellular anaerobic processes and a possible reduction of oxidative damage. In general, the antioxidant improvement of defenses seems to be happening for both thymol and menthol.

Though further studies are needed to understand the concentration-time relationship and the physiological changes at these development stages of *Danio rerio* as the literature is scarse, the use of these natural compounds to avoid stress in teleost fish seems to be a very interesting way and with great benefit for both animals and the environment.

Acknowledgement

The authors wanted to thank the FCT - Foundation for Science and Technology, I.P., under the PhD grant (SFRH/BD/144904/2019), R. Vieira and Projects IDB/04033/2020 and POCI-01-0145- FEDER-029542 (PTDC/CVT-CVT/29542/2017).

MODULATION OF FATTY ACID PROFILES IN THE NEREID POLYCHAETE *Hediste diversicolor* BY NUTRITIONAL AND ENVIRONMENTAL FACTORS

Andrea Villena-Rodríguez*[1], Óscar Monroig[1], Francisco Hontoria[1], Andreas Hagemann[2], Arne M. Malzahn[2] and Juan C. Navarro[1]

1 Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain
andrea.villena@csic.es
2 Department of Fisheries and New Biomarine Industry, SINTEF Ocean, 7010 Trondheim, Norway

Introduction

Marine invertebrates in general and polychaetes in particular, are attracting a great deal of attention as promising candidates for their use as alternative ingredients for aquaculture. Polychaetes are good sources of long-chain (C20-24) polyunsaturated fatty acids (LC-PUFA), especially the so-called "omega-3" (ω3 or n-3) eicosapentaenoic acid (EPA, 20:5n-3) and, to a lesser extent, docosahexaenoic acid (DHA, 22:6n-3). Their nutritional value, along with their detritivorous feeding habits enabling their culture on sidestreams from agri-food industries, has prompted interest to explore circular economy strategies for production of polychaete meals as alternative ingredients to fishmeal for aquafeed formulation [1]. Fatty acid (FA) composition of animals is accounted for by diet and endogenous production (biosynthesis). Interestingly, LC-PUFA biosynthesis can be modulated by diet (nutritional regulation) and environmental factors such as temperature [2]. This offers a great opportunity to establish culture protocols that enhance ω3 LC-PUFA biosynthesis and therefore result in production of highly nutritious polychaete biomasses. The present study aimed to assess the combined effects of diets varying in LC-PUFA composition and temperature on the FA profiles of the nereid polychaete *Hediste diversicolor*.

Materials and methods

To evaluate the combined effects of diet and temperature, an experiment with a multifactorial design testing five diets (see below) and five temperatures (5.8, 9.5, 12.5, 14.7 and 17.1 °C) (25 treatments) was carried out with three worms (biological replicates) each. Five worms from the stock were analysed as a reference at the beginning of the trial (START). Four diets were made up of a mix of two sidestreams. Sludge from salmon farming was used as a base and progressively substituted at 0, 33, 66 and 100 % (AS100, AS66, AS33 and AS0, respectively) by solid phase digestate from biogas production (SBD). Fish feed (FF) was used as control treatment. The worms were placed in individual 800 ml beakers with 8 cm thick sediment layer and were fed for two weeks. Survival and growth data (specific growth rate, SGR) were recorded. At the end of the experiment, the guts were emptied for 4 h before the worms were weighed individually and freeze dried for further lipid and FA analyses. Total lipids were extracted and quantified gravimetrically, and an aliquot was transmethylated to fatty acid methyl esters (FAME), which were analysed using gas chromatography. The results were chemometrically processed using principal component analysis (PCA) (SPSS® Statistics version 27).

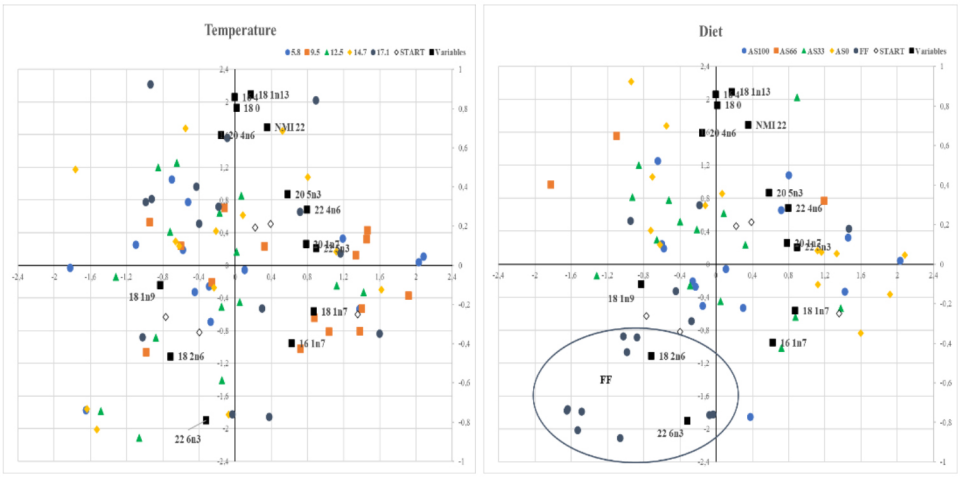


Figure 1. Biplots of the PCA analysis of the fatty acid profiles of *H. diversicolor*. Identification of the scores is based on temperature (left) and diet (right). Temperatures: 5.8, 9.5, 12.5, 14.7 and 17.1 ºC. Diets are named according to the % substitution of biogas sidestream on salmon farming sludge (AS100, AS66, AS33, AS0), FF: fish feed; START: worms at the beginning of the trial.

(Continued on next page)

Results

At the end of the trial no significant differences in SGR were found, with an average value of 1.61 being recorded. Survival was high (88 %) throughout the experiment. Our results did not show any clear effect of temperature on the FA composition of *H. diversicolor*, whereas a clear segregation between the FF group and the other dietary treatments was found. This segregation is mostly associated to the levels of 18:2n-6 (linoleic acid, LA) and 22:6n-3 (DHA) (Figure 1).

Discussion and conclusions

No temperature-driven segregation patterns could be identified in the FA profiles of *H. diversicolor*, which is in agreement with former reports [3]. The present work thus confirms that diet is the major factor influencing lipid composition in this species as reported by [4]. The segregation of the FA patterns of the control (FF) group was associated to LA and DHA, major constituents of fish aquafeeds [1,4]. In fact, the lack of a clear effect of sidestream diets on the FA composition of *H. diversicolor* could be due to the similar profile of the sludge and SBD components, along with their low lipid and FA contents (4-7% and 6-37%, respectively). Worms fed with sludge and SBD contained a high amount of monounsaturated (26%) and unsaturated FA (59%), close to those of the START treatment (27% and 62%), and to values reported by [5] in *H. diversicolor* fed with sludge (25% and 45%, respectively). These results suggested a lipid homeostatic capacity of *H. diversicolor*, based on its biosynthetic capacity, leading to a certain level of trophic upgrading. Dietary effects would be then more evident when the homeostatic threshold is surpassed, for example with lipid and PUFA-rich diets such as FF. The cultivation of *H. diversicolor* using sidestreams is thus promising, and further studies on gene expression underway in our laboratories will contribute to clarify these preliminary results.

References

1. Tacon, A.G.J.; Metian, M. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 2008, *285*, 146-158
2. Monroig, Ó.; Kabeya, N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: A comprehensive review. *Fish. Sci.* 2018, *84*, 911–928.
3. Sandmann, P. Utilization of sediments from the culture of African catfish (*Clarias gariepinus*) for cultivating the polychaete *Hediste diversicolor* (Müller, 1776). [Master Thesis 2019, SINTEF Ocean]. https://www.sintef.no/en/publications
4. García-Alonso, J.; Muller, C.T.; Hardege, J.D. Influence of food regimes and seasonality on fatty acid composition in the ragworm. *Aquat. Biol.* 2008, *4,* 7-13.
5. Wang, H.; Seekamp, I.; Malzahn, A. M.; Hagemann, A.; Carvajal, A.K.; Slizyte, R.; Standal, I.B.; Handa, A.; Reitan, K.I. Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Müller, 1776) cultivated on waste from land-based salmon smolt aquaculture. *Aquaculture* 2019, *502*, 232-241.

BIOSYNTHESIS OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN THE NEREID POLYCHAETA *Hediste diversicolor*

Andrea Villena-Rodríguez*[1], Juan C. Navarro[1], Francisco Hontoria[1], L. Filipe C. Castro[2] and Óscar Monroig[1]

1 Instituto de Acuicultura de Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain
andrea.villena@csic.es
2 Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, CIIMAR, Avenida General Norton de Matos, S/N, Portugal

Introduction

Aquaculture of carnivorous species is greatly dependent on the supply of marine ingredients, which provide adequate levels of essential nutrients including long-chain (C_{20-24}) polyunsaturated fatty acids (LC-PUFAs), particularly the "omega-3" (ω3) including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [1]. Primary production of these compounds was thought to take place exclusively by the action of photosynthetic microalgae, heterotrophic protists and bacteria [2]. However, a study has revealed that multiple aquatic invertebrates including polychaetes possess enzymes that enable them to produce ω3 LC-PUFAs *de novo* and, consequently, their culture with an adequate supply of biosynthetic fatty acid (FA) precursors and enhancing such pathways emerges as a highly innovative strategy to produce nutritious and sustainably sourced ingredients for aquaculture [3]. The capacity by which an animal species can biosynthesise LC-PUFAs depends upon the complement and function of two types of enzymes, namely fatty acyl elongases and desaturases. The aim of the present study was to characterise molecularly and functionally two elongases (Elo) and one front-end desaturase (Fed) involved in the LC-PUFA biosynthesis of the common ragworm *Hediste diversicolor*.

Materials and methods

To obtain the full-length sequences of the *H. diversicolor* Elo and Fed open reading frames (ORFs), BLAST searches were carried out on a raw transcriptome prepared from one single individual. The ORF sequences were identified and then isolated by PCR using *H. diversicolor* complementary DNA (cDNA) as template, with primers containing specific restriction sites for further cloning into the yeast expression vector pYES2. The *H. diversicolor* Elo and Fed were functionally characterised by heterologous expression in yeast. Transgenic yeast containing the different *H. diversicolor* Elo and Fed ORFs were grown in the presence of a series of exogenously supplemented polyunsaturated fatty acid (PUFA) substrates to test their elongation and desaturation capacity, respectively. After 2 d of incubation, yeast were harvested and washed prior extraction of total lipids. Total lipids were used to prepare fatty acid methyl esters for analysis by gas chromatography with mass spectrometry detection (GC–MS). Conversions of PUFA substrates to the corresponding elongation or desaturation products were calculated by the proportion of substrate PUFA converted to PUFA product(s) as [areas of all products /(areas of all products + substrate area)] × 100.

Results

Our results show that *H. diversicolor* has at least two Elo and one Fed with putative roles in the LC-PUFA biosynthetic pathways. We termed them as Elovl2/5 and Elovl4 based on their phylogenetic relationship with well characterised elongases from other animals. Functional characterisation results showed that the *H. diversicolor* Elovl2/5 is indeed a PUFA elongase since elongated PUFA substrates with C_{18} and C_{20}. Similarly, the *H. diversicolor* Elovl4 showed elongation capacity towards C_{18} and C_{20} PUFA substrates but, additionally, C_{22} PUFA substrates (Table 1). Functional characterisation of the *H. diversicolor* Fed1 confirmed its role in the LC-PUFA biosynthesis as it showed this enzyme has Δ5 activity converting 20:3n-6 and 20:4n-3 into 20:4n-6 (arachidonic acid) and 20:5n-3 (EPA), respectively (Table 1). No activities as Δ4, Δ6 or Δ8 were detected (Table 1). Along PUFA substrates, the *H. diversicolor* Fed1 also had Δ5 activity towards saturated substrates such as 18:0, which was converted to 18:1n-13.

Discussion and conclusions

The present work demonstrates that *H. diversicolor* possess at least two distinct elongase genes, namely Elovl2/5 and Elovl4, with putative roles in the biosynthesis of LC-PUFAs. Molecular and functional characterisation of the Elovl sequences from *H. diversicolor* revealed that Elovl2/5 have C_{18} and C_{20} PUFA as preferred substrates for elongation, that is very common in invertebrates [4]. The Elovl4 is able to elongate C_{22} substrates to C_{24} products, and thus compensates for the lack of C_{22} activity within Elovl2/5, as occurs in molluscs [4]. Functional characterisation of the *H. diversicolor* Fed1 Δ5 confirm its role in LC-PUFA biosynthesis. Δ5 desaturases have been previously demonstrated in some molluscs as well as echinoderms [4]. The herein reported results on the functions of the Elovl2/5, Elovl4 and Δ5 desaturase, along with those of the previously characterised methyl-end desaturases [5], clearly show that *H. diversicolor* has a highly diverse repertoire of LC-PUFA biosynthesising genes with complementary activities enabling an active production of these essential compounds.

(Continued on next page)

Table 1. Functional characterisation of the *H. diversicolor* Elovl2/5, Elovl4 and Fed1

FA substrate	FA product	Elovl2/5	Elov4	Activity	FA substrate	FA product	Fed1	Activity
18:3n-3	20:3n-3	20.08	1.67	C18→C20	18:2n-6	18:3n-6	nd	Δ6
18:2n-6	20:2n-6	45.85	1.12	C18→C20	18:3n-3	18:4n-3	nd	Δ6
18:4n-3	20:4n-3	29.56	1.28	C18→C20	20:2n-6	20:3n-6	nd	Δ8
18:3n-6	20:3n-6	69.39	0.39	C18→C20	20:3n-3	20:4n-3	nd	Δ8
20:5n-3	22:5n-3	24.46	2.37	C20→C22	20:3n-6	20:4n-6	34.43	Δ5
20:4n-6	22:4n-6	41.50	0.28	C20→C22	20:4n-3	20:5n-3	16.42	Δ5
22:5n-3	24:5n-3	nd	0.25	C22→C24	22:4n-6	22:5n-6	nd	Δ4
22:4n-6	24:4n-6	nd	0.12	C22→C24	22:5n-3	22:6n-3	nd	Δ4
22:6n-3	24:6n-3	nd	0.40	C22→C24				

nd, not detected; FA, fatty acid

References

1. Tocher, D.R.; Betancor, M.B.; Sprague, M.; Olsen, R.E.; Napier, J.A. Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. *Nutrients* 2019, *11*(1):89.
2. Pereira, S.L.; Leonard, A.E.; Mukerji, P. Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes. *Prostaglandins Leukot. Essent. Fat. Acids* 2003, *68*, 97–106.
3. Kabeya, N.; Fonseca, M.M.; Ferrier, D.E.K.; Navarro, J.C.; Bay, L.K.; Francis, D.S.; Tocher, D.R.; Castro, L.F.C.; Monroig, Ó. Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci. Adv.* 2018, *4*, 1–9.
4. Monroig, Ó.; Kabeya, N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: A comprehensive review. *Fish. Sci.* 2018, *84*, 911–928.
5. Kabeya, N.; Gür, I.; Oboh, A.; Evjemo. J.O.; Malzahn, A.M.; Hontoria, F.; Navarro, J.C.; Monroig, Ó. Unique fatty acid desaturase capacities uncovered in Hediste diversicolor illustrate the roles of aquatic invertebrates in trophic upgrading. *Phil. Trans. R. Soc. B* 2020, *375*: 20190654.

DIETARY SUPPLEMENTATION OF NON-STARCH POLYSACCHARIDES AFFECTS DIGESTIBILITY AND GROWTH OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) IN BIOFLOC AND RAS SYSTEMS

Apriana Vinasyiam[ab*], Kazi A. Kabir[c], Julie Ekasari[b], Johan W. Schrama[a], Johan A. J. Verreth[a], and Marc C. J. Verdegem[a]

[a]Aquaculture and Fisheries, Animal Sciences Group, Wageningen University, the Netherlands
[b]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University
[c]CIRAD, France
Email: apriana.vinasyiam@wur.nl

Introduction

Energy transfer in aquaculture systems depends on the interactions between the biota contributing to the food web, e.g. the culture species, algae, bacteria, and fungi. The interacting biota are part of an ecological trophic cascade exchanging energy and nutrients. The efficiency of energy transfer through the food web can be influenced by the diet and the resulting fecal composition. In Pacific white shrimp culture, when starch dominates the dietary carbon input, the majority will leave the system as CO_2, limiting access for further carbon utilization in the system. Supplementing non-starch polysaccharide (NSP) in the diet results in more carbon retained in the shrimp feces due to its low digestibility in shrimp. However, the heterotrophic bacteria in the rearing tank can utilize this carbon and transfer part of its energy to the food web. Together with algae, heterotrophic bacteria are at the base of the food web. In a biofloc system both biotas are present. This research compared the effect of a high and a low NSP diets on biofloc performance and shrimp production. Furthermore, the study also estimated the contribution of biofloc to shrimp growth reared in biofloc systems by estimating the growth in a zero biofloc system - recirculating aquaculture system (RAS).

Materials and methods

This experiment compared two diets differing in the NSP level: high (NSP) and low (CON). The first mentioned diet contains wheat bran as the NSP source. Two experiments, a growth and a digestibility experiment, were carried out. In the growth experiment, two culture systems were compared (Table 1): biofloc and RAS (with clear water rearing tanks) system, for 6 weeks. The digestibility experiment lasted for 5 weeks and was done only in RAS only. The biofloc treatments were performed in circular 1000-L fiber mesocosm tank in triplicates. The clear water rearing tanks were 120-L aquaria all part of the same RAS, with 4 replicates per treatment. The stocking densities were 100 ind/tank in biofloc tanks, 30-25 ind/aquaria in RAS, both for the growth and digestibility experiment. The shrimp were fed iso proteinic, once daily.

Results

The results showed that NSP diet increased the carbon to nitrogen (CN) ratio of the shrimp feces from 11.8 in CON treatment to 20.5, which later stimulated more biofloc formation ($P<0.05$). The algae concentration in the biofloc at week-6 was higher when shrimp was fed with NSP diet ($P<0.05$). The NSP diet had a lower apparent digestibility coefficient (ADC) of dry matter, protein, energy, carbohydrate, and minerals ($P<0.05$). The experimental diet did not show any effect on the shrimp production ($P>0.05$), but the system did ($P<0.05$), due to the higher stocking density in tanks (Table 2). Feed conversion ratio was higher with NSP diet ($P<0.05$). Biofloc presence as an additional natural food that contributed to an increase of the shrimp biomass by 32% in NSP and 46% in CON diet in BF tanks. The fraction of egested energy stored in shrimp biomass based on biofloc consumption was higher BF tanks fed the CON diet than in BF tanks fed the NSP diet ($P<0.05$). The NSP diet resulted in a 24% higher carbohydrate content in shrimp biomass at harvest ($P<0.05$).Future research should focus on the effect of NSP type in the diet on shrimp performance in biofloc tanks.

Conclusions

- Increasing the NSP content in the diet increases the C:N ratio in the feces.
- Although the NSP diet is less digestible than the CON diet, when fed isoproteinic, the final weight reached was similar, in either RAS or BF system.
- In BF tanks fed the NSP diet, more biofloc was present than in BF tanks fed the CON diet, but shrimp did not realize more growth based on biofloc in NSP fed BF tanks than in CON fed BF tanks.

(Continued on next page)

Table 1. Experimental design

	Diet	BF	RAS
Growth	NSP	NSP-BF	NSP-RAS
Experiment	CON	CON-BF	CON-RAS
Digestibility	NSP		Dig-NSP
Experiment	CON		Dig-CON

NSP: high NSP diet, CON: low NSP (control) diet, BF: biofloc system, RAS: recirculating aquaculture system with clear water aquaria, Dig: digestibility experiment.

Table 2. Shrimp growth performance at week-6

Parameters	Unit	BF		RAS		Pooled SEM	P-Value		
		CON	NSP	CON	NSP		System	Diet	System* Diet
Initial weight	g/ind	0.50	0.50	0.44	0.44	0.01			
Final weight	g/ind	6.0**a**	5.8**a**	3.9**b**	4.4**b**	0.08	**0.000**	0.473	**0.021**
SGR	% /day	5.9	5.8	5.2	5.5	0.05	**0.000**	0.376	0.068
FCR		1.2	1.7	2.1	2.5	0.04	**0.000**	**0.000**	0.632
PER	%	2.5	2.2	1.4	1.5	0.22	**0.000**	0.453	0.090
Production	kg/m^3	0.64	0.59	0.68	0.75	0.02	**0.008**	0.631	0.086
Survival	%	87	86	82	78	1.95	0.126	0.536	0.835
Feed contribution to shrimp biomass	(%)	54±6.6	68±6.9	100	100			0.065	
Biofloc contribution to shrimp biomass	(%)	46±6.6	32±6.9	0	0			0.065	
Egested energy stored in shrimp biomass by biofloc consumption	(%)	74±4.9**a**	32±11**b**	0	0			**0.004**	

NSP: high NSP diet, CON: low NSP (control) diet, BF: biofloc system, RAS: recirculating aquaculture system with clear water aquaria, SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio, P-value: probability value. Different letters following mean values within the same row indicate significant differences ($P<0.05$).

