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TABLE OF CONTENTS

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

aquaculture europe 20

ABSTRACTS

BREEDING STRATEGY INFLUENCES THE BACTERIAL COMMUNITIES OF FEMALE NILE TILAPIA (*Oreochromis niloticus*) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

Yousri Abdelhafiz[1*], Jorge M. O. Fernandes[1], Simone Larger[2], Davide Albanese[2], Omid Jafari[1,3], Artem V Nedolizhko[1], Claudio Donati[2], Viswanath Kiron [1]

[1] Faculty of Biosciences and Aquaculture, Nord University, Norway
[2]Unit of Computational Biology, Fondazione Edmund Mach, Italy
[3] International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization, Rasht, Iran

Presenting author: Yousri Abdelhafiz
Email: yousri.abdelhafiz@nord.no

Introduction

Livestock breeding started a long time ago by focusing on desired phenotypic traits. New technologies have endowed mankind with better understanding of genetics, and this knowledge has enabled us to improve breeding practices and produce high-quality offspring of farmed animals. Nevertheless, fish breeding has not kept pace with the breeding of other farmed animals.

Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species and by far the most farmed tropical fish. Here, we report for the first time the differences in mouth and intestine bacterial communities of female Nile tilapia that were produced through inbreeding and outbreeding.

Materials and methodologies

Eggs that were obtained from female wild-caught Nile tilapia were transported from Egypt to the Research Station of Nord University, Norway. They were then hatched and reared in a recirculating aquaculture system. Thereafter, the inbred and outbred study groups were produced by breeding fish from the same family and different families, respectively. Sample collection was performed based on the guidelines for research using experimental animals, suggested by the Norwegian Animal Research Authority.

In the present study, we employed whole genome sequencing technique to analyse the genetic structure; to understand the genetic differences between the inbred and outbred groups. Moreover, we used a 16S rRNA gene sequencing technique to analyse the microbial community composition in the mouth and intestine of female Nile tilapia.

Results

Analysis of single nucleotide polymorphisms (SNPs; 6825083) obtained from whole-genome shotgun sequencing uncovered the genetic similarity of the inbred and outbred groups. On the other hand, sequences of the 16S rRNA genes revealed the differences in the diversities of the bacterial communities of the inbred and outbred groups. Inter-individual variability and the plausible presence of beneficial and opportunistic bacteria in the intestine of the inbred and outbred groups, respectively were also evident. Taken together, through selective breeding we can manipulate the composition of the oral and intestine microbial community in Nile tilapia. The less variation in the intestine microbiome of the inbred group could be exploited for controlled studies that examine the maternal transfer of microbiome to offspring.

Conclusion

We report for the first time the effect of inbreeding and outbreeding on the mouth and gut microbiome in Nile tilapia. Our findings suggest that the breeding strategy can shift Nile tilapia bacterial communities.

Acknowledgement

This study has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme [grant 683210] and from the Research Council of Norway under the Toppforsk programme [grant 250548/F20].

PERFORMANCE EVALUATION OF BIO-INTEGRATED FOOD PRODUCTION SYSTEM - AQUAPONICS WITH ORNAMENTAL FISH, ORNAMENTAL AQUATIC PLANTS AND LEAFY VEGETABLES IN FLOATING NET CAGES

Rajan Abisha*, Kiran Dube, S P Shukla, Paromita B, Sawant, M H Chandrakant, Jacob Jane, K D Raju

ICAR- Central Institute of Fisheries Education, Mumbai, India
Email: abisha.aqcpa907@cife.edu.in

Introduction

The world population is rising and the need to satisfy the growing demand is gaining focus. Agriculture and aquaculture play a major role in satisfying the demand. On the other hand, there are underutilized resources to be brought to light and make a utilized one. India is endowed with 19,370 reservoirs, spreading over 15 states with an estimated area of 3.15 million ha (Kumar *et al*., 2015). Strategies to enhance their utilization are diverse. One of the strategies is cage culture which not only provides animal protein but also bestows farmers with income utilizing the same area and space without any ecological effect. Integrated Agriculture Aquaculture System (IAAS), a form of sustainable intensification is perceived as effective utilization of water, which increases water productivity and reduces risks associated with water scarcity (Godfray *et al*., 2010). Increasing water productivity by IAAS is a key strategy for achieving food security and could assume a significant job and key role in feeding the world (Ahmed *et al*., 2014). Different forms of IAAS exist such as FIMTA (Freshwater Integrated Multi-Trophic Aquaculture) and aquaponics, nonetheless, both anticipate a similar idea in aquaculture. Aquaponics, a combination of conventional aquaculture and hydroponics, through a microbial link with a symbiotic relationship, has been gaining interest recently as it represents a valuable option to overcome the food needs of the overwhelming population. Thus, integrating the agriculture aquaculture system as a prototype of aquaponics in cages might be a well-suited one providing food security and improving the economic status of the farmers.

Materials and Methods

The present study was undertaken integrating ornamental fish, aquatic plants, and leafy vegetables in floating net cages to determine its efficiency in the open water resource. The experiment was conducted in floating net cages in Dimbhe reservoir, Pune District, Maharashtra, India. The experimental period was 90 days following a Completely Randomized Design (CRD). The components used in the study comprise angelfish, *Pterophyllum scalare*, aquatic ornamental plant *Ceratophyllum demersum,* and leafy vegetable *Spinacia oleracea*. Angelfish and ornamental aquatic plant *Ceratophyllum demersum* were stocked inside the floating net cages of size 3mx3mx3m, while leafy vegetable, *Spinacia oleracea* were grown on rafts floating on the surface of cages. Three treatments with angelfish of different stocking densities 20, 25, 30/m3 along with uniform stocking densities of *Ceratophyllum* and spinach of 20 bundles/ cage and 144 numbers/cage respectively and two controls, one with only *Pterophyllum scalare* and the other with *Ceratophyllum* and spinach were used to compare the efficiency of the system. The initial size of angelfish was 1.02±0.07cm and 0.24±0.06g; *Ceratophyllum* was 414.17±5.12 g/cage and spinach was 8.69±0.52 cm height.

Results

At the end of the experiment, the average length and average weight of angelfish were higher in T3 with 4.05±0.03cm and 1.60±0.09g respectively. The SGR, FCR, FCE, and survival rate was higher in T3 with 2.11±0.01%/day, 3.15±0.01, 0.32±0.001, and 87.04±0.98% respectively. The aquatic plant achieved higher total biomass of 1038.33±8.35g but there was no significant difference ($p>0.05$) among the treatments and control. Similarly, the height gain percentage of spinach achieved was 66.91±2.56% and showed no significant difference ($p>0.05$) between the treatments and control. The physicochemical parameters of water were also within the optimal range. Apart from growth performance, the physiological parameters like digestive enzymes (amylase, protease, and lipase) and stress enzymes (SOD and catalase) were analyzed. The obtained results provide no significant difference ($p>0.05$) between the treatments and control.

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Discussion

Thus, the present study indicates that the integration of ornamental fish, aquatic plants, and leafy vegetables could be an efficient way of improving food security as well as the livelihood of the people. This integration improves the growth and health status of fish effectively without any stress compared to aquaria reared fishes and produces more crops by utilizing the same area without any ecological effects. Petrea *et al*., (2013) stated that one of the reasons for integrating different components is obtaining an extra profit from a second crop culture (plants). This bio-integrated food production system contributes not only to the food production system but also to rural development by developing their social and economic status. It is also suggested that adopting a bio-integrated food production system would effectively utilize the resource and provides income to the farmers.

References

Kumar Vikash, Gunjan Karnatak, Mishal P., A.K. Das, M.A.Hassan, A.P.Sharma.,2015. Potential species for Cage Aquaculture in Indian Reservoirs. World Aquaculture.pp 46-48.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. Science, 327, 812-818.

Ahmed, N., Ward, J.D. and Saint, C.P., 2014. Can integrated aquaculture-agriculture (IAA) produce "more crop per drop"?. *Food security*, *6*(6), pp.767-779.