REAL-TIME CONTROL OF WATER QUALITY IN AQUACULTURE FARMS – DESCRIPTION OF MONITORING SYSTEM AND PRELIMINARY RESULTS

N. Vladimir[1]*, M. Koričan[1], D. Omanović[2], V. Soldo[1], N. Hadžić[1]

[1] Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Ivana Lučića 5, 10002 Zagreb, Croatia
[2] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
Phone: +385 1 61 68 114
E-mail: nikola.vladimir@fsb.hr

Introduction

Aquaculture is a process of controlled cultivation of freshwater and marine organisms, mainly for human consumption and the renewal of fish stock [1]. It is highly dependent on the water quality which should be continuously monitored to undertake preventive actions in case of anomalies. Also, special attention should be paid to the environmental effect of aquaculture systems with an aim to reduce it as much as possible [2]. The water quality analysis includes control of several parameters, such as oxygen level, pH, chemical substances and others. Marine pollution is caused by various sources, from ships (e.g. oil spills) to onshore industries (e.g. toxic waste, chemical dissolutions). In case of aquaculture production, the decomposition of fish feed has the greatest impact but the infrastructures themselves also contribute. Materials immersed in saltwater can cause chemical anomalies by their decomposition. This is emphasized in case of ships where various antifouling coatings dissolve and release harmful substances, such as copper [3]. These substances enter the biosystem of marine organisms and can, by consumption of fish and mussels, affect human health. The deposition of uneaten feed on the seabed slows down the growth of marine flora and their degradation creates a chemical imbalance. The level of harmful substances, e.g. nitrogen and phosphorous, increases and leads to eutrophication of the marine area [4]. To reduce the negative impact of aquaculture production on the environment, greater funds are invested in monitoring systems and sensor networks to control the water quality and ensure a healthier environment.

Methodology and equipment

This study contributes to the preservation of the ecosystem, by installing a stationary multiparameter probe in an aquaculture farm, that collects real-time data about seawater quality and enables issuing alerts if a sort of anomaly appears. The aquaculture farm is located in the lower part of the Krka River estuary, characterised by a frequent inflow of freshwater that alters the salinity and a higher level of copper concentration harmful for marine organisms [3]. The specific characteristics make the location a valuable source of data for an environmental study. The multiparameter probe (EXO2, YSI, Xylem) has 7 ports, one port reserved for a central wiper and 6 ports for holding the sensors for temperature, conductivity, pH/ORP (Oxidation-Reduction Potential), dissolved oxygen, turbidity and depth. Other parameters that cannot be directly measured (such as total dissolved solids) are calculated through built-in correlations. The central wiper automatically cleans the surface of the sensors to prevent the growth of fouling and marine organisms, e.g. algae and mussels. The probe ensures a sampling rate of at least 1 Hz and data export in various formats (MIS, CSV, ASCII,…). The probe is located in an aquaculture farm at depth around 2 m (within the halocline) near an onshore workstation, which facilitates data transfer due to easy access to energy and an Internet connection. In-house program was developed to collect the data from the probe every 10 minutes.

Results

The first measurements were conducted during July and August 2021. The data were analysed following the FAO guidelines for aquaculture production [5]. The temperature ranges from 24°C to 28°C, as expected for the summer months. The majority of fish species has the optimum performance at temperatures of about 15°C, while at temperatures above 20°C they consume less food [5]. The optimal pH range for fish is from 6.5 to 8.5 [5] and the results show good values in a range from 7.74 to 7.97. Oxygen requirements depend on the cultivated species and increase at a higher temperature [5]. The results show a range from 5.6 mg O_2/L to 9.1 mg O_2/L. Salinity results show a greater range due to the inflow of freshwater and its position at the halocline, which harms marine organisms and needs to be further analysed. Turbidity shows low, controlled values and occasional peaks.

(Continued on next page)

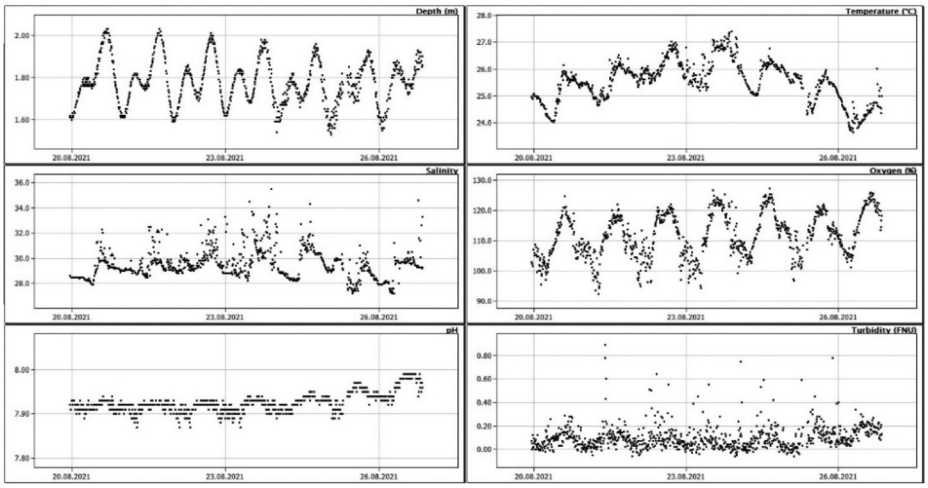


Figure 1. Water quality parameters – weekly results

Concluding remarks

Early results obtained by the described real-time monitoring system indicate that it is fully operable and appropriate for application in any type of aquaculture farm. Further measurements will give a better overview of the water quality during different seasons when greater precipitation and colder weather could have an effect on the water parameters. A larger database will enable more accurate analysis and preparation of guidelines for improving the work of aquaculture farms to reduce the environmental impact and establishing a balanced marine ecosystem.

Acknowledgements

This investigation has been funded by the European Maritime and Fisheries Fund of the European Union within the project "Integration of a high share of renewables into aquaculture systems – IN AQUA", granted by the Ministry of Agriculture, Directorate of Fisheries, Republic of Croatia (Award No. 324-01/19-01/1178) as well as was by a European transnational programme INTERREG V-B Adriatic-Ionian ADRION Programme 2014-2020, under the project Sector Adaptive Virtual Early Warning System for marine pollution (SEAVIEWS), (Project No. ADRION-951).

Literature

[1] Food and Agriculture Organization (FAO), 1987. Introduction to aquaculture, http://www.fao.org/3/ac169e/ac169e00.htm#ch5. (access: 08 July 2021).

[2] Bohnes F.A., Laurent A., 2020. Environmental impacts of existing and future aquaculture production: Comparison of technologies and feed options in Singapore, Aquaculture 532, 736001.

[3] Cindrić, A.M., Garnier, C., Oursel, B., Pižeta, I., Omanović, D., 2015. Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: the Krka River estuary (Adriatic, Croatia). Marine pollution bulletin. 94, 199-216.

[4] UNESCO, 2021. Intergovernmental Oceanographic Commission, http://www.unesco.org/new/en/natural-sciences/ioc-oceans/focus-areas/rio-20-ocean/blueprint-for-the-future-we-want/marine-pollution/ (access: 24 August 2021).

[5] Food and Agriculture Organization (FAO), 1993. Water quality and fish health, http://www.fao.org/3/t1623e/t1623e.pdf (access: 24 August 2021).

LEITMOTIF 2.0: NEW TOOL FOR EFFICIENT COMPUTATIONAL DETECTION OF ENZYME FAMILIES AND SUBFAMILIES

D.Vujaklija[1,2*], S. Biđin[3], T. Paradžik[2], A. Bielen[4] and I. Vujaklija[3]

[1]Laboratory for Mass Spectrometry and Functional Proteomics, Ruđer Bošković Institute, Zagreb, Croatia
[2]Centre of Excellence for Marine Bioprospecting-BioProCro, Ruđer Bošković Institute, Zagreb, Croatia
[3]Faculty of Electrical Engineering and Computing, University of Zagreb, Unska 3, Zagreb, Croatia
[4]Faculty of Food Technology and Biotechnology, Pierottijeva 6, University of Zagreb, Zagreb, Croatia

*Email: vujaklij@irb.hr

Introduction

Rapid advances in NGS technology have resulted in remarkable accumulation of marine metagenomics data which is essential for better understanding marine biodiversity, anthropogenic influence and bioprospecting. Deep sequencing reveals exceptional biodiversity of marine microbes from various niches (including sediments, microbiomes of marine macroorganisms or hydrothermal vents) and their capacity to produce robust enzymes with novel properties (Birolli et al, 2019). In addition, microalgae are becoming very promising source of novel enzymes (Vingiani et al, 2019). Thus, accurate protein annotation is extremely important since HTP experimental assays to validate protein annotations poses a serious challenge. Our results showed that current profile sequence similarity search methods like HMMER and PSI-BLAST are inadequate to distinguish proteins that share profile similarity but lack catalytically active sites (Vujaklija et al, 2016). Functional variations between family subtypes often depend on specificity determining residues (Goldstein et al., 2009). Therefore, the ability to distinguish different residues at such positions is very important. Motif scanning (motif-HMM) possesses unique advantages over classical profile-sequence search methods to discriminate proteins with conserved overall profile but lacking essential residues or sequence variations within motifs (Biđin et al, 2020).

Methods

In this study we developed and used Leitmotif, a motif scanning web application. It provides many parameterization options and the possibility to define up to five different motifs. Leitmotif is briefly described below and in more detail on the web application's help pages https://leitmotif.irb.hr. It offers (1) setting immutable residues in selected motif by clicking the corresponding sequence logo letter; (2) setting motif distances and motif distance penalties; (3) setting transition probabilities within motifs; (4) choosing one of the four offered algorithms (Simple pseudocounts, Dirichlet mixture, Henikoff and Modified ancestral) for computation of match state emission probabilities and choosing sequence weighting and BLOSUM/PAM matrices.

Results and discussion

To illustrate the benefits of Leitmotif's novel parametrizations (IR –immutable residues, DP – distance penalties) we used a manually curated dataset of GDSL liypolytic family. Since GDSL lipases are characterized by three most conserved motifs we have tested new parametrizations using three, two and one motif(s) as input. To model the situation where limited information is available, only two essential residues were set as IR (Table1). Ser and His and motif distances were chosen based on expert knowledge (Vujaklija et al 2016). Note that for any other family, information about conserved residues and motif distances is readily available from the seed sequence alignments, even when the total number of sequences is low (Biđin et al, 2020). All transition probabilities were set to 0.99 to enable motif-HMM to make insertion(s) or deletion(s) within the motif(s). This was applied since in our previous study we found a certain number of GDSL sequences with deletions in motifs. (Vujaklija et al 2016). Table 1 shows how novel parameterizations significantly improves protein annotation.

In summary, here we show how conserved residues and motif distances that characterize some protein families can be used as an additional information to improve protein annotation. Our results pointed out that the best results can be achieved when implementing both, immutable residues and selected motif distances combined with penalties. Leitmotif is freely available web application at https://leitmotif.irb.hr

(Continued on next page)

Table 1. ROC scores for different IR and DP values on GDSL datasets

GDSL	IR	DP	ROC
3 motifs	[6S, ,4H]	Weak	0.9902
	[, ,]	/	0.9784
2 motifs	[6S,4H]	Medium	0.9879
	[,]	/	0.9665
1 motif	[6S]	Strong	0.8916
	[ ]	/	0.7517

References

Birolli WG, Lima RN, Porto AL (2019) Application of Marine-Derived Microorganisms and their enzymes in biocatalysis and biotransformation, the underexplored potential. Front. Microbiol., 20 August 2019 | https://doi.org/10.3389/fmicb.2019.01453

Vingiani GM, De Luca P, Ianora A, Dobson ADW, Lauritano C (2019) Microalgal enzymes with Biotechnological Applications. Mar. Drugs 2019, 17(8), 459;

Goldstein,P. Zucko J, Vujaklija D, Križko A, Hranueli D, Long PF et al. (2009) Clustering of protein domains for functional and evolutionary studies. BMC Bioinformatics, 10, 335.

Vujaklija,I. Bielen A, Paradžik T, Biđin S, Goldstein P, Vujaklija D (2016) An effective approach for annotation of protein families with low sequence similarity and conserved motifs: identifying GDSL hydrolases across the plant kingdom. BMC Bioinformatics, 18, 17–91.

Biđin S, Vujaklija I, Paradžik T, Bielen A, Vujaklija D (2020) Leitmotif: protein motif scanning 2.0 .Bioinformatics 36(11): 3566-2020

VIRGIN SALMON- BROODSTOCK CARRYING STERILTY TO NEXT GENERATION

Hilal Güralp[1,2], Kai O. Skaftnesmo[1], Erik Kjærner-Semb[1], Fernanda Almeida[1,3], Anne Hege Straume[1], Rüdiger W. Schulz[1,4], Eva Andersson[1], Per Gunnar Fjelldal[1], Lene Kleppe[1], Rolf B. Edvardsen[1], Anna Wargelius[1*]

[1] Institute of Marine Research, Bergen, Norway
[2] University of South Bohemia in České Budějovice, Czech Republic
[3] Embrapa, Manaus, Brazil
[4] University of Utrecht, Netherlands
[*]Presenting author anna.wargelius@hi.no

Genetic introgression of escaped farmed Atlantic salmon (*Salmo salar*) into wild populations is a major environmental concern for the salmon aquaculture industry. Using sterile fish in commercial aquaculture operations is, therefore, a sustainable strategy for bio-containment. So far only the methodology used commercially for producing sterile salmon is triploidization; however, triploid fish are less robust. A novel approach to achieve sterility is to produce germ cell-free salmon. One way to accomplished this is to knock out the *dnd* (*dead end*) gene using CRISPR-Cas9. The lack of germ cells in the resulting *dnd* crispants, thus, prevents reproduction and subsequent large-scale production of the sterile fish. We have therefore sought to develop an approach suitable to obtain sterility, but combined with a method to inherit sterility in *dnd* knockout broodstock (Patent WO2020/070105): Inheriting sterility in broodstock salmon can be achieved by rescueing *dnd* gene function (by *dnd* mRNA co-injection) of *dnd* crispant salmon embryos. In one-year old rescued *dnd* crispant salmon we found germ cells, type A spermatogonia in males and previtellogenic primary oocytes in females. We have further followed rescued *dnd* crispants through oogenesis and spermatogenesis and obtained an F1 generation from incrosses. This method allows the large-scale production of Atlantic salmon broodstock that can inherit the sterility trait, but are able to produce 100% germ-cell free offspring. There may be partial, but not critical, limitations of the method due to potential functions of *dnd* during gametogenesis, something we are currently exploring in rescued F1 fish showing complete or partial *dnd* loss-of function, and in gametes obtained from rescued F0 crispants. This approach may solve the problems of both, genetic introgression and precocious maturation in farmed salmon, while ensuring a stable production of 100% sterile fish, and thus represents a significant commercial potential. Use of this sterility technology may also pave the way for safe genome editing of other traits, such as disease resistance, which would be contained in sterile individuals and hence presented a negligible risk of passing edited alleles on to wild stocks.

LOSS OF *FSHR* INHIBITS MATURATION IN MALE ATLANTIC SALMON

Eva Andersson [(1*)], Fernanda Almeida [(2)], Lene Kleppe[(1)], Kai Ove Skaftnesmo [(1)], Erik Kjærner-Semb [(1)], Diego Crespo [(1)], Rüdiger W. Schulz [(1,3)], Per Gunnar Fjelldal [(1)], Tom Hansen [(1)], Birgitta Norberg [(1)], Petra Vogelsang [(1)], Rolf B. Edvardsen [(1)], Anna Wargelius [(1)]

[(1)] Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817 Bergen, Norway
[(2)] Embrapa, Manaus, Brazil
[(3)] Utrecht University, The Netherlands
Email: evaa@hi.no

Introduction

Early puberty is a major problem in farmed Atlantic salmon males as it stunts growth and entails welfare problems due to the maturation-associated loss of osmoregulation capacity in seawater. A better understanding of the regulation of puberty is the basis for developing improved rearing protocols to avoid these problems. As puberty onset is controlled by activation of the brain-pituitary-gonad (BPG) axis, our aim here was to study if puberty is initiated when the gene encoding the follicle-stimulating hormone receptor (*fshr*) is rendered non functional. It is known that lack of *fshr* in male mice is associated with smaller testis and a reduced Sertoli cell number, but the mutants are still fertile. On the contrary, both medaka and zebrafish *fshr* KO males show no clear phenotype regarding testis size and fertility. Apparently normal spermatogenesis and fertility in these model fish may be related to Lh receptor-activation and the ensuing androgen production stimulating testis maturation. In salmon, this may be different since in other salmonids Fsh, like in mammals, cannot activate the Lh receptor and since Lh is usually not secreted until the spawning period. We therefore expected a phenotype different from *fshr* KO zebrafish and medaka males.

Material and methods

We made *fshr* mutants using CRISPR-Cas9 technology. In view of the long generation time, we first studied highly mutated F0 generation *fshr* crispants. *fshr* crispants and control males were reared in a common garden, and precocious maturation was induced by exposing the one-year old postsmolts to continuous light and 16º C water temperature for a period of three months. Samplings (testis, pituitary and plasma) took place 1, 2, 5 and 9 months post induction. For the F1 generation, the sampling times were 2, 7 and 11 months post induction.

Results

At the first sampling (1 month), all males displayed low GSI values and no effect of the *fshr* KO could be detected on plasma androgen levels or stage of spermatogenesis. However, in the samples collected during the last two samplings, we observed slightly (5 months) and clearly (9 months) lower GSI values in *fshr* KO mutants compared to control. The results in F0 crispants were variable, but we observed initially a delay in the start of maturation. The males then went through maturation but displayed a shorter cycle compared to control males. Later analysis suggested that the weak and variable phenotype in F0 crispants may be related to presence of in-frame mutations, which may leave some of the mutated protein at least partially functional. Most mutants reached maturity and we crossed four highly mutated fish (2 of each sex), to create an F1 generation. At one-year of age, we pit-tagged and genotyped 200 F1 fish for sex and *fshr* genotyping and were able to identify sibling fish having either wild-type, $fshr^{-/-}$ or $fshr^{+/-}$ genotypes with known mutations. When stimulating pubertal maturation in F1 fish, clear effects were observed. None of the $fshr^{-/-}$ (N=10) males entered puberty while 22% of the $fshr^{+/-}$ males (N=72) and 85% of the wild-type males (N=24) entered maturation.

Conclusion

We show that Atlantic salmon males do not enter puberty when lacking *fshr*, which is dissimilar to findings in zebrafish and medaka, but similar to findings in mouse.

EFFECT OF MACKEREL IN TOMATO SAUCE, MARINATED HERRING AND ANCHOVY TRIMMINGS ON GROWTH PHYSIOLOGY AND INTESTINAL HEALTH OF RAINBOW TROUT *(Oncorhynchus mykiss)*

Niklas Warwas[1,2]*, Marie Montjouridès[1], Markus Langeland[1,3], Jonathan A.C Roques[1,2], Henrik Sundh[1,2], Elisabeth Jönsson[1,2], Kristina Sundell[1,2]

[1]Dept. of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[2]Swedish Mariculture Research Center (SWEMARC)
[3]Swedish University of Agricultural Sciences, SLU, Uppsala, Sweden
Email: niklas.warwas@bioenv.gu.se

Introduction

The world's fish production reached 179 million tons in 2018 (FAO, 2020). About 70% of this fish undergoes further processing before reaching the consumer market (Ghaly et al., 2013). The processing steps create significant amounts of side streams ranging between 20 and 80% (Stevens et al., 2018). In addition to fish side streams, about 35% of the output from fisheries and aquaculture becomes food waste. Both food waste and processing side streams may be of high nutritional value. Today, 25–35% of the fishmeal and fish oil is produced using aquaculture side streams (FAO, 2020) but many high value side streams and food wastes, at different stages of the refining process, remain unused. Therefore, our aim is to screen three waste products, from different stages of the refining chain for their nutrient composition and suitability as feed ingredient in diets for rainbow trout *(Oncorhynchus mykiss)*.