Petrea, Ş.M., Cristea, V., Dediu, L., Contoman, M., Lupoae, P. and Mocanu, M., 2013. Vegetable Production in an Integrated Aquaponic System with Rainbow Trout and Spinach. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Animal Science & Biotechnologies*, *70*(1).

BURBOT REPRODUCTION IN CAPTIVITY: OUT-OF-SEASON VERSUS NORMAL SPAWNING USING DIFFERENT COMMERCIAL FEEDS

Jurgen Adriaen[1], Joachim Claeyé[1], Heidi Arnouts[1], Gilbert Van Stappen[2]
Aqua-ERF, University College Odisee, Sint-Niklaas (Belgium)
Artemia Reference Center, University Ghent, (Belgium)

E-mail: jurgen.adriaen@odisee.be

Introduction

Some commercial activities on burbot culture in Belgium redirected our research to burbot reproduction to obtain more stable and periodic production of burbot larvae around the year. The possibility for a farmer to obtain burbot fingerlings at different moments in the year results in a more cost-efficient use of their grow out installations. Maybe more important than quantity is the quality of the burbot larvae. Different studies have shown that nutrition has a very important role in the quality of the fertilization, embryonal and larval development (Izquierdo et al. 2001). As burbot is an upcoming aquaculture species a specific broodstock feed for this fish is not available for which we tested three different commercial broodstock feeds for their effect on reproduction parameters.

Materials and methods

Burbot broodstock (larval batch 2016 obtained at Bezirk Niederbayern) raised in RAS, that were used in an previous spawning trial (februari 2019), were divided in two groups mid of July 2019. One group (Regular) was stocked in cooling chambers with temperature and light regime following the regular temperature and light pattern. The other group (Out of Season) was stocked in a cooling chamber that followed a temperature and light regime in which the declination was faster aiming to forward ovulation period with three months. Figure 1 shows the temperature graph.

Distribution of individual tagged fish at stocking resulted in a male:female ratio of 5:4 in the tank with an average bodyweight of 634.8±189.9gram for males and 742.0±223.9gram for females at the start of the trial.

Each cooling chamber holds three fish tanks with their individual biofilter giving us the opportunity to test three different commercial feeds per cooling chamber. The broodstock feeds were specific for Mediterranean marine fish (Feed A), sturgeon (feed B) and freshwater RAS fish (feed C). The most remarkable difference between the feeds is the protein level being 50.5%, 52% and 46.5% respectively for feed A, B and C. Feed A contained a higher amount of omega-3 fatty acids while the other feeds had a higher content of linoleic acid.

Cathetering of the females was repeated every week, starting at the moment of the temperature drop (1.4°C), till oocyte showed to be ready for spawning. Females were then manually stripped to obtain the eggs. Obtained eggs were weighted and a working fecundity was calculated based on following formula: (Weight of eggs/Weight of fish) x100. Samples of the collected eggs were stocked in a -20°C freezer for total lipid and FAME-analyses. Males were checked every week manually if they were given sperm.

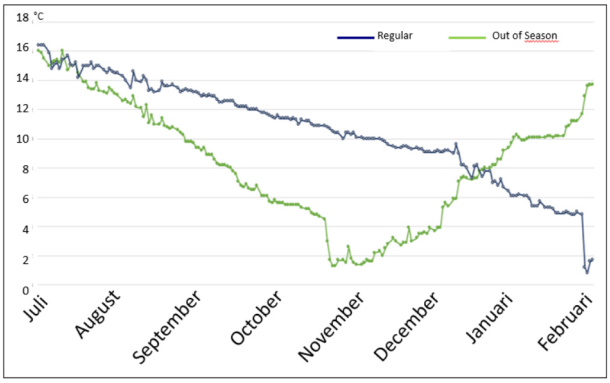

Figure 1: Temperature regime

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Results and Discussion

Out of season: Neither males nor females produced gametes in November 2019. There was no observation of oocyte going into final oocyte maturation stage. As for the regular season (Feburari 2020) we obtained gametes from 60% of the females and 71% of the males. Being this the first try to spawn this fish after the accelerated acclimation, the period between the previous spawning period (February 2019) and the latest (November 2019) was only nine months and maybe too short for optimal gonadal development. In a following attempt for out of season spawning (November 2020) these fish will not have followed an accelerated cycle, but a normal year, which could lead to normal spawning.