Material and Methods

The side streams tested included anchovy trimmings (A), marinated herring (H) and mackerel in tomato sauce (M). Three experimental diets were formulated to contain 50% of each test ingredient as the sole source of fish meal which resulted in 43% (A), 44% (H) and 44% (M) protein. The control diet (C) consisted of conventional ingredients including commercial fishmeal and 43% protein. All diets were produced via cold pelleting. Before the, experiment 180 rainbow trout were acclimatized to a land-based freshwater recirculating aquaculture system at 10°C. During the experiment, fish were fed one of the experimental diets in excess twice per day for 12 weeks. Measurements of weight and length were taken monthly. Tissue samples including blood, liver and intestine were taken at the end of the experimental period to assess the health and welfare of the animals. Additionally, samples of both proximal and distal intestine were used to assess intestinal nutrient uptake and barrier functions *in vitro*.

Results

During the 12-week feeding trial, most fish more than doubled their weight. However, fish fed diet C and diet A had significantly higher growth rates than fish fed diets M and H (Fig 1.A). Feed intake was reduced for the H treatment group compared to the diet A (Fig 1.B). In the proximal intestine, lysine transport and transepithelial potential (assessing barrier function) were significantly reduced in fish fed diet H. Lysine transport was also reduced for fish fed diet A. Additionally, a higher epithelial resistance was observed in the distal intestine of fish fed diet H. Plasma cortisol levels were higher in fish fed diets C and M compared to diets A and H. This coincided with an increase in plasma glucose in the fish fed diet M compared to the fish fed the diets C and A.

Discussion

The side streams included in the M and H diets were refined and contained tomato sauce and marinade respectively, which may be one reason for the reduced growth in these groups. For diet H, a reduction in feed intake was also observed indicating a lower palatability, which can contribute to the reduced growth. Reduced epithelial transport in the proximal intestine in fish fed diet H indicates a reduced capability for active nutrient uptake, potentially contributing to the reduced growth observed in this group (Vidakovic et al., 2016). The highest growth rates were observed in fish fed diet A even though it contained the lowest amount of protein and fat as well as high amounts of ash. However, the anchovy trimmings had not undergone any processing or refining which could have altered the natural taste, and were freshest, which may have resulted in the higher feed intake observed. Interestingly, diet A also led to a reduction in lysine uptake without compromising growth, indicating the presence of a compensatory mechanism such as increased feed intake (as observed) and longer residence time of chyme in the intestine.

(Continued on next page)

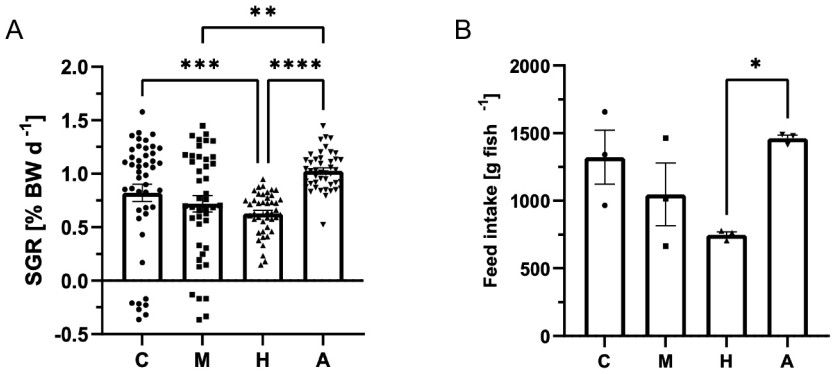


Fig 1. Growth and feed intake of rainbow trout fed 4 different diets, control (C), mackerel (M), herring (H) and anchovy (A). Left; specific growth rate (SGR), right; feed intake (FI), Bars indicate standard error of mean. Individual data point are individual fish for SGR and replicate tanks for FI. Symbols, ****, ***, **, * refer to p-values <0.0001, <0.001, <0.01, <0.05

Conclusion

The highest growth rates were observed in the treatment group fed with unprocessed anchovy trimmings (A). Thus, the refining processes of the herring and mackerel may introduce non-palatable and/or anti-nutritional factors leading to reduced growth and impaired barrier function of the proximal intestine. In this study the anchovy trimmings were the most promising side stream for the use as a feed ingredient in its raw form while the mackerel in tomato sauce (M) and marinated herring (H) may require additional treatment before they can be included in diets at high levels.

References

FAO, 2020. The State of World Fisheries and Aquaculture 2020. FAO. https://doi.org/10.4060/ca9229en

Ghaly, A.E., Ramakrishnan, V. V., Brooks, M.S., Budge, S.M., Dave, D., 2013. Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. J. Microb. Biochem. Technol. 5, 107–129. https://doi.org/10.4172/1948-5948.1000110

Stevens, J.R., Newton, R.W., Tlusty, M., Little, D.C., 2018. The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilisation. Mar. Policy 90, 115–124. https://doi.org/10.1016/j.marpol.2017.12.027

Vidakovic, A., Langeland, M., Sundh, H., Sundell, K., Olstorpe, M., Vielma, J., Kiessling, A., Lundh, T., 2016. Evaluation of growth performance and intestinal barrier function in Arctic Charr (Salvelinus alpinus) fed yeast (Saccharomyces cerevisiae), fungi (Rhizopus oryzae) and blue mussel (Mytilus edulis). Aquac. Nutr. 22, 1348–1360. https://doi.org/10.1111/anu.12344

EVALUATION OF THE EFFECT OF TEMPERATURE AND FOOD RESTRICTION ON COMPENSATORY GROWTH OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* IN BIOFLOC SYSTEM

W. Wasielesky*, E. Prates, M. Holanda, Luis Poersch and J.M. Monserrat

Marine Station of Aquaculture, Federal University of Rio Grande, Rio Grande City, Rio Grande do Sul State, Brazil
manow@mikrus.com.br

Introduction
Compensatory growth is defined as a phase of accelerated growth when favorable conditions are restored after a period of growth depression caused by a stressing factor. It is a strategy developed in some species due to frequent stress situations in the environment, assuming trade-offs in resource allocation among growth, reproduction and self-maintenance. In aquaculture, the use of feed restriction and low temperatures as a trigger for compensatory growth can be considered strategies to reduce feed supply, costs and to increase the period of production of tropical species in regions that growing seasons is limited by low water temperature. In addition, Biofloc Technology System (BFT), can also bring several production advantages compared to the traditional systems in ponds, as increase the stock density, and improvement in water quality and biosafety. Furthermore, microorganisms community besides removing nitrogen compounds, also acts as a food supplement for shrimps, providing a constant feed supply 24 h a day.

Thus, the present study aimed to evaluate the occurrence of compensatory growth in *Litopenaeus vannamei*, in three different temperatures (20, 24 and 28º C) under feed restriction reared in a biofloc system, and its effect energy reserves and immunological condition of shrimps.

Materials and Methods
The experiment lasted 64 days and was divided in two phases: (1) Restriction and (2) Recovery. *L. vannamei* were stocked with 1.78 g (±0.38) in a stocking density of 300 shrimps/m³. In the first phase (36 days), the experiment was performed using a 3 x 2 (three temperatures and two feed regimes) experimental design, totaling six treatments (in triplicate). Three temperatures were chosen as optimum (28 ºC), intermediate (24 ºC) and low (20 ºC) and two feeding regimes were established for each temperature: the control that received 100% of the calculated feed rate and feed restriction, where the feed rate was 40% of the control. In the second phase (28 days), all the experimental units (n=18) were exposed to favorable conditions (100% of feeding rate and 28 ºC). Three shrimps per tank (nine per treatment) was collected at days 0, 36 and 64 to determine energy reserves content (total protein, glycogen and triglycerides) in hepatopancreas and assess differential hemocyte count (DHC) in hemolymph.

Results
In the end of the experiment, previously restricted shrimps held at 24 and 28 ºC displayed complete body weight catch-up through compensatory growth following the restriction period with depressed growth ($P > 0.05$). Shrimps maintained at 20 and 24 ºC with no feed restriction did not reach 28ºC treatment body mass when favorable temperature (28 ºC) was established ($P < 0.05$). Protein levels in hepatopancreas were not affected in any treatment over the experiment ($P > 0.05$) and glycogen was used as a metabolic fuel in all restricted groups during phase 1 ($P < 0.05$), but fully recovered when total feed supply was offered ($P > 0.05$). Triglycerides were also used in restricted shrimps held at 24 and 28 ºC in phase 1 ($P < 0.05$), and after recovery period, only treatment previously maintained at 24º C presented total recovery ($P > 0.05$). DHC presented differences among treatments ($P<0.05$) but was maintained in the safe range for healthy shrimps reared in BFT system over the experimental period. Also, survival was not affected by feed restriction or low and intermediary temperatures ($P > 0.05$).

Conclusion
Therefore, it is possible to submit *L. vannamei* to partial feed deprivation with a later recovery period as a trigger for total compensatory growth, in order to improve feed efficiency and decrease of feed supply. Also, in regions that low temperatures are a limiting to shrimp growing seasons, it is viable to explore partial compensatory growth to increase annual production.

(Continued on next page)

Acknowledgements

The authors are grateful for the financial support provided by the European Union (ASTRAL Project – H2020 – Grant Agreement 863034), National Council for Scientific and Technological Development (CNPq), Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS). Special thanks to GUABI Animal Health and Nutrition SA for donating the commercial diets.

References

Ali, M., Nicieza, A., & Wootton, R. J. (2003). Compensatory growth in fishes: a response to growth depression. Fish and fisheries, 4(2), 147-190.

Fóes, G., Krummenauer, D., Lara, G., Poersch, L., & Wasielesky Jr, W. (2016). Long term storage and the compensatory growth of white shrimp *Litopenaeus vannamei* in aquaculture ponds. Latin American Journal of Aquatic Research, 44(3), 588-594.

Jory, D.E., Cabrera, T.R., Dugger, D.M., Fegan, D., Lee, P.G., Lawrence, A.L., Jackson, C.J., Mcintosh, R.P., Castaneda, J., 2001. A global review of shrimp feed management: status and perspectives. In: Browdy, C.L., Jory, D.E. (Eds.), The New Wave: Proceedings of the Special Session on Sustainable Shrimp Culture. The World Aquaculture Society, Baton Rouge, pp. 104–152.

Krummenauer, D., Samocha, T., Poersch, L., Lara, G., & Wasielesky Jr, W. (2014). The

reuse of water on the culture of Pacific white shrimp, *Litopenaeus vannamei*, in BFT system. Journal of the World Aquaculture Society, *45*(1), 3-14.

Lara, G., Hostins, B., Bezerra, A., Poersch, L., & Wasielesky Jr, W. (2017). The effects of different feeding rates and re-feeding of *Litopenaeus vannamei* in a biofloc culture system. Aquacultural engineering, 77, 20-26.

Steinberg, C. E. (2018). Dietary Restriction, Starvation, Compensatory Growth–'Short-Term Fasting Does Not Kill You: It Can Make You Stronger'. In Aquatic Animal Nutrition (pp. 137-287). Springer, Cham.

Wasielesky, W., Froes, C., Fóes, G., Krummenauer, D., Lara, G., & Poersch, L. (2013). Nursery of *Litopenaeus vannamei* reared in a biofloc system: the effect of stocking densities and compensatory growth. Journal of Shellfish Research, 32(3), 799-807.

INCREASING COMPETITIVENESS OF SWEET LUPINE AND FABA BEAN IN FEED VALUE CHAINS IN EUROPE

Monika Weiss*, Sinem Zeytin, Vanessa Fuchs, Christina Hörterer, Matthew J. Slater

Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
E-mail: monika.weiss@awi.de

Introduction

Although inclusion rates of fishmeal and fish oil have already been strongly reduced in aquadiets, they remain staple ingredients, and a main environmental and economic concern of aquaculture today (Schmidt et al. 2016). Terrestrial plant protein sources have been key to alternative feed protein but the main interest to date has been in soy products, which have come under public criticism due to the widespread use of transgenic seeds and deforestation for soy cultivation. Recent studies focus on additional terrestrial protein sources, mainly grain legumes like field pea, lupin and faba bean (Carter & Hauler, 2000; De Santis et al. 2015; Øverland et al. 2009), which are regionally and organically produced. Legumes provide nitrogen for themselves and subsequent plants (Blume, 2010; Sulieman, 2015) and thus reduce the overall fertilization needs during crop rotation. Here we present validated diets for 3 species relevant for European Aquaculture (*Litopanaeus vannamei*, *Salmo salar*, *Dicentrarchus labrax*) containing locally grown lupin and faba bean. The diets are formulated tightly aligned to the requirements of each species, which is necessary not only from the economic but also from the ecological point of view. These formulations and diets are valuable for producers aiming to make fed aquaculture of salmon, shrimp of seabass more sustainable in the future.

Material and Methods

Controlled feeding experiments have been conducted in a RAS device (8-12 weeks) with three species of high relevance for European aquaculture (*L. vannamei*, *S. salar*, *D. labrax*). Aquafeeds containing meal or protein concentrate of Blue lupin (*Lupinus angustifolius* Boregine) and Faba bean (*Vicia faba*) as fish meal and soy product replacement were formulated to accurately meet the species and stage nutritional requirements, i.e. equal energy content, protein and amino acid profile, lipid and fatty acid composition, vitamins and minerals. After an appropriate trial period, samples were taken for metabolic, histologic and immunological analyses.

Results and Discussion

Untreated lupine meal can be used as an alternative protein source at rates of up to 10% (-20%) of the total feed (= 30-40% of animal protein) without growth or metabolic impairment compared to a commercial shrimp feed (Fig. 1a, b). A moderate lupin inclusion obviously operated as bioactive compound stimulating the shrimp's immune system (Fig. 1b, d).

Faba bean protein concentrate as well as Lupin protein concentrate can be used at rates of 35% of the total feed as a regional alternative to soy and as a partial fish meal replacement in feed for postsmolt Atlantic salmon without any impairments of health and growth (Fig. 2a).

Lupine kernel meal can be used in high inclusion rates (-50%) as a replacement for fishmeal in diets for the carnivorous European Seabass, with appropriate pre-treatment effectively mitigating negative effects on growth in smaller individuals (<30g) (Fig. 2b).

In conclusion, grain legumes and their products have great potential as a main protein source in diets for carnivorous aquaculture species in Europe.

(Continued on next page)

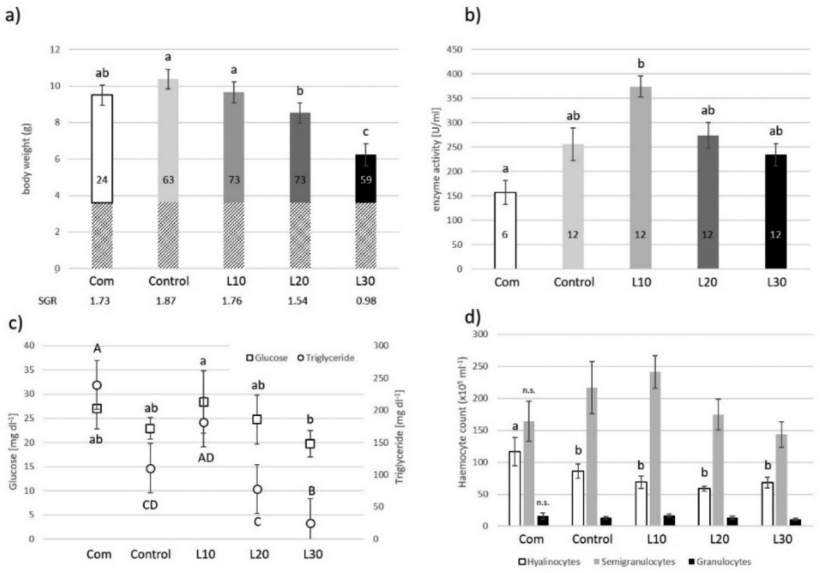


Figure 1: Results from feeding experiments with *L. vannamei*. Com – commercial diet, Control – control diet, L10, L20, L30 – diet with 10, 20 and 30 % lupin. Figures show means ± standard deviation, letters indicate significant differences (ANOVA) a) growth after 8 experimental weeks, striped bars show start weight, SGR – specific growth rate, number of measured individuals written in bar. b) Phaenoloxidase activity, replicate number in bar. c) metabolic measurements glucose and triglycerides d) Haemogram

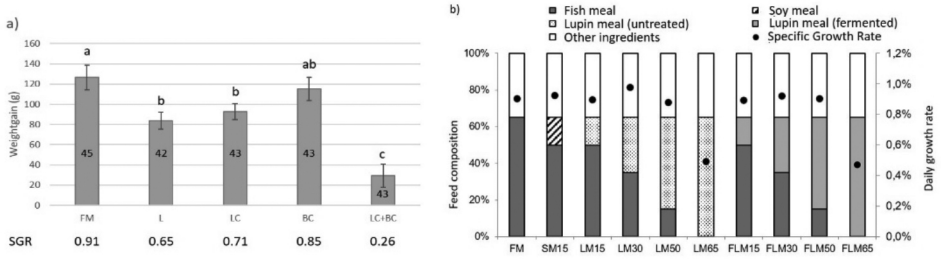


Figure 2: a) Weight gain of *S. salar* fed grain legume diets. Figure shows means ± standard deviation, letters indicate significant differences (ANOVA) FM – fish meal control, L – lupin meal, LC – lupin protein concentrate, BC – faba bean protein concentrate, LC+BC – only lupine protein concentrate + faba bean protein concentrate as protein sources. b) Main results of feeding *D. labrax* different diets containing lupin: feed composition (bars), Specific Growth Rate (% per day, dots), FM – Fish meal, SM – soy meal, LM – lupine meal untreated, FLM – fermented lupine meal

References

Carter, C. G., & Hauler, R. C. (2000). Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture, 185*, 299-311.

De Santis, C., Crampton, V. O., Bicskei, B., & Tocher, D. R. (2015). Replacement of dietary soy- with air classified faba bean protein concentrate alters the hepatic transcriptome in Atlantic salmon (*Salmo salar*) parr. Comp Biochem Physiol Part D Genomics Proteomics, 16, 48-58. doi:10.1016/j.cbd.2015.07.005

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., & Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture, 288(3-4), 305-311. doi:10.1016/j.aquaculture.2008.12.012

Schmidt, V., Amaral-Zettler, L., Davidson, J., Summerfelt, S., & Good, C. (2016). Influence of Fishmeal-Free Diets on Microbial Communities in Atlantic Salmon (*Salmo salar*) Recirculation Aquaculture Systems. Appl Environ Microbiol, 82(15), 4470-4481. doi:10.1128/AEM.00902-16

FULL-FAT MEAL AND FRACTIONS OF BLACK SOLDIER FLY *Hermetia illucens* LARVAE IN DIETS FOR ATLANTIC SALMON *Salmo salar*: EFFECTS ON NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE AND GUT HEALTH

Pabodha Weththasinghe[1*], Jon Øvrum Hansen[1], Sérgio DC Rocha[1], Mateusz Rawski[2], Damian Józefiak[3], Leidy Lagos[1], Byron Morales-Lange[1], Margareth Øverland[1]

[1]Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[2]Division of Inland Fisheries and Aquaculture, Institute of Zoology, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, ul Wojska Polskiego 71C, 60-644, Poznań, Poland
[3]Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, ul. Wołyńska 33, 60-627, Poznań, Poland
Email: pabodha.weththasinghe@nmbu.no

Introduction
Black soldier fly larvae (BSFL) (*Hermetia illucens*) have a great potential as a sustainable novel feed ingredient in fish feed. Numerous studies reported the effects of dietary inclusion of BSFL on fish species including Atlantic salmon (*Salmo salar*) (Belghit et al., 2018; Weththasinghe et al., 2021). These studies have proposed that the fractions of BSFL might differently affect the fish. However, to the best of our knowledge, the effects of different fractions of BSFL have still not been evaluated in a single study. Therefore, the present study investigated the effects of full-fat meal and fractions of BSFL in diets on nutrient digestibility, growth performance and gut health in Atlantic salmon pre-smolts.

Materials and methods
Six experimental diets were produced: a control diet based on fishmeal, plant protein sources and fish oil (CD); full-fat BSFL meal diet (IM), defatted BSFL meal diet (DFIM); de-chitinized BSFL meal diet (DCIM); BSFL oil diet (IO) and BSFL exoskeleton diet (EX). The full-fat, defatted and de-chitinized meals replaced 15% of the protein content of the control diet. A total of 900 Atlantic salmon pre-smolts with 28 g of mean initial weight were distributed into 18 fiberglass tanks (50 fish per tank), and fed with one of the six experimental diets. The growth performance parameters and nutrient digestibility in fish were estimated. The histological evaluations of pyloric caeca and distal intestine of fish were done. In addition, immunological parameters (IL-1 β and IgM) in distal intestine were evaluated by indirect ELISA. The RNA sequencing of distal intestine and 16S rRNA sequencing of gut microbiota of fish are ongoing.