Feeds: Ovulation was higher for females given feed B and C (63%) than for those given feed A (50%) with fecundity being higher for feed C (19.8%). FAME-analyses showed that the fatty acid composition of the feeds is not reflected in the eggs. Although feed A had higher omega-3 levels than the other feeds, this difference is much smaller between the eggs of the different treatments. Differences found in the results among the treatments were not significant. Due to mortality and spontaneous spawning, the number of replica were too low for strong statistical analyse for which no conclusion can be drawn towards the effect of the commercial feeds on egg quality.

References

*Izquierdo M.S., Fernandez-Palcios H., Tacon A.G.J. (2001) “Effect of broodstock nutrition on reproductive performance of fish”, Aquaculture 197: 24-42

EFFECT OF SILK NANOPARTICLES ON GILTHEAD SEABREAM (*Sparus aurata*) SKIN IMMUNITY AND HEALING

N. Albaladejo-Riad[1]*, C. Espinosa[1], G. Carissimi[2], F.G. Díaz Baños[2], G. Víllora[2] & M.A. Esteban[1]

[1]Immunobiology for aquaculture group. Department of Cell Biology and Histology. Faculty of Biology, University of Murcia, 30100 Murcia, Spain
[2]Department of Physical Chemistry, Faculty of Chemistry, University of Murcia, Murcia, Spain
E-mail: nora.albaladejo@um.es

Introduction

The silkworm *Bombyx mori* has been exploited for centuries throughout the world due to its numerous applications, ranging from cooking, industry, home textiles (Janani et al., 2019), to medicine (Holland et al., 2019). Fibroin from mulberry silkworm silk exhibits high biocompatibility and biodegradability, as well as low immunogenicity (Chouhan & Mandal, 2020). Due to these properties, fibroin been used in tissue regeneration engineering, with great results (Kamalathevan et al., 2018; Lee et al., 2016).

Thus, the aim of this work is to study the effects of dietary silk nanoparticles on skin wound healing. The current aquaculture carries out systems of intensive culture, in which there is a great overcrowding of the specimens, in order to generate a greater control and yield of the cultures (Xiao & Zhang, 2020)"type":"article-journal","volume":"615"},"uris":["http://www.mendeley.com/documents/?uuid=82222b92-6772-43dc-9866-a666a9a09497"]}],"mendeley":{"formattedCitation":"(Xiao & Zhang, 2020. This overpopulation present in marine cages leads not only to different stressor situations, such as overcrowding and starvation but also to the formation of skin wounds (Vijayan & Leatherland, 1988). When the fish's skin loses its integrity, the animals are more vulnerable to the great number of microorganisms present in the aquatic environment. In this situation, the use of natural substances able of accelerating or improving skin wound healing is mandatory. In the present study, the silk nanoparticles were used to know its effects of fish skin and gilthead seabream (*Sparus aurata*) was used as a fish model, due to its great importance in the Mediterranean.