Results and discussion
The full-fat and de-chitinized meals improved the growth rate of salmon, while defatted meal, oil and exoskeleton fraction supported similar growth performance in fish as the control. Furthermore, the improvement in the growth rate of fish fed the full-fat meal diet was accompanied by higher feed intake, whilst defatted meal gave a better feed conversion ratio than the full-fat meal. Replacement of dietary protein with defatted meal, de-chitinized insect meal or exoskeleton fraction reduced protein digestibility, whereas neither full-fat meal nor fractions affected lipid digestibility (Table 1).

In the pyloric caeca, fish fed de-chitinized insect meal diet showed low occurrence and severity of enterocyte steatosis compared to control and full-fat meal diets (Figure 1A). Evaluation of the distal intestine revealed normal and healthy morphology for most of the fish. The fish fed de-chitinized meal showed a higher level of pro-inflammatory cytokine IL-1β in the distal intestine compared to the fish fed the control diet (Figure 1B), whereas distal intestine IgM level showed no difference between the fish fed insect diets and the control diet.

Conclusions
In conclusion, the full-fat BSFL meal improved feed intake and growth rate in salmon when replacing 15% of dietary protein, however, defatted meal gave a better feed utilization than full-fat meal. Dietary inclusion of de-chitinized BSFL meal improved the histology of pyloric caeca of fish by reducing enterocyte steatosis and increased pro-inflammatory IL-1 β level in the distal intestine. These results contribute to establish a baseline for the study of insect-based diets that are capable of modulating the immune system of fish.

(Continued on next page)

Table 1: Growth performance and nutrient digestibility of fish fed experimental diets with full-fat meal or fractions of BSFL

	CD	IM	DFIM	DCIM	IO	EX	SEM	*p* value
SGR	2.01^{c}	2.26^{a}	2.10^{bc}	2.14^{b}	2.03^{bc}	2.11^{bc}	0.02	<0.001
Feed intake	53.1^{b}	68.8^{a}	55.9^{b}	59.4^{b}	55.7^{b}	57.1^{b}	1.31	<0.001
FCR	0.76^{ab}	0.80^{a}	0.74^{b}	0.76^{ab}	0.78^{ab}	0.75^{b}	0.006	0.013
ADC of protein	89.4^{a}	88.0^{abc}	87.4^{bc}	86.9^{c}	89.0^{ab}	86.7^{c}	0.28	0.001
ADC of lipid	97.6	97.6	97.1	95.7	96.9	97.1	0.23	0.16

SGR: Specific growth rate, FCR: Feed conversion ratio, ADC: Apparent digestibility coefficient

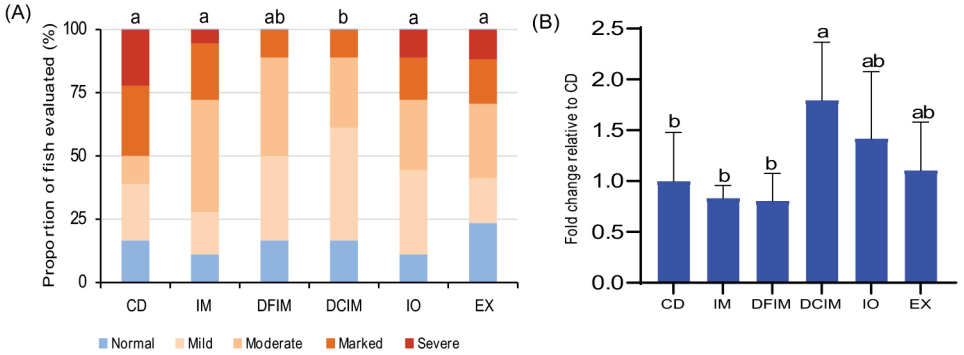


Figure 1: Histological evaluation of pyloric caeca and detection of immunological parameters in distal intestine in fish fed full-fat meal or fractions of BSFL. **(A)** number of pyloric caeca tissue sections scored as "normal", "mild", moderate", "marked" or "severe" for enterocyte steatosis and **(B)** Pro-inflammatory IL-1β level in distal intestine. Different superscript letters denote significant differences (p<0.05) among the dietary groups.

References

Belghit et al., 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture. 491, 72-81.

Weththasinghe et al., 2021. Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. Aquaculture. 530, 735785.

SUSTAINABLE WATER SOLUTIONS TO INCREASE YIELDS AND IMPROVE QUALITY

Diane White

diane.white@evoqua.com

Objectives
Discover the challenges and opportunities faced by global aquaculture including climate change, population growth, more mindful consumerism, and environmental regulations.

Learn why sustainable water solutions are essential for the protection and growth of this market.

Discover the new technologies that are supporting land-based hatcheries and the enterprising ways farmers can enhance their water treatment process, improve fish welfare and boost stock yields.

Method
New regulations and changes to sea-based aquaculture are driving the growth of land-based hatcheries. As our population grows, and together with climate change and more mindful consumerism, the demand for fish continues to evolve rapidly.

Evoqua uses sustainable water disinfection and filtration treatment technologies to help fish farmers address microbial management, produce larger fish, increase stock density and yields, improve water quality, and vastly improve fish welfare.

Results
Improved water quality in land-based hatcheries and sustainable treatment technologies that keep water clean and free from disease increases stock yields in existing infrastructures. Thanks to sustainable and non-chemical solutions for Recirculating Aquaculture Systems (RAS), the world now produces more seafood from fish farms than wild catch.

Conclusion
Evoqua provides sustainable solutions for productive fish growth and improved water quality that vastly reduces the risk of prevalent fish diseases like Infectious Salmon Anaemia and Gill disease. Our experts assist with regulatory compliance, and provide solutions that are simple to operate and easy to maintain, delivering optimal performance from a smaller footprint.

The speaker
The speaker is Diane White - Industrial Disinfection Sales Manager for the Aquaculture Industry at Evoqua Water Technologies. With over 25 years of experience within the water treatment industry & 12 years as a UV specialist, Diane is an expert in advanced treatment solutions to support the challenges facing the Aquaculture industry.

ECO-FORMULATION OF FISH FEEDS: A PROMISING EFFICIENT SOLUTION TO LIMIT AQUACULTURE IMPACTS ON THE ENVIRONNEMENT

Aurélie Wilfart[1*], Florence Garcia-Launay[2], Frederic Terrier[3], Espoir Soudé[3], Pierre Aguirre[3], Sandrine Skiba-Cassy[3]

1INRAE, Institut Agro, SAS, 35000 Rennes, France
2INRAE, Institut Agro, PEGASE, 35590 Saint-Gilles, France
3INRAE, Univ. Pau & Pays Adour, E2S UPPA, NUMEA, 64310 Saint Pée-sur-Nivelle, France

aurelie.wilfart@inrae.fr

Introduction

To meet the growing demand for seafood and the need for protein sources, aquaculture is expanding. It now provides more than 50% of fish for human consumption and this proportion is expected to continue to increase over the long term to meet the food needs of more than nine billion people by 2050. All animal production systems, including aquaculture, are widely criticized for their environmental impact and animal feed is responsible for the majority of these. Currently, feeds are formulated to fulfill fish nutritional requirements while minimizing feed cost and taking into account incorporation constraints of particular feed ingredients such as fishmeal and fish oil. To reduce environmental footprint of feed, feed formulation should take simultaneously into account the cost and the environmental impacts of raw materials. Recently, Garcia-Launay et al. (2018) developed a multiobjective formulation algorithm which uses the constraints of least-cost formulation and calculates a multi-objective function that includes both feed cost and environmental impact indicators obtained by life cycle assessment (climate change, non-renewable energy use, phosphorus demand, land occupation). The objective of the present study was to adapt and apply this algorithm to design eco-friendly diet (eco-diet) and assess their nutritional value in rainbow trout.

Material & Methods

In this study, two isoprotein, isolipid and isoenergetic diets were formulated: a control diet (C-diet), close to commercial feeds currently used in rainbow trout aquaculture and an ECO-diet, formulated using a multiobjective function to minimize both the environmental footprint (including climate change, phosphorus demand, acidification, NPPU and water demand) and price of feed . The digestibility of the diet was measured and a 12 weeks-growth trial was conducted (3 tanks per diet) on juvenile rainbow to assess the consequences of these diets on growth performance, body composition and nutrient utilization. Finally, the results were used in a life cycle approach to estimate the environmental impact of 1 kg of body-weight gain.

Results

Diets are presented in Table 1. Multiobjective formulation reduced the level of fishmeal and fish oil of 55 % but introduced animal proteins and fat as well as yeast proteins (Table 1). Environmental impacts estimated by life cycle assessment were all subjected to a reduction from 23 to 46%. Whereas diets exhibited similar protein digestibility, the eco-diet showed a significant lower apparent digestibility coefficient (ADC) of lipids, energy and ash but enhanced ADC of starch, even ADC values could be all considered as high. At the end of the growth trial, no significant differences difference in body weight was observed but analysis of the growth curves using a mixed linear models revealed a significant difference between the two diets in favor of the control diet (Figure 1). Feed efficiencies were similar but daily feed intake was significantly enhanced in fish fed the C-diet. Fish fed the Eco-diet showed a lower protein gain resulting from higher protein intake. Body composition was similar between the two diets. Producing 1 kg of weight gain induces less environmental impacts with the Eco-diet than with the commercial diet, whatever the impact considered. The reduction is more important for NPPU, water demand and phosphorus demand (Figure 2).

Discussion

The present study demonstrates that lowering environmental footprint of the feed reduces the use of fishmeal and fish oil with little even no consequence of growth performance, but it can significantly reduce the environmental impact of producing one kg of trout, depending of the impact considered. Development of eco-friendly aquafeed through multi-objective formulation appears as a promising solution to reduce the reliance of aquaculture on marine ingredient and thus promote the sustainable development of aquaculture.

(Continued on next page)

Table 1: diet composition and environmental impacts

% of the diet	**C-diet**	**Eco-diet**
Fishmeal	16.01	7.24
Fish oil	6.53	3.61
Plant sources	58.62	54.52
Processed animal protein	-	15.58
Plant oils	13.19	6.84
Animal fat	-	3.55
Yeast	-	1.82
Attractant Shrimp hydrolysate	2.00	2.00
Mineral and vitamin premix	2.32	2.37
Amino acids[1]	1.20	0.94
Dicalcium Phosphate	0.10	1.50
Carophyll pink (astaxanthine)	0.03	0.03
Environmental impacts of diets, per kg of feed		
CC (kg CO_2-eq)	1.39	0.75
NRE (MJ)	14.85	8.55
AC (molc H^+-eq)	0.02	0.01
EU (kg PO_4^{3-}-eq)	0.01	0.00
LO (m^2year)	1.62	1.24
NPPU (kg C)	21.59	12.15
WD (m^3)	10.32	5.76
PD (kg P)	0.01	0.01

[1]L-Lysine, L-Methionine, L-Threonine

[2]CC= climate change; NRE= non-renewable and fossil energy demand; AC= acidification; EU= eutrophication; LO= land occupation; NPPU= net primary production demand, WD= water demand; PD= P demand

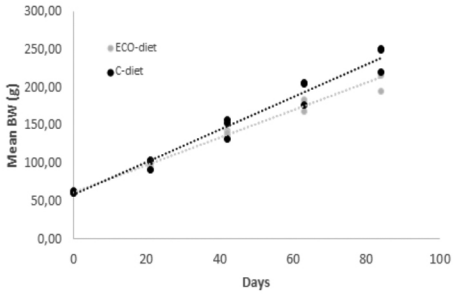


Figure 1: Adjusted growth curves for the fishes according to the diet

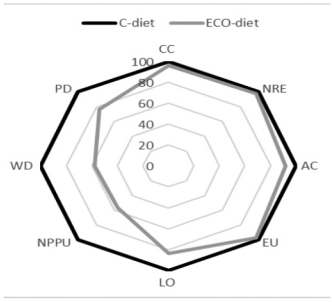


Figure 2: Environmental impacts of 1 kg of bodyweight gain expressed in % of the highest impact.

SCREENING OF POTENTIAL PROBIOTICS ISOLATED FROM NORMAL BACTERIAL FLORA OF *Litopenaeus vannamei* AGAINST VIBRIOSIS

F. Wulandari*, I. Herliana, T. Y. Prajitno A. Nurina and V. Anggara

Research & Diagnostic Departement of PT Vaksindo Satwa Nusantara, Indonesia
E-mail: febriana.wulandari@japfa.com

Introduction

Vibriosis is considered one of the most important bacterial diseases in shrimp farms of Indonesia. This disease is responsible for high mortality in aquaculture worldwide. Advancing the immune response through probiotics application is expected to be an environmentally friendly alternative to prevent the disease. The purpose of this study was to identify candidates for normal bacteria flora of the shrimp gut that has potency as probiotics to inhibit *Vibrio harveyi* infection.

Materials and methods

Sampling was conducted in Probolinggo and Banyuwangi, Indonesia. Gut samples from vannamei shrimp (*Litopenaeus vannamei*) that survived a vibriosis outbreak were diluted with serial dilution technique then cultured in SWC-agar at 30 ºC for 24 hours. The bacteria that grow are then coated with *Vibrio harveyi* strain MR5339 and incubated at 30 ºC for 24 hours. Bacterial colonies that appear clear inhibition zones for *V. harveyi* are then cultured and purified using SWC-agar only. These bacterial colonies were designated as probiotic candidates. This study used PCR and Sanger sequencing of the 16s gene to identify probiotic candidates. In vitro challenge tests using SWC agar were carried out on four identified probiotic candidates against *V. harveyi* isolate obtained from the field.

All of these probiotic candidates were challenged in vitro by culturing with *V. harveyi* on SWC agar. After that, the number of *V. harveyi* colonies that can still grow was counted. The challenges test were carried out with two doses ratio between probiotic candidates and V. harveyi,of 1:1 and 2:1. The inhibition percentage of *V. harveyi* bacteria was calculated by the formula:

$$x = \frac{V.\ harveyi \text{ without challenge (CFU)} - V.\ harveyi \text{ after challenge (CFU)}}{V.\ harveyi \text{ without challenge (CFU)}} \text{ x } 100\%$$

Results

Four probiotic candidates obtained and identified as *Bacillus cereus* (B1-02 & B1-04), *Bacillus albus* (B2-05) and *Enterococcus faecium* (E1-12). Our study showed that a dose of B1-02, B1-04 and B2-05 bacteria could inhibit V. harveyi above 99% while E1-12 was 43.85%. Furthermore, the challenge test with double dose of probiotic candidates showed that there was an increase in the percentage of inhibition by bacteria B1-02, B1-04 and B2-05 although not significant otherwise inhibition by E1-12 increased significantly more than 2 times. Details of inhibition data can be seen in Table 1 and Figure 1.

Conclusions

Our study showed that *B. cereus*, *B. albus* and *E. faecium* were found to be able to inhibit *V. harveyi* growth. All of that bacteria could inhibit V. harveyi more effectivelly at two-fold dosage. Presumably, *B. cereus*, *B. albus* and *E. faecium* could be used in the shrimp culture as the probiotic. Indeed, more research into the in vivo challenge test is required in the future.

References

Hannan MA, et all. 2019. Molecular identification of *Vibrio alginolyticus* causing vibriosis in shrimp and its herbal remedy. *Polish Journal of Microbiology Vol. 68, nNo 4*.

Sarida M, Harpeni E. 2010. Screening of potential probiotic *Vibrio* sp. against vibriosis in the *Litopenaeus vannamei*. *Biosfera (27) 2*.

(Continued on next page)

Table 1 The colony counting result of *V. harveyi* after challenge

Probiotic Candidates	Single dose challenge		Double dose of challenge	
	V. harveyi (CFU)	Inhibitoin (%)	*V. harveyi* (CFU)	Inhibitoin (%)
B1-02	2.24 X 10^7	99.80	1.00 X 10^7	99.91
B1-04	8.00 X 10^7	99.29	3.80 X 10^7	99.67
B2-05	4.60 X 10^7	99.59	2.00 X 10^4	99.99
E1-12	6.40 X 10^9	43.85	2,95 X 10^6	99.97

* Total count of *V. harveyi* without challenge: 1.14 x 10^{10}

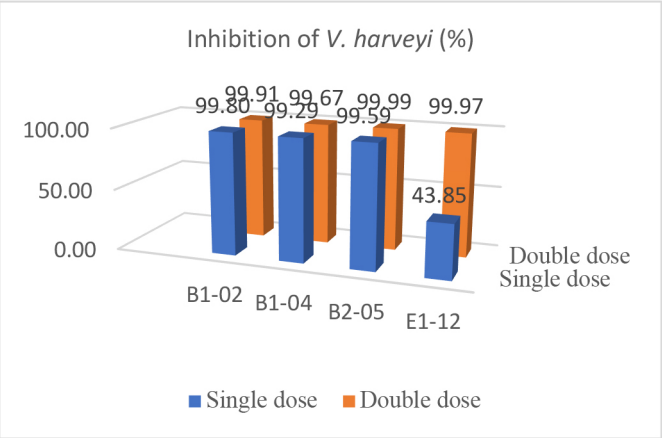


Figure 1 The inhibition percentage of *V. harveyi*

DIETARY CURCUMIN MODULATES DIGESTIVE CAPACITY AND OXIDATIVE STATUS IN DIFFERENT LARVAE STAGES OF GILTHEAD SEABREAM

Maria J. Xavier[*1,2,3,4], Luís E.C. Conceição[2], Luisa M.P. Valente[3,4], Sofia Engrola[1]

[1]CCMAR, Centro de Ciências do Mar, Universidade do Algarve, *Campus* de Gambelas, 8005-139 Faro, Portugal
[2]SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[3]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[4]ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313 Porto, Portugal
E-mail: mmxavier@ualg.pt

Curcumin (diferuloymethane) is a polyphenol extract from the rhizome of the plant *Curcuma longa* and it is known to possesses a strong antioxidant capacity, and anti-inflammatory, antimicrobial and immunostimulatory proprieties. The use of this natural plant extract as a feed additive for juvenile fishes have showed good results as an enhancer of growth, oxidative status, stress resistance and promoter of digestion capacity (e.g., Alagawany *et al*., 2021). However, knowledge on the impact that dietary curcumin may have in early larvae stages is still scare. Marine fish larvae are a transitory stage very vulnerable and highly prone to stress that exhibit a drastic metamorphosis involving several morphologic and metabolic changes to achieve the juvenile stage (Hamre *et al*., 2013). Therefore, the aim of this work was to assess if curcumin supplementation could promote gilthead seabream larvae robustness and digestive maturation to ultimately improve fish growth performance.

Two experimental trials were conducted to test the effects of different doses of curcumin (LOW and HIGH) in gilthead seabream larvae and postlarvae. In Experiment 1, 42 days after hatching (DAH) gilthead postlarvae were fed exclusively control or the supplemented diets for 20 days. At the end of the growth trial fish were sampled to analysed oxidative status, gut maturation, and morphology. In Experiment 2, 4 DAH larvae were fed the experimental diets for 27 days, in an early co-feeding regime until 24 DAH. Diet impact was assessed at several sampling points, to determine the oxidative status, digestive capacity, and feeding incidence of the larvae throughout ontogeny.

The first trial showed that fish from HIGH and LOW significantly improved the oxidative status compared to CTRL treatment, through a decrease in the content of protein oxidative damage and an increase in the total antioxidant capacity. Moreover, postlarvae fed curcumin supplemented diets also presented an upregulation of *nfr2* and *gr* in HIGH and *hsp70* in LOW treatments, when compared to non supplemented fish (CTRL). On the other hand, no differences were observed in the growth performance, intestine morphometry and digestive enzymes activities.

In the second trial, no differences were observed in growth performance at larvae from 4 to 24 DAH. However, at the end of the experiment (31 DAH) larvae fed LOW diet had a better condition factor than CTRL fish. Moreover, 31 DAH larvae fed HIGH diet showed higher trypsin and chymotrypsin activity levels when compared to CTRL fish. LOW and HIGH larvae were able to prevent an increase in the mtROS production during development, in contrast to non supplemented larvae (CTRL).

In conclusion, dietary curcumin supplementation seems to promote larvae digestive capacity and modulate the oxidative status during early ontogeny of gilthead seabream. In fact, in postlarvae this supplement was able to enhance fish oxidative status through an increase in the total antioxidant capacity and reduction in the content of protein oxidative damage. Both studies provide new evidence that dietary supplementation of natural compounds, could be a nutritional strategy to enhance marine fish larvae robustness at early life stages of development. Therefore, this study contributes to improve larvae quality production in marine hatcheries and promote a more sustainable industry.