Material & Methods

Thirty-six gilthead seabream were randomly distributed in 6 seawater aquaria (250 L, flow rate 900 L h^{-1}, 28 ‰ salinity, 20ºC, and photoperiod 12:12). After the acclimation period, fish in two aquaria fed one of the following experimental diets: 0 (control) 50 (SN1) and 100 (SN2) mg of silk nanoparticles per kg of feed, at a rate of 1.5% of its weight/day. After 30 days of feeding, 6 fish from each experimental group were sedated (MS222) and sampled. The remaining fish were then wounded on the right side, below the lateral line, with an 8 mm diameter punch. The fish were photographed and returned to their respective tanks to continue feeding for a further 7 days (7-d healing group). Afterwards, all the fish were sampled, and the wounds were photographed again. All sampled fish were sacrificed (MS222 100 mg L^{-1}). Skin mucus, and skin samples were collected (in wounded fish the skin surrounding the wound was sampled) (Chen et al., 2020). Wound images were analysed to determine wound perimeter, area or roundness. Peroxidase, protease, antiprotease and immunoglobulin M activities were determined in skin mucus. Some skin samples were included in paraplast and processed for light microscopy study while others were used for gene expression by PCRrt. The expression of three antioxidant genes [superoxide dismutase 1 (*sod),* catalase *(cat)* & glutathione-disulfide reductase *(gsr*)], seven genes involved in inflammation [transforming growth factor beta (*tgf-β1),* prostaglandin-endoperoxide synthase 2 *(ptgs2),* tumor necrosis factor alpha *(tnfα),* interleukin 8 *(il-8),* interleukin 10 *(il-10),* interleukin 1 beta *(il-1β),* myeloperoxidase *(mpo)* & colony stimulating factor 1 receptor (*csf1r*)] and eight genes involved in tissue regeneration [insulin like growth factor 1 (*igf-1),* proliferating cell nuclear antigen *(pcna*), collagen type X alpha 1 chain *(colXα),* matrix metallopeptidase 9 *(mmp9),* keratin 2 *(krt2),* sonic hedgehog signaling molecule *(shh),* cellular communication network factor 1 *(ccn1),* fibronectin 1 alpha *(fn1α)* & collagen type I alpha 1 chain (*col1α*)] was determined.

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Results and discussion

When carrying out the photographic analysis of the wounds, it was observed that there was a greater decrease in the area and perimeter of the wounds in the SN1 group, in addition to the fact that the wounds of these fish heal more irregularly.

Regarding the results of the immune activities measured in mucus, only the protease activity shows statistically significant differences according to diet, so that it decreases in the SN1 group. No significant variations were detected in the gene expression among fish fed the different experimental diets for one month. However, fish fed SN2 diet and sampled at 7 days post-wounded had up-regulated the expression of *il-10* (cytokine with potent anti-inflammatory properties) and *krt2, pcna,* and *fn1α* (proteins involved in tissue regeneration process) in the skin, respect to the data recorded in skin from fish fed control diet (without nanoparticles). The addition of 100 (SN2) mg of silk nanoparticles per kg of diet improved the healing process of gilthead seabream skin.

Acknowledgments

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References

Chen, Z., Ceballos-Francisco, D., Guardiola, F.A. & Esteban, M.A. (2020). Dietary administration of the probiotic *Shewanella putrefaciens* to experimentally wounded gilthead seabream (*Sparus aurata* L.) facilitates the skin wound healing. Scientific Reports 10:11029 https://doi.org/10.1038/s41598-020-68024-z

Chouhan, D., & Mandal, B. B. (2020). Silk biomaterials in wound healing and skin regeneration therapeutics: From bench to bedside. *Acta Biomaterialia*, *103*, 24–51. https://doi.org/10.1016/j.actbio.2019.11.050

Holland, C., Numata, K., Rnjak-Kovacina, J., & Seib, F. P. (2019). The Biomedical Use of Silk: Past, Present, Future. *Advanced Healthcare Materials*, *8*(1), 1800465. https://doi.org/10.1002/adhm.201800465

Janani, G., Kumar, M., Chouhan, D., Moses, J. C., Gangrade, A., Bhattacharjee, S., & Mandal, B. B. (2019). Insight into Silk-Based Biomaterials: From Physicochemical Attributes to Recent Biomedical Applications. *ACS Applied Bio Materials*, *2*(12), 5460–5491. https://doi.org/10.1021/acsabm.9b00576

Kamalathevan, P., Ooi, P. S., & Loo, Y. L. (2018). Silk-Based Biomaterials in Cutaneous Wound Healing: A Systematic Review. *Advances in Skin & Wound Care*, *31*(12), 565–573.