(Continued on next page)

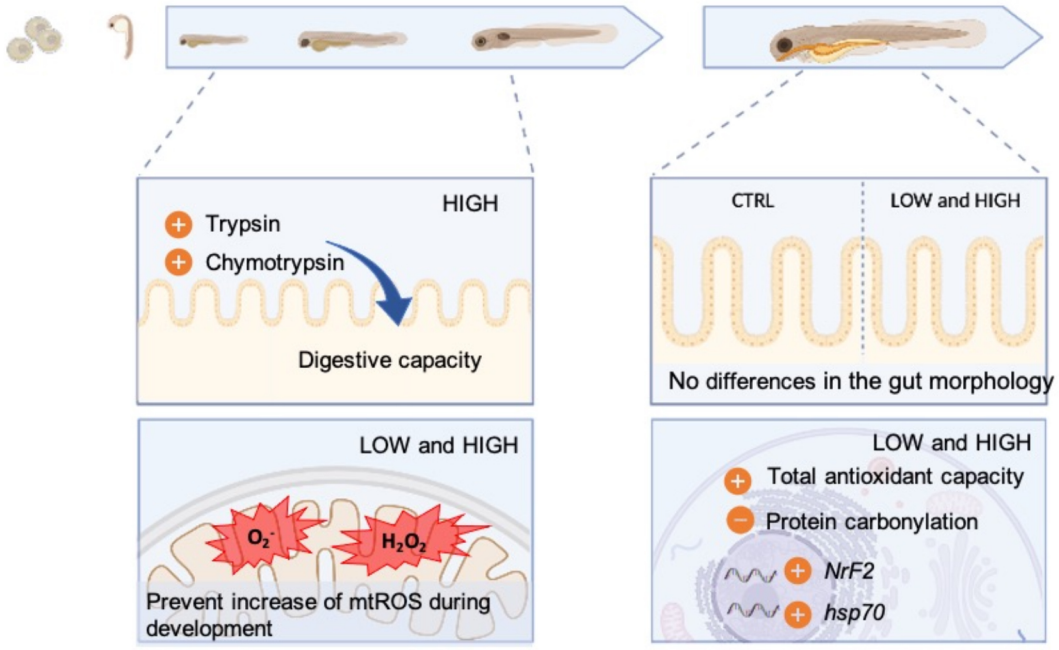


Figure 1: Schematic representation of the main results of two experimental trials conducted in gilthead seabream larvae and postlarvae fed different doses of curcumin supplementation (LOW and HIGH).

Acknowledgements

The present study was supported by projects ALG-01-0145-FEDER-029151 "PROLAR – Early metabolic programming in fish through nutritional modulation", and UIDB/04326/2020 financed by the FCT (Portugal). Maria J. Xavier was supported by Grant PDE/0023/2013 (SANFEED Doctoral program, with support by FCT and SPAROS Lda., Portugal).

References

Alagawany, M., Farag, M. R., Abdelnour, S. A., Dawood, M. A., Elnesr, S. S., & Dhama, K. (2020). Curcumin and its different forms: A review on fish nutrition. *Aquaculture*, 736030.

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L. E., & Izquierdo, M. (2013). Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture*, *5*, S26-S58.

LIVE FEED ENRICHMENTS USING ALGAE TECHNOLOGY FOR PIKEPERCH (*Sander lucioperca*) LARVAL CULTURE

Carlos Yanes-Roca*, Karolina Ranglova[2], Michal Bures[2], Tomas Policar[1], Jiri Masojidek[2].

[1] South Bohemia University, Českých Budějovicích, Faculty of Fisheries and Protection of waters Vodňany, Czech Republic
cyanesroca@jcu.cz
[2] Institute of Microbiology of the Czech Academy of Sciences, Algatech Center, Novohradská 237, Třeboň, Česká republika

The use of *Chlorella vulgaris* and *Trachidiscus minitus* as live feed enrichments for rotifer and artemia fed to Pikeperch (*Sander lucioperca*) larvae was tested during this trial. Larvae were fed a combination of rotifers (until day 15 post hatchting) and artemia (from day 12 post hatchting) under seven different enrichments: a) *Nannochloropsis occulata (Nanno 3600 reed Mariculture), b) Chlorella vulgaris cultured at 20 degrees Celsius in BG117 media*, c) *Chlorella vulgaris cultured at 30 degrees Celsius in BG117 media, d) Chlorella vulgaris cultured at 20 degrees Celsius in Urea media, e) Chlorella vulgaris cultured at 30 degrees Celsius in Urea media, f) Trachidiscus cultured at 15 degrees Celsius and g) Trachidiscus cultured at 25 degrees Celsius*. After 21 days from the trial initiation significant differences were found between treatments on total length (TL), myomere height (MH), and fatty acid composition. In terms of growth parameters, larvae from treatment c had significantly higher values in total length (11.48 mm) and myomere height (2.04 mm). Furthermore, larvae from treatment c) showed significant higher concentration of Docosahexaenoic acid (DHA) (%), and Linoleic acid (LA) (%) and larvae from treatment g) had significant higher concentration of Eicosapentaenoic acid (EPA) (%) and Arachidonic acid (ARA) (%).

Overall, larvae from treatments c and g, performed better than the other treatments, likely due to the difference in Essential Fatty Acids (EFA) concentration. The results from this trial, will help to optimize the pikeperch larvae nutritional requirements and diversify the live feed enrichments used during first feeding.

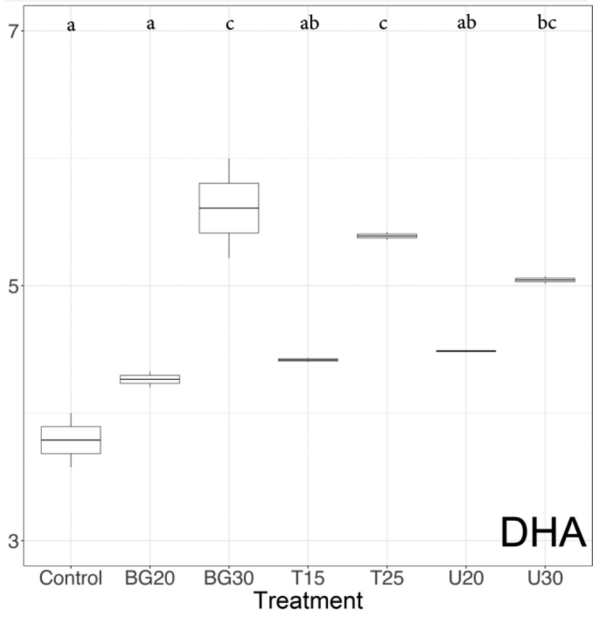


Figure 1. Pikeperch larvae DHA concentration after 21 days from 7 different enrichment diets

THE INTERACTION BETWEEN GROWTH HORMONE AND REDOX SYSTEM IN DEVELOPING ATLANTIC SALMON (*Salmo salar*) SMOLT

Peng Yin[1,2], Bjørn Thrandur Bjørnsson[3], Tu Ahn Vo[4], Sofie Remø[1], Per Gunnar Fjelldal[5], Takaya Saito,[1] Tomasz Furmanek[1], Rolf B. Edvardsen[1], Tom Hansen[5], Sandeep Sharma[6], Rolf Erik Olsen[4], Elin Kjørsvik[4], Kristin Hamre[1]

[1] Institute of Marine Research, Bergen (Norway)
[2] University of Bergen
[3] University of Gothenburg (Sweden)
[4] NTNU, Trondheim, (Norway)
[5] Institute of Marine Research, Matredal, (Norway)
[6] Biomar AS, Trondheim, (Norway)

*E-mail: peng.yin@hi.no

Introduction and objective

Reactive oxygen species (ROS) are becoming increasingly appreciated as signaling molecules, regulating varying cellular processes, such as proliferation and differentiation, beyond the role as damage signal [1, 2]. The key mechanism of redox signaling and control involves is impacting the structures, functions, activities or trafficking of associated proteins via the reversible or irreversible redox modifications of redox-sensitive amino acids [3, 4]. Accumulating evidence indicates a strong relationship between increased oxidative stress and periods of high growth rates in fish [5-7]. However, whether and how the redox system changes during the growth stimulation seen in Atlantic salmon, and do the growth hormone (GH) signals interact with redox signaling directly to increase metabolism or is the oxidative stress a secondary function of a higher metabolic rate and oxidative phosphorylation in the mitochondria caused by growth hormone stimulation remain unclear.

The objective in this study is to elucidate the relationship between growth stimulation and redox environment of Atlantic salmon and the potential role of ROS in contribution to the effects on GH.

Material and methods

This study had two sequential experimental phases (EP) termed EP1 and EP2, both lasting for 6 weeks. Atlantic salmon (initial weight: 38.7 ± 0.6 g) were divided into two groups and reared in 6 replicate tanks at stable temperature (around 12°C) and continuous light. We provided salmon with a diet low vitamins (L) with 230mg/kg ascorbic acid as stay C and 120mg/kg α-tocopherol using the acetate form, while diet high vitamins (H) had 380mg/kg ascorbic acid and 210mg/kg α-tocopherol, respectively. At the beginning of EP2, half of the fish from each group was implanted with formulation of recombinant bovine GH (Posilac®), while the other half was sham-implanted with same volume of the sesame-seed oil vehicle. We evaluated the liver and muscle redox environment and health condition of Atlantic salmon via accessing the total glutathione (tGSH) levels, the redox potential (Eh), ascorbic acid (AA), α-tocopherol (α-TOH), malondialdehyde (MDA) concentrations, the activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) and cataract score compared between treatments in EP1 and EP2.

Results

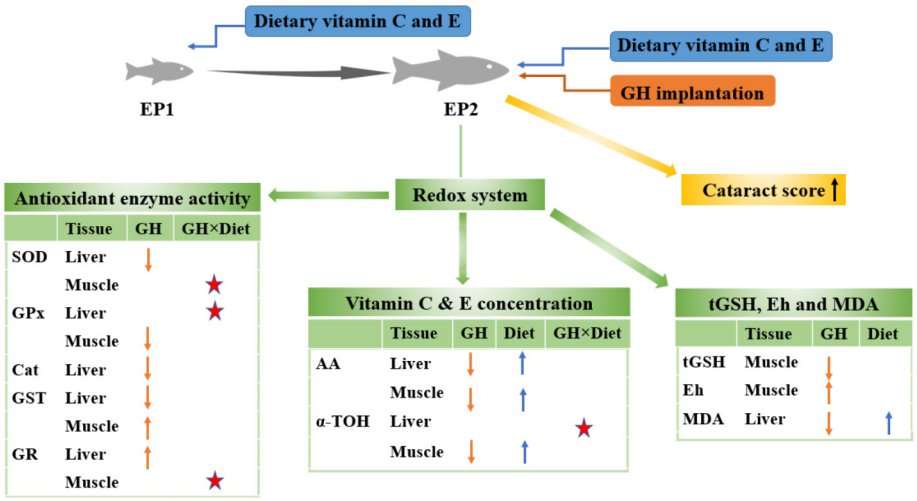
Here we show that redox-related parameters measured in the EP2 were influenced by growth hormone and diet to varying degrees. Notably, GH-treated salmon had lower levels of reduced glutathione, a more oxidized redox potential of muscle, and higher MDA concentrations of liver. We found that fish fed diet H had higher ascorbic acid and α-tocopherol of liver and muscle than those fed diet L, whereas implantation GH significantly decreased their concentrations. The activities of antioxidant enzymes of liver and muscle were differently regulated by GH implantation, which may be associated with the difference in tissue antioxidant capacity and regulation. There were interactive effects between GH and diet on GPX of liver, and SOD and GR of muscle. Furthermore, we found GH-treated salmon had higher cataract score in the EP2, which suggested cataract is more severe in fish implanted with GH.

Conclusion

Our results demonstrated GH has an oxidative effect on liver and muscle of Atlantic salmon and that there is an interaction between the effects of antioxidant and GH on redox signaling. The present study is intended as a starting point to further understand the potential interactions between growth and redox signaling of fish.

(Continued on next page)

Graphical abstract



References

1. Diebold, L.; Chandel, N. S., Mitochondrial ROS regulation of proliferating cells. Free Radic Biol Med 2016, 100, 86-93.
2. Ray, P. D.; Huang, B. W.; Tsuji, Y., Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 2012, 24 (5), 981-90.
3. Corcoran, A.; Cotter, T. G., Redox regulation of protein kinases. FEBS J 2013, 280 (9), 1944-65.
4. Jones, D. P.; Sies, H., The Redox Code. Antioxid Redox Signal 2015, 23 (9), 734-46.
5. Nordgarden, U.; Ornsrud, R.; Hansen, T.; Hemre, G. I., Seasonal changes in selected muscle quality parameters in Atlantic salmon (Salmo salar L.) reared under natural and continuous light. Aquaculture Nutrition 2003, 9 (3), 161-168.
6. Hamre, K.; Sissener, N. H.; Lock, E. J.; Olsvik, P. A.; Espe, M.; Torstensen, B. E.; Silva, J.; Johansen, J.; Waagbo, R.; Hemre, G. I., Antioxidant nutrition in Atlantic salmon (Salmo salar) parr and post-smolt, fed diets with high inclusion of plant ingredients and graded levels of micronutrients and selected amino acids. PeerJ 2016, 4, e2688.
7. Carney Almroth, B.; Johnsson, J. I.; Devlin, R.; Sturve, J., Oxidative stress in growth hormone transgenic coho salmon with compressed lifespan--a model for addressing aging. Free Radic Res 2012, 46 (10), 1183-9.

EFFECT OF DIETARY RETINOL CONCENTRATION ON ASTAXANTHIN UTILISATION IN ATLANTIC SALMON AND IMPLICATIONS OF STRESS

T. Ytrestøyl[1*], Morken[2], T., Mullins[2], J., Dikiy[3], A., Skavang[3], P., Østbye[4], T.K., Hatlen[1], B., Krasnov[4], A., Ruyter[4], B.

[1] Nofima, Sjølsengvegen 22, NO-6600 Sunndalsøra, Norway
[2] Skretting ARC, Sjøhagen 3, NO-4116 Stavanger, Norway
[3] NTNU, Institute for biotechnology and food science, NO-7034 Trondheim, Norway
[4]Nofima, Osloveien 1, NO-1433 Ås, Norway
E-mail: trine.ytrestoyl@nofima.no

Introduction

The red flesh color is an important quality trait for farmed Atlantic salmon. The color is due to the salmon's ability to deposit the carotenoid astaxanthin in the muscle. The retention of astaxanthin in the muscle is not very efficient, normally less than 10% of the ingested astaxanthin is retained in the muscle. There are indications that the fillet color has decreased in recent years. Reduced inclusion of marine ingredients in the modern salmon diet (Ytrestøyl et al., 2015, Aas et al., 2019) has led to reduced levels of several nutrients (phospholipids, vitamin A and cholesterol) that may affect flesh pigmentation in Atlantic salmon, both during absorption in the gut and through interference with metabolic conversion of astaxanthin to vitamin A. In addition to its provitamin A function (Schiedt et al., 1985), astaxanthin is a powerful antioxidant that reacts with free radicals and singlet oxygen (Naguib et al., 2000, Dose et al., 2016). Increased oxidative stress has been suggested as a possible cause of decreased flesh pigmentation in salmon, but experimental evidence supporting this hypothesis is limited. In this project, the effect of dietary retinol and astaxanthin concentrations in combination with stress on astaxanthin deposition and metabolism were tested in Atlantic salmon.

Materials and methods

Diets with two concentrations of astaxanthin (30 and 60 mg/kg) combined with three levels of vitamin A (6500, 35 000, 100 000 IU/kg), six diets in total, were fed to A. salmon for 16 weeks (start weight 190g: final weight:1000g). The trial was done in tanks with flow through seawater and feed intake were measured for calculation of astaxanthin retention in the muscle. After the 16-week feeding trial, four of the diets (high/low astaxanthin and retinol) were fed for a period of 5 weeks and split in a group that were stressed and a control group without stress. The stress was induced by lowering the water level and oxygen concentration in the tanks three times/week. Samples of muscle, liver and intestine were sampled for analysis of astaxanthin and breakdown products by HPLC and NMR. Visual color was assessed by a Minolta Chroma Meter and SalmoFan™. Hepatocytes and enterocytes were isolated and incubated with ^{14}C-labelled astaxanthin to study the metabolism of astaxanthin to retinol *in vitro*. Samples of liver, muscle and intestine were taken for analysis of gene expression by microarray.

Results

Growth rate was not significantly affected by dietary astaxanthin or retinol concentration. The flesh color measured by Minolta redness (a*-value) and Salmofan scores was lower at the highest dietary vitamin A concentration. This was confirmed by analysis of astaxanthin concentration in NQC by NIR and HPLC. A decreasing astaxanthin concentration with increasing vitamin A in the diet was also found in liver and plasma, but not in intestine. The retention of astaxanthin in the muscle was highest in the salmon fed the diet with 30 ppm astaxanthin and medium vitamin A concentration. Analysis of samples from the stress trial and *in vitro* trial as well as gene expression are currently on-going and will be presented.

References

Aas, T.S., Ytrestøyl, T. & Åsgård, T. (2019) Utilization of feed resources in the production of Atlantic salmon (Salmo salar) in Norway: An update for 2016. Aquaculture Reports, 15, 100216.

Dose, J., Matsugo, S., Yokokawa, H., Koshida, Y., Okazaki, S., Seidel, U., Eggersdorfer, M., Rimbach, G., Esatbeyoglu, T. Free Radical Scavenging and Cellular Antioxidant Properties of Astaxanthin. Int. J. Mol. Sci. 2016, 17, 103.

Naguib, Y.M., 2000. Antioxidant activities of astaxanthin and related carotenoids. J. Agric. Food Chem. 48, 1150-1154

Schiedt, K., Leuenberger, F.J., Vecchi, M., Glinz, E. 1985. Absorption, retention and metabolic transformation of carotenoids in rainbow trout, salmon and chicken, Pure and Applied Chemistry 57, 685-692.

Ytrestøyl, T., Aas, T.S. & Åsgård, T. (2015) Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. Aquaculture, 448, 365-374.

GENOMIC SELECTION AND INTROGRESSION SIGNATURES RESULTING FROM ADAPTATION TO HIGH SALINITY: A CASE STUDY ON AN INDONESIAN FARMED TILAPIA (SUKAMANDI) STRAIN

Xiaofei Yu[1*], Hendrik-Jan Megens[1], John W.M. Bastiaansen[1], Langqing Liu[1], Martien A.M. Groenen[1] and Hans Komen[1].

1. Animal Breeding and Genomics, Wageningen University & Research, The Netherlands.
2. Email: xiaofei.yu@wur.nl and hendrik-jan.megens@wur.nl.

Background
Tilapia is currently the most important fish in aquaculture in the tropics and subtropics. Originally a freshwater species, it is cultured in a wide range of conditions. Among the most challenging is when high salinity is involved, e.g. culturing in estuaries or high salinity ponds in polyculture with shrimp. Optimized tilapia strains generally do not tolerate high salinity well. Although the physiological characteristics for osmoregulation are reasonably well understood, it is less clear how selection results in salinity tolerance. Here we investigate one such strain that was bred to perform well in brackish water. Specifically, we infer signatures of selections in the genome. In addition, since the salinity tolerance in the strain was hypothesized to be derived from another species, we also inferred signatures of introgression between species.

Materials and methods
Indonesian farmed tilapia (Sukamandi) strain was derived from the BRPI research institute (Sukamandi, Java), and has been selected for growth for four generations under salinity levels varying from 15 to 58 ppt. Fin clips from a total of 20 fish were sampled from the nucleus population in 2019. In order to understand the genomic architecture of Sukamandi strain, whole genome sequencing data of blue tilapia were downloaded from the Sequence Read Archive (NCBI bio-project number PRJNA358089 and PRJEB23203). Nile tilapia, originating from the Genetically Improved Farmed Tilapia (GIFT) population, were collected by the Genomar company. We performed a joint-calling strategy for genetic variants, genetic differentiation and diversity analysis, selection signatures and Identity by descent detection.

Results
We compared the genome of this Sukamandi strain to that of Nile tilapia (*Orechromis niloticus*) and blue tilapia (*Orechromis aureus*), the latter a putative donor of the salinity tolerance. Our results indicate that the Sukamandi strain is genetically much more similar to Nile tilapia (*Orechromis niloticus*) (Fst=0.042) than to blue tilapia (*Orechromis aureus*) (Fst=0.386). By two pairwise comparisons (as shown in Figure 1), 33 salinity adaptive genes involved in MAPK3 activity, potassium ion homeostasis, ATPase activity (coupled to transmembrane movement of ions), calcium ion binding pathway were identified. Combining genome-scale scanning for selection and introgression, revealed that salinity tolerance related genes, such as *slc25a24* and *cdh1* were under selection (as shown in Figure 2).

Conclusion
Salinity adaptive genes disclosed by genetic differentiation comparison with blue and Nile tilapia strain were partially under selection. The genome of Sukamandi strain appears to be overwhelmingly of Nile tilapia origin but introgression from blue tilapia may have conferred some of the observed salinity tolerance. We provide the genetic basis for breeding resilient tilapia -Sukamandi strain.

(Continued on next page)

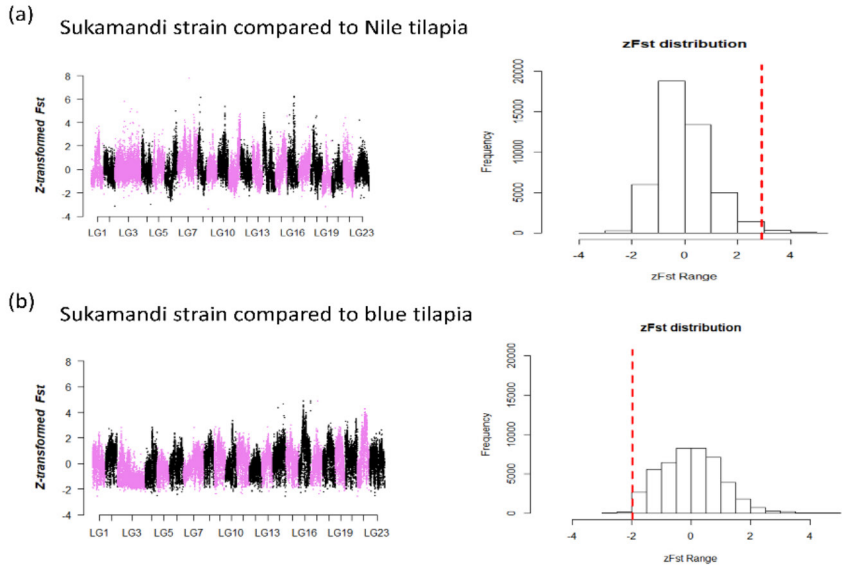


Figure 1. Manhattan Plot of Z(Fst) value of Sukamamdi strain versus Nile tilapia (a) or blue tilapia (b).

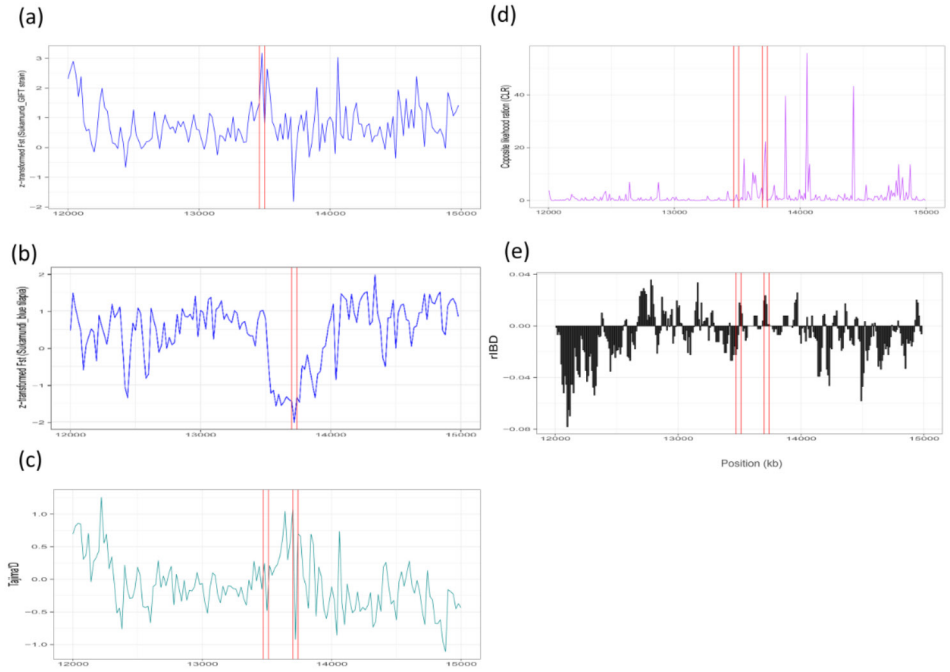


Figure 2. Examples of genomic regions with Fst (a) and (b), Tajima'D (c), Composite likelihood ratio (CLR) (d) and relative identify-by-descent (rIBD) (e).

EVALUATION OF THE NUCLEAR ESTROGEN RECEPTORS OF SEA BASS (DICENTRARCHUS LABRAX) AS TARGET SITES FOR ENDOCRINE DISRUPTING COMPOUNDS: POTENTIAL EFFECTS ON REPRODUCTION AND THEIR USE FOR RISK ASSESSMENT

C. Zapater[1*], G. Molés[1], C. Moreira[2], T. Monsinjon[2], P. I. S. Pinto[3], A. Gómez[1]

[1]Instituto Acuicultura de Torre de la Sal, CSIC, 12595 Torre de la Sal, Castellón, Spain
[2]Environmental Stress and Aquatic Biomonitoring (SEBIO), Université Le Havre Normandie, F-76600 Le Havre, France
[3] Centro de Ciências do Mar, Universidade do Algarve, 8005-139 Faro, Portugal
*E-mail: cinta.zapater@csic.es

Introduction

Estrogens are involved in the regulation of a wide range of processes in teleost reproduction (Lubzens et al, 2010; Schulz et al, 2010), and exert their functions mainly through ligand-activation of their specific cognate receptors. Nuclear estrogen receptors are transcription factors that bind to estrogen response elements (EREs) on gene promoters to regulate their expression (Tsai et al, 1994), and three subtypes of these receptors have been identified in teleosts. Differences in binding affinity and seasonal expression patterns in reproductive tissues among estrogen receptor subtypes suggest different roles during oogenesis, vitellogenesis and testicular development along the brain-pituitary-gonad axis. It is known that endocrine-disrupting compounds (EDCs) may act as agonists or antagonists of regulatory actions of steroid receptors or of the production of the receptors themselves. Thus many different points in the endocrine control of fish reproduction can be potential targets for the actions of EDCs. In aquaculture, the presence of estrogenic compounds in water is as worrying as its presence in new formulation of commercial fish diets (Arpin-Pont et al, 2016; Nacher-Mestre et al, 2013; Quesada-García et al, 2012). Nevertheless, the lack of information available on the role of each nuclear estrogen receptor in teleosts, including European sea bass, makes it difficult to have an accurate knowledge on the impacts of EDCs on reproduction. This study focused on investigating the role of the three nuclear estrogen receptor subtypes in European sea bass, on evaluating the impacts of endocrine-disrupting compounds on nuclear estrogen receptors functions and on their use as tools for risk assessment.

Material and methods

To investigate the role of the three nuclear estrogen receptors subtypes in European sea bass, analysis of the expression of their coding genes during a whole reproductive cycle was investigated by qPCR in testis, ovary and pituitary. Localization of the nuclear estrogen receptors along the brain-pituitary-gonad axis was performed by immunohistochemistry using specific antibodies for each subtype.
Using the human embryonic kidney cell line HEK293, we have performed transient transfections (1) to characterize nuclear estrogen receptors (Esr1, Esr2a and Esr2b) by using transactivation of an ERE- luciferase reporter gene assay; (2) to study the effect of two possible EDCs, genistein – a phytoestrogen also present in fish meals – and fluoxetine – an antidepressant, mainly constituent of Prozac - on all nuclear estrogen receptors from sea bass.

Results

The coding genes of the three nuclear estrogen receptor subtypes of sea bass are highly expressed in reproductive-related tissues such as pituitary and gonad. Quantification of *esr1*, *esr2a* and *esr2b* expression in the gonad and pituitary during a whole reproductive cycle showed different expression patterns depending on stage and subtype. Localization of the three nuclear estrogen receptors along the pituitary-gonad axis showed differences among subtypes depending on the gonadal stage.

The functional characterization of the nuclear estrogen receptors showed that there are different ligand affinities among the nuclear estrogen receptor subtypes, which also translate into differential receptor responses when we evaluate the effect of potential EDCs, such as genistein and fluoxetine.

(Continued on next page)

Conclusion

The results suggest that the three nuclear estrogen receptors of European sea bass are not redundant and have differential roles in the regulation of gametogenesis, as proposed also in other teleosts, which means that the effects produced by EDCs can induce potential adverse effects at reproductive level.

The results also show that *in vitro* bioassays using nuclear estrogen receptors are a good tool for risk assessment of potential endocrine-disrupting compounds.

Acknowledgements
Supported by funds from MICINN (AGL2015-67477-C2-1-R, RTI2018-094667-B-C22). PISP. received Portuguese national funds from FCT (Foundation for Science and Technology) through project UIDB/04326/2020 and researcher contract DL57/2016/CP1361/CT0015 with the Univ.Algarve.

Bibliography

Lubzens E, Young G, Bobe J, Cerdà J. Oogenesis in teleosts: how fish eggs are formed. General and comparative endocrinology 2010; 165:367-389.

Schulz RW, de Franca LR, Lareyre JJ, Le Gac F, Chiarini-Garcia H, Nobrega RH, Miura T. Spermatogenesis in fish. Gen Comp Endocrinol 2010; 165:390-411.

Thomas, P., et al., *Progestin, estrogen and androgen G-protein coupled receptors in fish gonads*. Steroids, 2006. **71**(4): p. 310-6.

Tsai, M.J. and B.W. O'Malley, *Molecular mechanisms of action of steroid/thyroid receptor superfamily members*. Annu Rev Biochem, 1994. **63**: p. 451-86

Arpin-Pont, L., et al., *Occurrence of PPCPs in the marine environment: a review*. Environ Sci Pollut Res Int, 2016. **23**(6): p. 4978-91.

Nacher-Mestre, J., et al., *Qualitative screening of undesirable compounds from feeds to fish by liquid chromatography coupled to mass spectrometry*. J Agric Food Chem, 2013. **61**(9): p. 2077-87.

Quesada-García, A., et al., *Assessment of estrogenic and thyrogenic activities in fish feeds*. Aquaculture, 2012. **338-341**: p. 172-180.

EFFECTS OF GARLIC *Allium sativum* POWDER ON NUTRIENTS, HAEMATOLOGY, AND IMMUNE AND STRESS RESPONSE IN EURASIAN PERCH *Perca fluviatilis* JUVENILRS

M. Zare*, H. Q. Tran, M. Prokešová, V. Stejskal

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Na Sádkách, České Budějovice, Czech Republic
Email: mzare@frov.jcu.cz

Introduction

Herbal derivatives and extracts have been used in fish diets as natural growth promoters and immune stimulants (Abdelwahab et al., 2020). The aim of present study was to investigate the effects of garlic powder in feed on growth performance, body proximate composition, apparent nutrient digestibility, selected blood and immune parameters, and resistance to overcrowding stress in Eurasian perch juveniles.

Materials and Methods

After 14-day acclimation, 110 fish per tank were distributed randomly into twelve 185 L tanks in recirculation aquaculture system (RAS). Feed formulation was extruded at Exot Hobby s.r.o. (Černá v Pošumaví, Czech Republic). European perch (*Perca fluviatilis*) (initial weight 25.0±0.4 g) were fed a diet including 0 (Control), 10 (G10), 20 (G20), and 30 (G30) g kg^{-1} garlic powder. Fish were fed manually based on apparent satiation at 08.00, 12.00, and 16.00 for 87 days.

Results and Discussion

No significant differences in growth performance, viscerosomatic and hepatosomatic index were observed among groups ($p > 0.05$). Condition factor was significantly lower in the G30 diet compared to other groups ($p < 0.05$). This agrees with Sahu et al. (2007) who reported that garlic powder in rohu diet did not significantly improve SGR or FCR. Moreover, no significant differences were observed among groups in whole body dry matter, fat, or ash ($p > 0.05$). But the level of protein in the G30 diet was significantly higher than G10 ($p < 0.05$). Significantly higher dry matter digestibility was observed in all garlic groups compared to control ($p < 0.05$). Significantly higher fat digestibility was found in G10 and G30 compared to control and G20 ($p < 0.05$). No differences in protein digestibility were observed among groups ($p > 0.05$). The number of RBC and WBC in G10 was observed significantly higher than other groups ($p < 0.05$). Lymphocyte and myeloid cell percent did not differ among groups ($p > 0.05$).

No significant differences in blood serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, triglycerides, or total protein were observed among groups ($p > 0.05$). Garlic powder at all levels was associated with significantly lower levels of cholesterol ($p < 0.05$). Significantly higher levels of albumin were detected in G10 and G20 compared to other groups ($p < 0.05$). In agreement with our result garlic powder did not show significant effect on ALT and AST activity in Asian sea bass (*Lates calcarifer*) (Abdelwahab et al., 2020). Garlic powder reduced cholesterol in rainbow trout (Mohebbi et al., 2012), and increased blood serum albumin in rainbow trout (Nya and Austin 2009). We did not find blood serum total protein to differ among groups the same as (Nya and Austin 2011) who observed in rainbow trout. Garlic powder inclusion did not affect respiratory burst and phagocytic activity ($p > 0.05$). Immediately after stress, all garlic diet groups showed significantly higher levels of cortisol compared to the control ($p < 0.05$). At 24 h, significantly higher and lower cortisol levels were detected in control and G30 groups, respectively ($p < 0.05$) while glucose was significantly higher in controls than in garlic-fed groups ($p < 0.05$).

Acknowledgments

This study was financially supported by the Ministry of Agriculture of the Czech Republic by NAZV project (QK1810296).

References

Abdelwahab, A.M.; El-Bahr, S.M.; Al-Khamees, S. (2020) Influence of dietary garlic (*Allium sativum*) and/or ascorbic acid on performance, feed utilization, body composition and hemato-biochemical parameters of juvenile Asian Sea Bass (*Lates calcarifer*). Animals. 10, 2396.

Mohebbi, A.; Nematollahi, A.; Dorcheh, E.E.; Asad, F.G. (2012) Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research. 43, 1184–1193.

Nya, E.J and Austin, B (2009). Use of garlic, *Allium sativum*, to control control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases, 32, 963-970.

Nya, E.J.; Austin, B. (2011) Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish and Shellfish Immunology. 30, 845-850.

Sahu, S.; Das, B.K.; Mishra, B.K.; Pradhan, J.; Sarangi. N. (2007) Effect of Allium sativum on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Journal of Applied Ichthyology. 23, 80–86.

THE APPLICATION OF ULTRASOUND AS CAGE ANTIFOULING METHOD AND ITS IMPACT ON EUROPEAN SEA BASS, *Dicentrarchus labrax*

Sinem Zeytin[1]*, Desislava Bögner[1], Meeno Mathes[1], Hilal Tolasa Gündogdu[2], Ertugrul Gündogdu[2], Julien Jost[3], Pierre-Oliver Jost[3], Camille Blanc[3], Matthew J. Slater[1]

[1] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
E-mail: sinem.zeytin@awi.de
[2] Nesne Elektronik Tasarım ve Danışmanlık Ltd. Erzene Mahallesi, Ankara Cad. Ege Üniversitesi Kampüsü, Bornova-İzmir, Turkey
[3] Sofchem Sarl, Rue du Gue 9, Reuil Malmaison, 92500, France

Introduction
Aquaculture faces a major issue to reach its full potential, due to biofouling, which is affecting products quality, quantity and environment- labour- equipment integrity. According to market experts, current treatment measures represents from 10 to 20% of farm exploitation costs (costs of chemicals, nets cleaning and repair, and fish escapees). Therefore, this study aims to prevent micro-fouling layers apparition on offshore aquaculture fish farm cage nets with ultrasonic (US) waves to increase not only the aquaculture productivity and operational efficiency but also to verify the implementation and performance in the field. However, there is a lack of knowledge about the effects of applying US on fish growth, feeding behavior and health. Therefore, in order to facilitate the adoption of the system, the innocuity of the technology on fish's welfare and health has to be evaluated. In this study, we determined and defined the maximum level of power and frequency after an adequate trial period with US to get the most effective anti-fouling system without harming the commercially important European sea bass (*Dicentrarchus labrax*).

Material and Methods
Field trial (1) was conducted in an offshore mini-cage system (one test and one control cage; diameter: 3 m, depth: 5 m) without fish and the effectivity of US treatment was measured by video recording of the cages to evaluate biofilm formation for 1-month. Full (continuous ultrasound signaling) and pulse/intermittent fire were tested in order to observe the power consumption and its effectivity on the algal accumulation on cage nets. Both cages were installed nearby the Barge at the offshore fish farm.

Field trial (2) was conducted in an offshore cage system (one test and one control cage; diameter: 20 m, depth: 8 m, stocking density: 30.000 to 50.000 fish) with various (full-fire/intermittent fire) configurations. The effectivity of US treatment was measured by video recording of the cages to evaluate and compare biofilm formation after 1-month. In addition, fish mortality and behaviour (feed intake, swimming behaviour) as stress marker were observed in order to select the best frequencies and power.

Field trial (3) will be conducted in operational cages (two test and two control cages; diameter: 30 m, depth: 20 m, stocking density: >50.000) with US treatment (fixed frequency and operational mode full or pulsed fire will selected after analysing the Field trial (2) assay). In order to evaluate the effect of a specific power/treatment configuration on full or pulse fire on feed intake, growth and health of European sea bass, five sampling will be performed at the offshore fish farm. These are initial sampling (d0; before US application, d2: two days after US application, d32, d62, d92: one-month interval between sampling). For the impact on feed intake and growth: weight, length, FCR, feeding behavior; for the impact on health and stress response: histological studies on skin, liver and gills, Cortisol level, LDH, Glucose, Na+, K+, Cl- ions in blood, and additionally skin mucus, blood smears, haematocrit levels and gene expression in skin, liver and gills will be analysed.

Results and Discussion
Results from the preliminary Field trial (1) offer an initial glance on the impact of different levels of frequencies on the accumulation of algae on mini-cage nets. Hereby, enhancing the wave density with close proximity positioning of the transducers exhibits clearly the highest impact area thus confirming that the placement and combination of transducers induced the most effective during 1-month of trial. Field trial (2) showed that algae do not grow near the transducers and high frequency transducers have more impact for preventing the anti-fouling on the cages compare to low frequency transducers. However, the system set up needs to improve power and performance to reduce algae growth on the whole area of the cages. Nevertheless, no stress response was observed on fish feeding and behavior during the US application.

(Continued on next page)

Final results are pending! However, we expect that the US application being tested for preventing of anti-fouling will be new to the aquaculture industry, and can be implemented as a standardized application in offshore fish farms. However, as stress response can be observed in different levels in different fish 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 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 H2020-EIC-FTI-2018-2020 programme under grant agreement no. 958776.

PRELIMINARY RESULTS OF EXPERIMENTS TESTING THE FEASIBILITY OF CO-CULTURE OF SHRIMP AND ALGAE IN RAS SYSTEM

A. Zgrundo*, H. Gawrysiak, K. Czmajduch, O. Bogusławski, B. Dmochowska, , H. Łądkowska

University of Gdańsk, Bażyńskiego 8, Gdańsk, 80-309, (Poland)
Email: aleksandra.zgrundo@ug.edu.pl

Introduction

In Europe, the majority of animals considered alien species are farmed in closed systems and a great deal of emphasis is placed on their sustainability. One of the requirements for such farms is to minimise their impact on the environment, which in the context of aquaculture means that farm water discharged into the environment must meet very stringent standards for various types of pollution, including nutrients. Thanks to the high levels of nutrient uptake, high photosynthesis level, and high growth rate algae have recently come into focus for their application potential in RAS systems.

Therefore, 2 experiments were conducted to demonstrate that native algae can be used in RAS cultures as biofilters to purify the culture water of *Paneus vannamei* (whiteleg shrimp). In the first experiment the idea of "algae scrubber" on a trickling filter was tested. In the second experiment, native filamentous green algae of the genus *Chaetomorpha* were tested.

Materials and methods

Experiments testing the simultaneous culture of algae and whiteleg shrimp were carried out between May 2021 and September 2021 in two RAS-500 systems designed by AquaMedic Poland located at the Institute of Oceanography, University of Gdansk. Both RAS are identical and have 500 dm^{-3} cultivation pools and system operation is controlled and monitored by a dedicated computer. For algae cultivation, additional flow-through tanks with a maximum volume of 125 dm^{-3} equipped with Aqua Illumination Prime HD lamps were connected to the original systems. In both algae tanks the same light exposure time was set (10h light and 14h dark cycle), and the same default light parameters were used: 60% blue light, 60% green light, red light, 60% warm white light, 60% cold white light. The values of the main water parameters (temperature, pH, salinity, redox potential) were continuously monitored during the experiments. Additionally, the concentration of nitrogen (NH_4^+, NO_3^- and NO_2^-) and phosphorus compounds (PO_4^{3-}) as well as Fe ions were measured every 2 days with a Hach Lange DR5000 spectrophotometer using sachet tests during the first 2 weeks and then once a week.