Lee, O. J., Kim, J.-H., Moon, B. M., Chao, J. R., Yoon, J., Ju, H. W., Lee, J. M., Park, H. J., Kim, D. W., Kim, S. J., Park, H. S., & Park, C. H. (2016). Fabrication and characterization of hydrocolloid dressing with silk fibroin nanoparticles for wound healing. *Tissue Engineering and Regenerative Medicine*, *13*(3), 218–226. https://doi.org/10.1007/s13770-016-9058-5

Vijayan, M. M., & Leatherland, J. F. (1988). Effect of stocking density on the growth and stress-response in brook charr, Salvelinus fontinalis. *Aquaculture*, *75*(1–2), 159–170. https://doi.org/10.1016/0044-8486(88)90029-4

Xiao, J., & Zhang, Y. (2020). Marine factory farming techniques and equipment. *IOP Conference Series: Earth and Environmental Science*, *615*, 012013. https://doi.org/10.1088/1755-1315/615/1/012013

DISEASE DYNAMICS AND PARASITIC TRANSMISSION BETWEEN *Cerastoderma edule* AND SHOREBIRDS IN THE IRISH COAST

S. Albuixech-Martí [a,*], S.A. Lynch [a,b], S.C. Culloty [a,b,c]

[a] School of Biological, Earth & Environmental Sciences
[b] Aquaculture & Fisheries Development Centre
[c] MaREI Centre for Climate, Energy and Marine, Environmental Research Institute, University College Cork, The Cooperage, Distillery Fields, North Mall, Cork, Ireland
Email: saraalbuixechmarti@ucc.ie

Introduction

In the marine food web, common cockles *Cerastoderma edule*, hosts of a diverse range of parasites, are an important food source and are a link between primary producers and consumers, playing a crucial role in the ecosystem. One of the main consumers feeding on up to 300 cockles by day are marine bird populations, whose feeding ecology may shape their diverse and multiple endoparasitic fauna, responsible for significant effects on shorebird populations. In turn, birds may play an important role as a reservoir of infection and/or pathogen carrier influencing the pathogen transmission and disease dissemination. However, the trophic transmission of microbial and parasitic infections in shorebirds and their influence on disease dynamics and dissemination are difficult to establish. Therefore, our objective is to assess the differences in the transmission between a number of parasites previously associated to cockles (*Vibrio* spp., Haplosporidia spp., Ostreid herpesvirus type 1, Microsporidia spp.) in the Irish coast. The role of the shorebirds, feeding on cockles, in the parasitic transmission was also investigated. Likewise, the site influence and seasonality as well as the role of the environment in the disease dynamics and its influence in the pathogen persistence was examined.

Material and Methods

Cockles (n=735) from the intertidal were sampled from April/July 2018 to April 2019 at four sites with no commercial fishing activity on the south coast (Celtic Sea) and two sites on the northeast coast (Irish Sea) with an active commercial fishery. Video recording of the bird community on those areas were done to identify the species present and examine their foraging behaviour. Moreover, bird faecal samples (n=204) were collected as near as possible to the cockle collection area, the same number of samples were also taken from the sediment nearby the stool (n=204). Screening of the cockle, faecal and sediment samples by molecular techniques (PCR, qPCR, Sanger sequencing) was carried out.

Results and Discussion

A variety of waders, actively feeding on bivalve molluscs, were identified in the recordings along with mainly several species of gulls and hooded crows, both groups with a wide-ranged diet. According to the bird feeding behaviour observed, *C. edule* DNA was found in over 22% of the bird faecal samples analysed. As expected, cockle DNA was also found in sediment samples (34%), given cockles inhabit the sediment and are bioturbators [1]. Of the various pathogen groups screened for, only vibrios were found both in sediment (63%) and faecal samples (14%), apart from in cockle samples (25%) (Figure 1). Identical strains of *Vibrio splendidus* were found in cockles and bird faecal samples, providing the first evidence of trophic transmission of *V. splendidus* to birds through cockle consumption. *V. splendidus* detection in bird faeces further support the carrier role that migratory birds play in the dissemination of *Vibrio* species [2,3].

The *Vibrio* infection in faecal samples showed spatial variability, with the highest *Vibrio* spp. prevalence and the lowest level of cockle DNA found in the same site, Cuskinny (Celtic Sea). All together it may be indicative of a potential cockle mortality due to highly infected individuals. In sediments samples, however, *Vibrio* spp. prevalence remained fairly high through the sites regardless of the different levels of cockle DNA found, which seems to support the *Vibrio* reservoir role of the sediment [4]. There was a higher incidence of *C. edule* DNA in the faecal and sediment samples from spring and summer, when more screened cockles were found dead or in a poor condition. This is related to the fact that *Vibrio* prevalence also have a peak in summer, due to the higher temperature [4]. Consequently, infected cockles may have been more susceptible and accessible for birds at that time of the year, promoting also its consumption by wide-ranged feeders as gulls and crows, mainly seen in spring and summer.

Trophic transmission, therefore, seems a good strategy for dissemination of *Vibrio* parasites. Nevertheless, trophic transmission was not detected in the case of haplosporidians, found in cockle samples but not in bird faecal samples. No ostreid herpesvirus type 1 (OsHV-1) neither Microsporidia spp. were found in the analysed compartments.

Findings provide an insight of the transmission modes and connectivity of micro-parasites of bivalves through the different compartments considered: *C. edule* and shorebird populations and sediment.

References:

[1] Rakotomalala, C., Grangere, K., Ubertini, M., Foret, M., Orvain, F., 2015. Modelling the effect of *Cerastoderma edule* bioturbation on microphytobenthos resuspension towards the planktonic food web of estuarine ecosystem. Ecological Modelling. 316, 155-167. 10.1016/j.ecolmodel.2015.08.010.

[2] Fernandez-Delgado, M., Sanz, V., Giner, S., Suarez, P., Contreras, M., Michelangeli, F., Alexandra Garcia-Amado, M., 2016. Prevalence and distribution of *Vibrio* spp. in wild aquatic birds of the southern Caribbean Sea, Venezuela, 2011-12. Journal of wildlife diseases. 52, 621-626. 10.7589/2015-06-154.

[3] Laviad-Shitrit, S., Izhaki, I., Halpern, M., 2019. Accumulating evidence suggests that some waterbird species are potential vectors of *Vibrio cholerae*. Plos Pathogens. 15, e1007814. 10.1371/journal.ppat.1007814.

[4] Vezzulli, L., Pezzati, E., Stauder, M., Stagnaro, L., Venier, P., Pruzzo, C., 2015. Aquatic ecology of the oyster pathogens *Vibrio splendidus* and *Vibrio aestuarianus*. Environmental microbiology. 17, 1065-1080. 10.1111/1462-2920.12484.

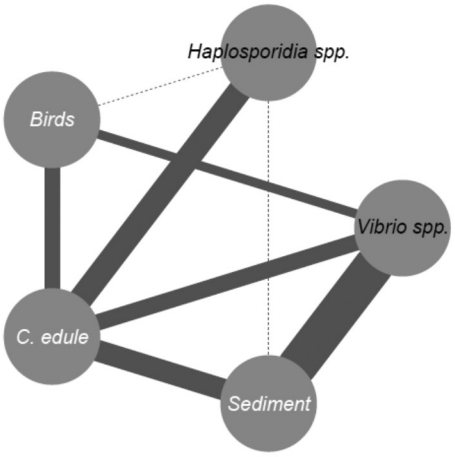

Figure 1: Network association plot displaying the links between the different compartments studied: *Cerastoderma edule* and shorebird populations and sediment. Black text refers to pathogens and white text relates to hosts/reservoirs. A solid line represents target DNA presence and a dashed line represents absence. Line thickness indicates the prevalence (%) of affected individuals.