For the first experiment testing the development of algae biofilm on a trickling filter, in the algae culture tank, three 50 x 35 cm nets were mounted on the water supply pipes in such a way that water flowed down them continuously along their entire length.

In the second experiment, filaments of green algae *Chaetomorpha linum* collected locally from the coastal waters of the Baltic Sea were used in variants with nitrogen gas and distilled water pre-treatment, with and without acclimatization.

In both cases, intensive observations of biofilm and *Ch. linum* filaments were carried out. Additionally, *Ch. linum* biomass was measured on a SBS-LW Balance Scale with a measurement accuracy of 0.001 g. As in the case of water parameters, during the first 2 weeks analyses were performed every 2 days and then once a week.

Results and discussion

In both experiments, the basic properties of the culture water in RAS (i.e. temperature, pH, salinity, redox potential) and concentration of NH_4^+ crucial for shrimp growth were kept stable at desirable levels. However, the concentration values of nitrate (NO_3^-) and nitrite (NO_2^-) and orthophosphate (PO_4^{3-}) increased with shrimp culture age to values that significantly exceeded the environmental values. For example, after 21 weeks, NO_3^- concentrations reached 25 $mg \times dm^{-3}$, and PO_4^{3-} – 9.8 $mg \times dm^{-3}$. In both experiments, culture waters were supplemented with Fe ions because they initially lacked this key element for photosynthesis.

Initially, in the first experiment, no biofilm was formed to act as a biofilter, confirming the initial assumption that in the case of a RAS system meeting the high purity requirements no algal colonization of the system would occur. Only after the introduction of *Ch. linum* filaments into the algae tank a biofilm formed, consisting mainly of diatoms and cyanobacteria.

(Continued on next page)

In the experiment with *Ch. linum* it was shown that native algae without and after acclimatization process show intensive growth only for a period of several weeks (6-9 depending on the variant of the experiment). It was also observed that irrespective of the method used to purify *Ch. linum* filaments obtained from the environment, microalgae were always introduced into the system along the host algae. Hence, only the culture of specially prepared and purified strains e.g. from unialgal cultures can ensure the purity of the RAS system. Furthermore, the decline of the growth of *Ch. linum* after a couple of weeks indicates suboptimal conditions for its development. This is probably due to inadequate light conditions or lack of key micronutrients, which were not measured.

Based on the experiments, no significant effect of algal co-culture on the reduction of nutrients in RAS systems was recorded.

Conclusions

Both microalgae and macroalgae can be cultured in RAS with shrimps. However, in order to achieve satisfactory levels of water purification and algal biomass growth, the introduction of selected and pre-adapted algal species is required along with the provision of suitable growth conditions.

Acknowledgement

Scientific work published is a part of an international project AquaVIP, INTERREG South Balltic, co-financed by the of the Minister of Science and Higher Education program funds entitled “PMW” in the years 2020-2022; Agreement No. 5126 / SPB 2014-2020/2020/2.

MOLECULAR MONITORING OF TWO BETANODAVIRUS- SUSCEPTIBLE SPECIES IN THE NORTHERN SHORES OF THE PERSIAN GULF AND PHYLOGENETIC ANALYSIS OF ISOLATED

M Ziarati[1], M.J Zorriehzahra[2*] and F. Hassantabar[3]

[1.]Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Jahrom, Iran.
[2.]Department of Information and Scientific Communication, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
[3.]Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

Corresponding author: m.zorriehzahra@areeo.ac.ir

Introduction

Viral Nervous Necrosis (VNN) is identified as an emerging agent in wide variety of wild and farmed fish. Rising temperatures on the shores of the Persian Gulf, imported fish, and the onset of some nonspecific signs of the virus in wild grouper (*Epinephelus* spp.) and farmed Sea bass (*Lates calcarifer)* fish, prompted fish screening hypothesis by molecular methods. This is the first report on VNN monitoring in fish from the Persian Gulf of Iran.

Material and Methods

This study monitored from two species of fish susceptible to VNN using PCR and Nested PCR. Furthermore, Water temperature was measured during random collection of visibly healthy and asymptomatic fish.

Results

The results of present study, the PCR showed no positive cases but the Nested PCR revealed some positive results. Ultimately, the phylogenetic analysis of the isolates was performed.Isolated sequence analysis showed a high homology of 98% with Red Spotted Grouper Viral Nervous Necrosis (RGNNV) genotype. According to the importance of emerging viral diseases and their irreparable damages, continuous investigation and epidemiological studies of VNN were recommended.

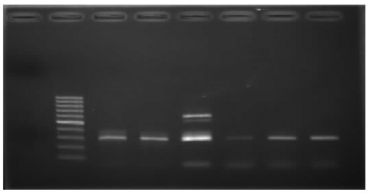


Fig1. Result of Nested-PCR method

-Bandin I, Souto S (2020) Betanodavirus and VER disease: a 30-year research review. Pathogens 9:106.

References:

Banerjee D, Hamod MA, Suresh Th, Karunasagar I (2014). Isolation and characterization of a nodavirus associated with mass mortality in Asian Sea bass (Lates calcarifer) from the west coast of India. Virus Dis 25(4):425–429.

Kumar S, Stecher G, Tamura K (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874.

NaveenKumar S, Hassan MA, Mahmoud MA, Al-Ansari A, Al-Shwared WK (2017). Betanodavirus infection in reared marine fishes along the Arabian Gulf. Aquacult Int. https ://doi. org/10.1007/s1049 9-017-0134-1

Nishizawa T, Mori KI, Nakai T, Furusawa I, Muroga K (1994). Polymerase chain reaction (PCR) amplification of RNA of striped jack nervous necrosis virus (SJNNV). Dis Aquat Organ 18:103–107.

Nishizawa T, Furuhashi M, Nagai T, Nakai T, Muroga K (1997). Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Appl Environ Microbiol 63:1633–1636.

Rajan JJS, Praveena PE, Bhuvaneswari T, Jithendran KP (2016). Design and evaluation of reverse transcription nested PCR primers for the detection of betanodavirus in finfish. Virus Dis 27(2):123–129.

Thiery R, Raimond JC, Castric J (1999). Natural outbreak of viral encephalopathy and retinopathy in juvenile Sea bass, Dicentrarchus labrax: study by nested reverse transcriptase polymerase chain reaction. Virus Res 63:11–17.

Zorriehzahra MJ, Nakai T, Sharifpour I, Gomez DK, Shau-Chi C, Soltani M, Saidi AA (2005). Mortality of wild golden grey mullet (Liza auratus) in Iranian waters of the Caspian Sea associated with viral nervous necrosis-like agent. Iran J Fish Sci 4(2):43–58.

Zorriehzahra MEJ, Ghasemi M, Ghiasi M, Haghighi Karsidani S, Nazari A, Sharifpour I, Rohani MS (2013). Study of Viral Nervous Necrosis (VNN) in Caspian Sea grey mullet Liza auratus and the evaluation of its infection and a transition probability to other fish (sturgeon, Rutilus frisi and cultural fish) in Iran. Final report of a national research project, Iran Fish Res Org, 186 p.

INFLUENCE OF SOLVENT AND ULTRASOUND-ASSISTED EXTRACTION ON PIGMENTS RECOVERY AND ANTIOXIDANT ACTIVITY FROM ALGA *Ulva lactuca*

R. Raos[a], A. Dobrinčić[b], Z. Čošić[b], P. Lisica[b], S. Pedisić[b], V. Dragović-Uzelac[b], R. Čož-Rakovac[c,d] and Z. Zorić[b*]

[a]University of Zadar, Department of Ecology, Agronomy and Aquaculture, Trg Kneza Višeslava 9, 23000 Zadar, Croatia
[b]Faculty of Food Technology & Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia
[c]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[d]Center of Excellence for Marine Bioprospecting (BioProCro), Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
E-mail: zzoric@pbf.hr

Introduction

Ulva lactuca is a green macro alga also known as "sea lettuce" belonging to the genus Ulva in which about 50 other species have been described. Due to the high content of biologically active molecules (BAM), *Ulva lactuca* is considered not only as food but also as a dietary supplement.

Extraction is a fast and efficient method of separation and concentration of BAM which differ in chemical structure. For each matrix it is necessary to optimize the extraction conditions considering all the extraction parameters: the availability of the extraction technique, the purity of the extract, the extraction yield and the energy consumption (Rostagno & Prado, 2013). The advantages of new extraction techniques manifest in the extraction rate and yield, lower energy consumption and environmental acceptability when compared to conventional techniques.

Therefore, the aim of this study was to optimize the parameters of ultrasound-assisted extraction (UAE) in order to achieve the maximum yield of carotenoids and chlorophylls as well as antioxidant activity (AOA) from alga *U. lactuca* using two solvents (hexane and 80% acetone) at two temperatures (30 and 50 ºC) and three different times (10, 15 and 20 min).

Materials and methods

U. lactuca samples were collected from the Adriatic Sea, washed with distilled water, frozen at -60 ºC, freeze-dried (CoolSave 55-9 PRO, Labogene Denmark) and ground. UAE was performed using ultrasound bath (Elmasonic 40H, Elma, Germany) according to above mentioned experimental conditions. Determination of carotenoids and chlorophylls was performed using HPLC system (Agilent Infinity 1260 system) with UV/VIS PDA detection. The solvent composition and gradient conditions used were previously described by Castro–Puyana et al. (2017). Identification was carried out by comparing retention times and spectral data with those of the authentic standards (α- and β-carotene, lutein, chlorophyll *a* and *b*). Quantifications were made by the external standard calculation, using calibration curves of the standards. The AOA of the extracts was assessed by ORAC assay according to Elez Garofulić et al. (2020). The ORAC procedure included an automated plate reader (BMG LABTECH, Offenburg, Germany) with 96-well plates, and data were analysed by MARS 2.0 software. The experimental design and statistical analysis were done using STATISTICA v.10 Experimental design (DOE) software (StatSoft Inc., Tulsa, OK, USA).

Results

Results showed that *U. lactuca* contained high amounts of carotenoids and chlorophylls. Lutein was the predominant carotenoid in all obtained extracts, followed by α- and β-carotene. The highest concentration of lutein was determined in extract obtained at 30 °C and 20 min (91.98 mg 100 g^{-1}), while the lowest concentration was detected in hexane at UAE parameters of 30 °C and 10 min (0.03 mg 100 g^{-1}). Chlorophylls were determined only in acetone extracts and the most represented one was chlorophyll *a* with the highest amount (78.11 mg 100 g^{-1}) detected in extract obtained at 30 °C and 20 min of extraction time. All extracts obtained using acetone had higher ORAC values (286.66 – 329.74 μmol TE L^{-1}) in comparison with hexane extracts (77.22 – 117.00 μmol TE L^{-1}).

Discussion and conclusion

The effects of UAE parameters were assessed on pigments recovery from *U. lactuca* by using acetone and hexane as extraction solvents. Obtained results showed that the yields of all pigments, except β-carotene, were significantly higher when acetone was used. According to Martins et al. (2021), this is due to the higher affinity of chlorophylls and xanthophylls for polar *vs*. non-polar solvents and the ability of acetone to better dissolve cell walls.

(Continued on next page)

Statistical analysis showed that the highest concentration of carotenoids was achieved in acetone extraction at 30 °C for 20 min and in hexane extraction at 50 °C for 15 min. This is in accordance with Abd El-Bakya et al. (2008) results. Although Razi Parjiklaei et al. (2014) concluded that temperature increase has a positive effect on the amount of extracted carotenoids, it was observed in this study that temperature of 30 ºC had higher extraction potential for all pigments than temperature of 50 ºC.

ORAC values were significantly influenced by extraction solvent. The highest ORAC values were observed in acetone extracts, what can be linked with higher carotenoids and chlorophylls amounts present in these extracts.

The highest yields and greater stability of pigments during UAE were achieved under 30 °C, 20 min of extraction time and using acetone as solvent.

References

Abd El-Baky, H.H., El Baz, F.K., I Baroty, G.S.E. (2008). Evaluation of Marine Alga *Ulva lactuca* L. as A Source of Natural Preservative Ingredient. American-Eurasian J.Agric. & Environ.Sci., 3(3), 434-444

Castro-Puyana, M., Pérez-Sánchez, A., Valdés, A., Ibrahim, O.H.M., Suarez-Álvarez, S., Ferragut, J.A., Micol, V., Cifuentes, A., Ibáñez, E., García-Cañas,V. (2017). Pressurized liquid extraction of *Neochloris oleoabundans* for the recovery of bioactive carotenoids with anti-proliferative activity against human colon cancer cells. FoodRes. Int., 99, 1048–1055.

Dominguez, H., Loret, E. (2019). *Ulva lactuca, A Source of Troubles and Potential Riches*. Marine Drugs. 17(6), 357.

Elez Garofulić, I., Kruk, V., Martić, A., Martić, I., Zorić, Z., Pedisić, S., Dragović, S., Dragović-Uzelac, V. (2020) Evaluation of Polyphenolic Profile and Antioxidant Activity of *Pistacia lentiscus* L. Leaves and Fruit Extract Obtained by Optimized Microwave-Assisted Extraction. Foods, 9(11), 1556-1572.

Martins, M., Oliveira, R., Coutinho, J.A.P., Faustino, M.A.F., Neves, M.G.P.M.S., Pinto, D. C.G.A., Ventura, S.P.M. (2021). Recovery of pigments from *Ulva rigida*. Separation and Purification Technology, 255, 117723.

Razi Parjikolaei, B., Casas Cardoso, L., Fernandez-Ponce, M.T., Mantell Serrano, C., Bruhn, A., Christensen, K.V., Fretté, X. (2014). Supercritical fluid extraction of carotenoids from *Ulva lactuca* (Chlorophyta). Poster session presented at 62nd International Congress and Annual meeting of the Society for Medicinal Plants and Natural Products, Portugal.

Rostagno, M.A., Prado, J.M. (2013) Natural product extraction: Principles and Applications, Royal Society of Chemistry, Cambridge.

SPERMATOGENESIS IN WILD AND CAPTIVE-REARED GREATER AMBERJACK *Seriola dumerili* (RISSO, 1810)

R. Zupa[1*], A. Corriero[1], C. Pousis[1], I. Fakriadis[2], M. Papadaki[2], L. Passantino[1] and C. C. Mylonas[2]

[1]Department of Emergency and Organ Transplantation, Section of Veterinary Clinics and Animal Production, University of Bari Aldo Moro, Valenzano 70010, Bari (Italy)
[2]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, Heraklion 71003, Crete (Greece)
E-mail: rosa.zupa@uniba.it

Introduction

Greater amberjack *Seriola dumerili* (Risso, 1810) is an excellent candidate for aquaculture thanks to its rapid growth, excellent flesh quality and worldwide market appreciation. As other captive-reared fishes exhibiting reproductive dysfunctions (Zohar and Mylonas, 2001), greater amberjack males produced reduced sperm volume with a decreased sperm quality. The present work represents an overview of the results obtained in a comparative research study on the spermatogenesis of wild and captive-reared greater amberjack carried out in the framework of the EU FP7 project Diversify (www.diversifyfish.eu) (Zupa et al, 2017a, b).

Material and Methods

Twelve greater amberjack males caught from the wild and reared in captivity for three years in a sea cage in Salamina Island (Greece) and 14 males caught from the wild around Pelagie Islands (Sicily, Italy), were sampled during three phases of the reproductive cycle: early spermatogenesis (late April-early May), advanced spermatogenesis (late May-early June) and spawning (late June-July). For each fish, biometric data (fork length, FL, in cm; body mass, BM, in kg; testis mass, TM, in g) were registered and gonadosomatic index (GSI = 100 × TM/BM) calculated. Testis samples were chemically fixed and destined to basic histological analysis and to the identification of proliferating and apoptotic germ cells through the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA) and the TUNEL method, respectively. Blood samples were centrifuged and plasma was collected and stored at -20°C for the analysis of 17β-estradiol (E_2), testosterone (T), 11-ketotestosterone (11-KT) and 17,20β-dihydroxypren-4-en-3-one (17,20β-P) by ELISA assays.

Results and Discussion

Captive-reared fish showed lower GSI and smaller seminiferous lobules compared to wild fish in all the three phases of the reproductive cycle.

Anti-PCNA immunostaining was observed in the nuclei of spermatogonia and primary spermatocytes (Fig. 1a). All the captive-reared and most of the wild greater amberjack showed TUNEL-positive germ cells (Fig. 1b). Individuals reared in captivity showed a gradual decrease of germ cell proliferation throughout the three reproductive phases, which led to a precocious cessation of the spermatogenesis as well as a higher germ cell apoptosis in early spermatogenesis. In all three reproductive phases, captive-reared fish showed lower T, 11-KT and 17,20β-P plasma concentrations compared to wild fish; however, captive-reared fish showed a many-fold higher E_2 plasma levels during the early spermatogenesis.

The occurrence of a severe endocrine dysfunction was described in captive-reared greater amberjack males, including low T, 11-KT and 17,20β-P plasma levels during all the examined spermatogenesis phases. Abnormally high E_2 plasma concentrations were associated to an increased germ cell apoptosis during early spermatogenesis. The observed reproductive dysfunction finally led to a lower sperm concentration and quality (Zupa et al., 2017a). A severe impairment of the reproductive function was observed also in females of the same broodstock and involved low steroid plasma concentrations and extensive atresia of late vitellogenic oocytes (Zupa et al., 2017b). Preliminary data obtained within the H2020 project NewTechAqua indicate that hatchery-produced greater amberjack males reared in sea cages in Salamina (Greece) have similar GSI compared with wild fish sampled in the same period of the reproductive cycle (early June 2021). Although further analyses are required, the available data seem to suggest that the reproductive function might be less affected by captivity-induced stress in hatchery-produced greater amberjack than in wild-caught breeders.

(Continued on next page)

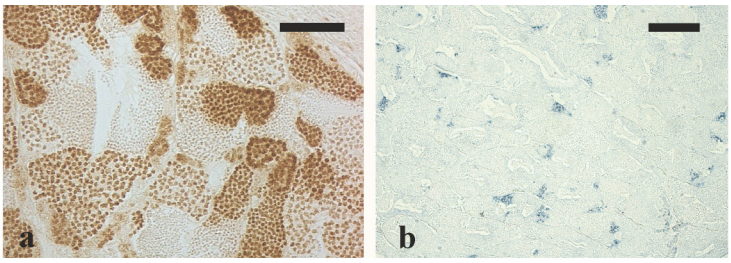


Fig. 1. Micrographs from greater amberjack testis sampled during early spermatogenesis. (a) Testis section immunostained with anti-PCNA antibodies showing the nucleus of proliferating cells stained in brown. (b) Testis section stained with the TUNEL method showing apoptotic cells labelled in dark blue. Magnification bar = 150 μm.

Financial grant provided by the European Union´s Programmes FP7 (GA 603121, DIVERSIFY) and H2020 (GA 862658, NewTechAqua).

References

Zohar, Y., Mylonas, C. C. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture, 197: 99–136.

Zupa, R., Fauvel, C., Mylonas, C. C., Pousis, C., Santamaria, N., Papadaki, M., Fakriadis, I., Cicirelli, V., Mangano, S., Passantino, L., Lacalandra, G. M., Corriero, A. 2017a. Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack *Seriola dumerili* (Risso, 1810). Journal of Animal Science, 95: 4085-4100.

Zupa, R., Rodríguez, C., Mylonas, C. C., Rosenfeld, H., Fakriadis, I., Papadaki, M., Pérez, J. A., Pousis, C., Basilone, G., Corriero, A. 2017b. Comparative study of reproductive development in wild and captive-reared greater amberjack *Seriola dumerili* (Risso,1810). PLoS ONE 12(1): e0169645.

ADDENDUM

DOES HATCHERY ENVIRONMENT AFFECT SKIN AND BONE DEVELOPMENT IN ATLANTIC SALMON?

J. Kolarevic*[1,2], C. Karlsen[1], E. Ytteborg[1], Krasnov A[1], Gerwins J[1], Johnsen, H[1,3], Robinson N[1,4]

[1]Nofima AS, Muninbakken 9-13, Breivika, Tromsø, Norway
[2]The Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics UiT The Arctic University of Norway, N-9037, Tromsø, Norway
[3]Norwegian Polar Institute, Fram Centre, N-9296 Tromsø, Norway
[4]Sustainable Aquaculture Laboratory- Temperate and Tropical (SALTT), School of BioSciences The University of Melbourne, Parkville 3010, Australia
E-mail: jelena.kolarevic@uit.no

Introduction

Production of Atlantic salmon in recirculating aquaculture systems (RAS) is increasing worldwide. RAS provides better environmental control and higher biosecurity compared to traditional flow-through systems (FT). However, RAS is a much more complex system in which metabolites, organic material, hormones, metals, and other elements can accumulate (Davidson et al., 2009; Good et al., 2014; Schumann and Brinker, 2020). In addition to this, salmon is sharing the RAS environment with a dynamic microbial community dependent on physiochemical water properties and nutrient availability (Fossmark et al., 2020). RAS can be used for production of all life stages; however, we know very little about how this environment affects early salmon development. In this study we aimed to assess the effect of hatchery environment (RAS vs. FT system) on early development of salmon skin and bones.

Methods

The experiment was performed at the Nofima Centre for Recirculation in Aquaculture (NCRA), Sunndalsøra, Norway. The experimental trial was approved by the Norwegian Animal Research Authority. Total of 7200 eggs (AquaGen Atlantic QTL-innOva SHIELD) were incubated at 7.8 ◦C and transported to NCRA at 322 degree days (◦d). Eggs were split and stocked into 12 hatchery trays, each with 600 eggs, and continued with incubation on either FT (9 hatchery trays) or RAS (3 hatchery trays) at a mean temperature of 7.7 ◦C. To examine the effect of biologically active water, we chose to use a bioreactor as a part of RAS water treatment. The same freshwater from ground wells was used in the FT system and in RAS. Detailed description of system design and operation is provided in Robinson et al, 2021. Biological sampling was done at three time points prior to start feeding: at 650◦d, 807◦d and 875◦d. Fish from each tank were randomly netted and euthanized (MS-222; FINQUEL®vet, ScanAqua AS, Årnes, Norway) at every time point. Fish were added to RNAlater (Invitrogen) and stored at -20◦C until RNA extraction or fixed in 10% buffered formalin (CellStor™ pots, CellPath) and stored at 4◦C. Length and weight were measured at 875◦d. In total, 40 alevins per hatching tray were weighed, and the length of 9 alevins from each hatching tray was measured. Following analyses were done: histology, micro-CT scanning, RNAseq, differential gene expression analysis and gene ontology enrichment analysis. Detailed description of methods used is provided in Robinson et al., 2021.

Results and discussion

The use of two different production systems affected the expression of genes in pathways related to skin and skeletal development. Genes known to be involved in bone development and mineralisation were significantly up-regulated (inner ear specific collagen, transforming growth factor-beta-inducible early growth response protein 3 and salmon calcitonin) or down-regulated (metalloproteinase inhibitor 2-like, 25-hydroxyvitamin D3 1-alpha-hydroxylase and elongation of very long chain fatty acids protein 7-like) with RAS relative to FT treatment. Genes known to affect skin development, including keratin type I cytoskeletal 13-like and angiopoietin-related protein 7, were down-regulated in RAS compared to FT treated fish. The image analysis revealed less mineralisation of the skeleton (Fig. 1), thinner skin and more pronounced mucous gland development toward the skin surface in fish developing in the RAS.

How environmental factors, or the interaction between abiotic and biotic components in RAS and FT systems influence the development of skin and mineralisation of the skeleton, remain unclear. Further research will be needed to determine potential causes for observations documented in this study.

(Continued on next page)

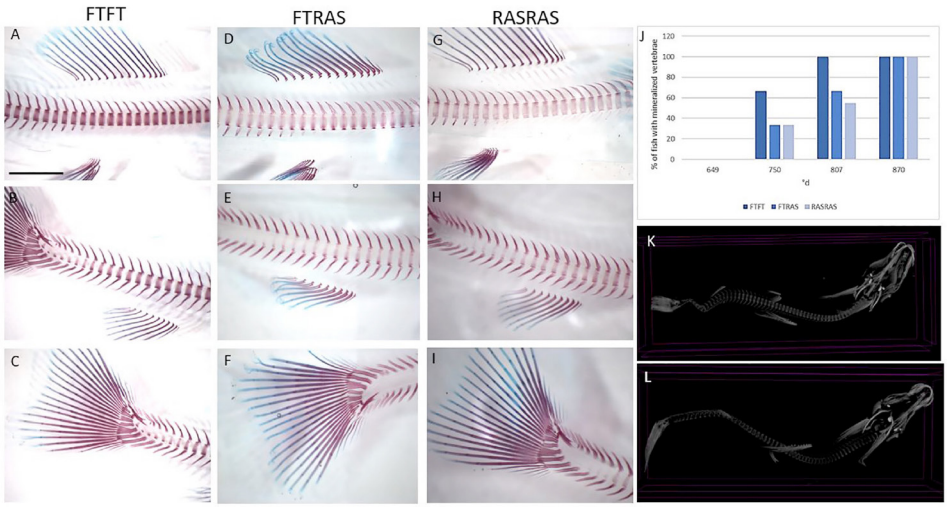


Fig. 1. Wholemount Alizarin red S/Toluidine blue staining of fish at 807◦d from FTFT (A-C), FTRAS (D–F) and RASRAS (G-I). Percentage of fish staining Alizarin red S positive in the vertebrae at different developmental stages (J). CT-scanning of FTFT (K) and RASRAS (L) fish at 807 ◦d, with intensity showing the degree of mineralisation of the vertebrae. n = 9 per treatment. Scale bar = 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of Robinson et al., 2021)

Acknowledgements

This work was a part of the strategic institute project "Farmed Animals Welfare Toolbox (FARMWELL)" supported by The Norwegian Research Council, grant number 194050.

References

Davidson, J., Good, C., Welsh, C., Brazil, B., Summerfelt, S., 2009. Heavy metal and waste metabolite accumulation and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. Aquacult. Eng. 41 (2), 136–145.

Good, C., Davidson, J., Earley, R.L., Lee, E., Summerfelt, S., 2014. The impact of water exchange rate and biofiltration on water-borne hormones in recirculation aquaculture systems containing sexually maturing Atlantic salmon Salmo salar.J. Aquacult. Res. Dev. 5 (5), 1–7.

Fossmark, R. O., Vadstein, O., Rosten, T.W., Bakke, I., Koseto, D., Bugten, A.V., Helberg, G., Nedberg, A., Nesje, J., Jørgensen, N.O.G., Raspati, G. S., Azrague, K., Østerhus, S. W., Attramadal, K. 2020. Effects of reduced organic matter loading through membrane filtration on the microbial community dynamics in recirculating aquaculture systems (RAS) with Atlantic salmon parr (Salmo salar). Aquaculture, 524, https://doi.org/10.1016/j.aquaculture.2020.735268

Schumann, M. and Brinker, A. 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Reviews in Aquaculture 12, 2109-2139.

Robinson, N., Karlsen, C., Ytteborg, E., Krasnov, A., Gerwins, J., Johnsen, H., Kolarevic, J. 2021. Skin and bone development in Atlantic salmon (Salmo salar) influenced by hatchery environment. Aquaculture, 544, https://doi.org/10.1016/j.aquaculture.2021.737155

EUROPEAN SEA BASS *(Dicentrarchus labrax)* FED ANIMAL FATS: FILLET QUALITY BASED ON FATTY ACID PROFILE, COLOUR AND TEXTURE

A. Marques[1*], E. Matos[2], T. Aires[3], M.B.P.P. Oliveira[4], L.M.P. Valente[1,5]

[1] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeirosdo Porto de Leixões, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[2] B2E, Associação para a Bioeconomia Azul – Laboratório Colaborativo, Avenida da Liberdade S/N, 4450-718 Leçada Palmeira, Portugal
[3] SORGAL, Sociedade de Óleos e Rações, S.A., Estrada Nacional 109, Lugar da Pardala, 3880-728 S. João de Ovar,Portugal
[4] REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de JorgeViterbo Ferreira 228, 4050-313 Porto, Portugal
[5] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228,4050-313 Porto, Portugal

*Presenting author: amarques@ciimar.up.pt

Introduction

The diets for carnivorous fish species like European sea bass *(Dicentrarchus labrax)* are still heavily dependent on fish oil (FO) as main source of essential fatty acids (FA), such as eicosapentenoic (EPA; 22:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids. But such high levels of FO used by the expanding aquaculture industry have led to major economic and environmental concerns, due to the lack of sustainability of this limited marine resource, making the use of alternative sources imperative. Up to now,the use of animal fats (AF) has still been poorly explored in Europe, but previous studies in European sea bass have shown that their incorporation into aquafeeds could be a sustainable alternative to the conventional FO (Monteiro et al., 2018; Campos et al., 2019). However, since these fats present low levels of EPA and DHA, their inclusion levels should be carefully assessed in order to produce feeds capable of meeting the fish dietary requirements while preserving the fillet nutritional value. In this context, the maingoal of this work was to assess the effect of two distinct AF, poultry fat (PF) and mammal fat (MF), with or without an emulsifier, on European sea bass growth performance and fillet quality.

Materials and methods

Four extruded isoproteic (45%) and isolipidic (21%) diets were formulated. The lipid fraction of all diets comprised 55% FO and either 45% PF or 45% MF. For each experimental diet, containing each of the aforementioned fat types, an emulsifier was added at 0.01%, resulting in the following experimental diets: FOPF (PF without emulsifier), FOPFe (PF with emulsifier), FOMF (MF without emulsifier) and FOMFe (MF with emulsifier). A growth trial was carried out with European sea bass (initial body weight of 255.7 ± 15.2g), in an open system aquaculture facility, for 12 weeks. At the end of the trial, growth performance,feed efficiency, whole body composition, fillet FA profile, instrumental texture and colour were evaluated.

Results

All diets were equally well-accepted by European sea bass and fish reached a final body weight between 380-417g, without significant differences in daily growth index or feed efficiency among groups. The inclusion of the emulsifier led to increased whole body lipid and energy contents, regardless of the type offat, but retention and nutrient gain remained unaffected by the dietary treatments. EPA and DHA retentionand gain were not significantly affected by the type of fat or the use of emulsifier, though there was a trendtowards increased gain of these fatty acids in fish fed mammal fat diets.

The dietary fat source did not affect significantly muscle total SFA, total MUFA and n-3/n-6 PUFA ratio. In fish fed diets including MF, muscle lipid content reached the highest levels (Fig.1A), whilst total PUFA,namely n-6 PUFA, were the lowest; The use of MF in the diets resulted in a significantly higher amount ofEPA and DHA in the muscle (0.26-0.27 g/100g fillet), when compared to the diets comprising PF (0.21-0.24 g/100g fillet) (Fig.1B). Fish fed diets with MF without emulsifier also demonstrated an increase of

(Continued on next page)

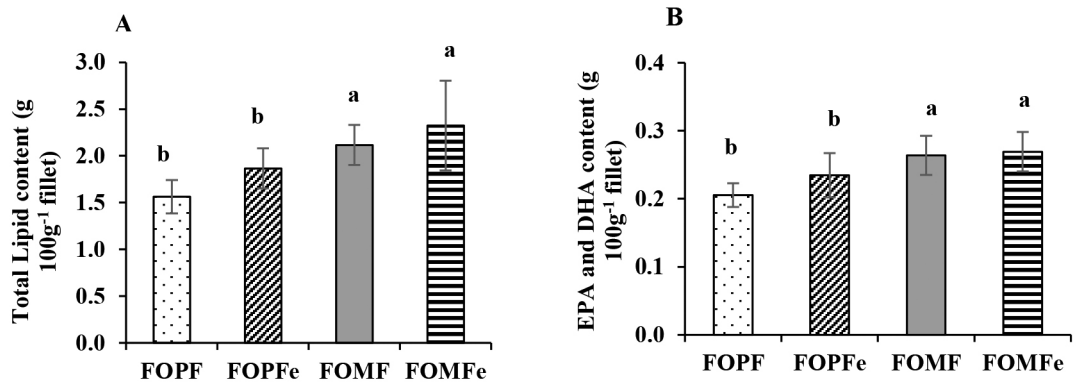


Fig. 1. Total lipid content (A) and muscle EPA and DHA (B) of European sea bass fed the experimental diets for 12 weeks

Discussion and Conclusion

The present study shows that is equally feasible to include poultry fat or mammal fat in diets for Europeansea bass without compromising fish growth performance or feed utilisation. Although diets did not affect muscle SFA and MUFA content, total PUFA were lowest in fish fed MF, suggesting a fillet that will be less subject to rancidity, since PUFAs are more prone to lipid peroxidation. European sea bass fed with MFproduced fillets with a higher lipid content, and had also a tendency to retain higher levels of EPA + DHA.This resulted in muscle EPA + DHA values above those recommended by WHO for human consumption (> 0.25g $100g^{-1}$), but the same could not be observed in fish fed with PF.

Overall, this study suggests that land mammal fat should be considered a sustainable lipid source for European sea bass diets, contributing to a circular economy concept, and diminishing the carbon footprintof the aquafeed sector, since this ingredient can be obtained by rendering locally produced by-products.

Acknowledgements

This work was supported by the Project ANIMAL4AQUA, funded by Portugal 2020, financed by the European Regional Development Fund (FEDER) through the Operational Competitiveness Program(COMPETE) - POCI-01-0247-FEDER-017610 and by FCT – Foundation for Science and Technology toCIIMAR (UIDB/04423/2020, UIDP/04423/2020).

References

Campos, I., Matos, E., Maia, M.R.G., Marques, A., Valente, L.M.P., 2019. Partial and total replacement offish oil by poultry fat in diets for European seabass *(Dicentrarchus labrax)* juveniles: Effects on nutrient utilization, growth performance, tissue composition and lipid metabolism. Aquaculture. 502, 107-120.

Monteiro, M., Matos, E., Ramos, R., Campos, I., Valente, L.M.P., 2018. A blend of land animal fats can replace up to 75% fish oil without affecting growth and nutrient utilization of European seabass. Aquaculture. 487, 22-31.

SEX DIFFERENTIATION AND EARLY SEX IDENTIFICATION OF GREATER AMBERJACK *Seriola dumerili* REARED IN SEA CAGES

Maria Papadaki[1,2,*], Manolis Mandalakis[1], Thekla I. Anastasiou[1], Marina Pouli[2], Michalis Asderis[1], Pantelis Katharios[1], Nikos Papandroulakis[1], and Constantinos C. Mylonas[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research
P.O. Box 2214, Iraklion, Crete 71003, Greece
mpapadak@hcmr.gr
[2]University of Crete, Department of Biology, P.O. Box 2208, Heraklion 71409, Crete, Greece

Introduction

The greater amberjack *Seriola dumerili* is a fast-growing fish, for which commercial production has been recently achieved in the Mediterranean. It is a gonochoristic species, that completes sex differentiation at the end of the first year of life (Marino et al., 1995). The study of its sex differentiation process during on-growing in sea cages is essential, in order to investigate for possible sex-related growth differences and eliminate the possibility that the rearing method would have an effect on the final sex ratio of the populations. Moreover, the study of the plasma levels of sex steroids and corticosteroids can help reveal the role of these hormones in the process of sex differentiation. Differences in the hormonal levels between sexes can be used for sex identification in greater amberjack before puberty or during the non-reproductive period, in order to ensure optimal broodstock sex ratios and facilitate selective breeding programmes.

Materials and Methods

Fish used in the present study were produced from eggs obtained after spawning induction of wild-caught breeders with gonadotropin releasing hormone agonist (GnRHa) implants (Fakriadis et al., 2020). Eggs were transferred to the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (Hellenic Center for Marine Research, HCMR) and reared until 50 days post-hatching (dph). At 50 dph, fish were moved to the pilot sea cages of HCMR at Souda Bay, Chania, Crete, Greece, where they were maintained until the end of the experiment.

Seven samplings were conducted from 101 until 408 dph (n=17-23), during which total length (TL) and wet weight (WW) were measured, and gonads and blood were collected, the former for histological analysis and the latter for the measurement of sex steroid (adrenosterone (Ad), androstenedione (Δ4), 11-ketotestosterone (11-KT), testosterone (T), estradiol (E_2), progesterone (P4) and 17,20β-dihydroxyprogesterone (DHP)) and corticosteroid (cortisol and cortisone) plasma levels with the use of liquid chromatography/mass spectrometry (LC-MS/MS).

Results

Growth of greater amberjack was similar for both sexes and females had the same size as males at all samplings. Moreover, the sex ratio in all the samplings was 1:1.

As regards the histological description of sex differentiation, in females at 101 dph (14.98 ± 6.20 cm TL), the ovarian cavity was already formed, and germ cells were visible around it. At 198 dph (25.8 ± 0.14 cm TL) the first primary oocytes were visible. Complete ovary differentiation was achieved at 408 dph (41.22 ± 3.83 cm TL). In males, at 101 dph the gonads contained mostly somatic cells and connective tissue and no germ cells were present (14.47 ± 6.60 cm TL). The first germ cells were apparent at 150 dph, when spermatocytes could be found in the gonads (24.71 ± 3.09 cm TL). The typical testicular structure featuring all types of male germ cells, was observed at 260 dph (28.63 ± 2.85 cm TL). This structure was obviously maintained until the last sampling (357 and 408 dph, 34.25 ± 2.98 cm and 41.7 ± 1.99 cm TL, respectively).

Of the nine measured hormones in greater amberjack females and males during the sex differentiation process, the ones that exhibited statistically significant changes in time were cortisol, Ad, Δ4, 11-KT, T and P4 in females and Ad, 11-KT, T, P4 and DHP in males. Cortisol and Ad exhibited lower values as the sex differentiation period was progressing in females; on the other hand, Ad, 11-KT, T and P4 in both sexes and DHP in males presented higher values at the end of the sex differentiation period.

The 11-KT/E_2 ratio was significantly different between the sexes from the second sampling at 150 dph until the last sampling at 408 dph (Figure 1).

(Continued on next page)

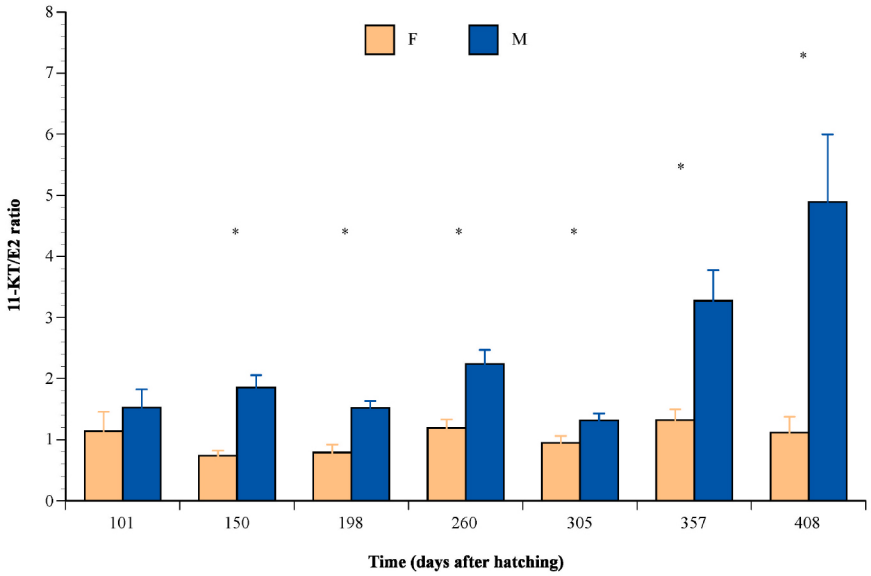


Figure 1. Evolution of the 11-ketotestosterone (11-KT)/estradiol (E2) ratio in hatchery produced greater amberjack in relation to time. Differences in the ratio between males and females are indicated with an asterisk (one-way ANOVA, Tukey HSD, $P < 0.05$)

Discussion

The absence of sex-related growth dimorphism and the constant 1:1 sex ratio in greater amberjack populations reared in sea cages suggest that both sexes can be equally preferable in aquaculture production and that there is no effect of the rearing method on the sex differentiation of the species. No sex ratio fluctuations are to be expected in cage-reared populations. Moreover, using LCMS/MS, nine sex- and cortico-steroid hormones of the cholesterol metabolism pathway can be detected, enabling the study of the biochemical pathway involved in the teleost sex differentiation process. Sex identification was possible in juvenile fish from 150 dph, with the use of the 11-KT/E_2 ratio.

References

Fakriadis, I., Miccoli, A., Karapanagiotis, S., Tsele, N., Mylonas, C.C., 2020. Optimization of a GnRHa treatment for spawning commercially reared greater amberjack *Seriola dumerili*: Dose response and extent of the reproductive season. Aquaculture 521, 735011.

Marino, G., Mandich, A., Massari, A., Andaloro, F., Porrello, S., 1995. Aspects of reproductive biology of the Mediterranean amberjack (*Seriola dumerilii* Risso) during the spawning period. Journal of Applied Ichthyology 11, 9-24.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement n° 862658 (NEWTECHAQUA).