SEX DIMORPHISM IN EUROPEAN SEA BASS (*Dicentrarchus labrax* L.): ULTRA-SMALL RFID TAGS TO INVESTIGATE SEX-RELATED GROWTH PATTERNS DURING VERY EARLY LIFE STAGES

François Allal[1*], Sara Faggion[1], Alain Vergnet[1], Frédéric Clota[1,2], Marie-Odile Blanc[1], Pierre Sanchez[1,2], François Ruelle[1], Marc 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

mail : francois.allal@ifremer.fr

Introduction

The European sea bass (*Dicentrarchus labrax*) displays female-biased sexual size dimorphism (SSD) early in development (Saillant et al., 2001). To investigate the link between growth and SSD at early stages, we evaluated a new tagging technique with ultra-small RFID transponder microchips (500 × 500 × 100 µm, 82 µg) and monitored individual sex-related growth during the post-larval and juvenile stages.

Material and methods

For this study, we produced an experimental population composed by 50:50 normally pigmented larvae and albino larvae through artificial fertilization and followed a rearing-temperature protocol (~16 °C from hatching to 112 days post-hatching, dph; ~20 °C from 117 to 358 dph) targeting a roughly balanced sex ratio (Vandeputte et al., 2020). To evaluate the impact of the tagging procedure (Figure 1), 5 trials were performed over 35 days, in fish aged 61, 75, 83, 89 and 96 dph. Each time, 50 normally pigmented fish were tagged intraperitoneally with p-Chips® RFID transponders (PharmaSeq, Inc., Monmouth Junction, New Jersey), while 50 untagged albino fish were used as controls. Mortality was recorded daily, while biometric measurements were performed at 75, 83, 89, 96, 103 and 110 dph via image analysis. At 117 dph, the fish were tagged with RFID microtags (Nonatec,) and regularly measured for SL and BW until 335 dph. The experiment ended at 358 dph with the sexing of the fish.

Results and discussion

Microchip tagging was possible in larvae from an age of 75 dph (standard length ~20 mm), with satisfactory performance in terms of survival rate (between 84 and 98% 24 h after tagging) and growth rate, and without significant differences in comparison with the untagged controls.

The sex-ratio at the end of the experiment was significantly in favor of females (65.6% vs. 34.4%).The females were significantly longer and heavier than the males from 103 dph (~30 mm SL, ~440 mg BW) to 165 dph, but the modeling of the growth curves suggests that differences in size already existed at 83 dph (~23 mm SL, ~160 mg BW, Figure 2). A significant difference in the daily growth coefficient (DGC) was observed only between 96 and 103 dph, suggesting a physiological or biological change occurring during this period.

The female-biased SSD pattern in European sea bass is thus strongly influenced by very early growth differences between sexes, in any case long before gonadal sex differentiation has been started. This leads to the hypothesis that early growth might be a cause rather than a consequence of sex determination in sea bass.

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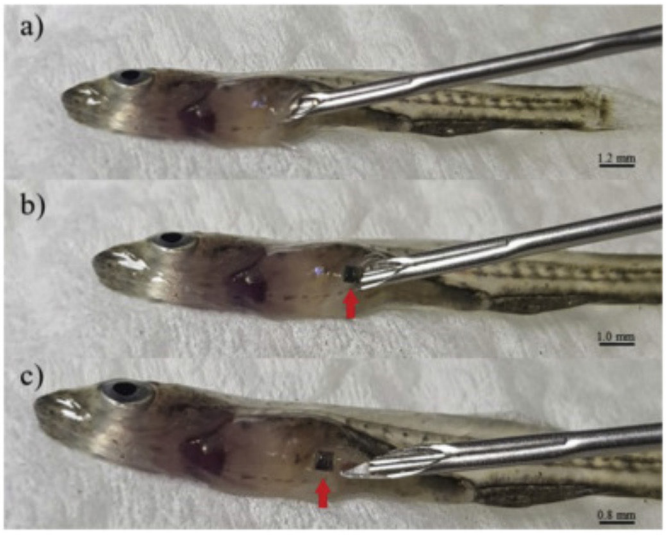

Fig. 1. Intraperitoneal implantation of the microchip in a 75 dph larva: a) insertion of the injector needle; b) ejection of the microchip; c) withdrawal of the injector needle.

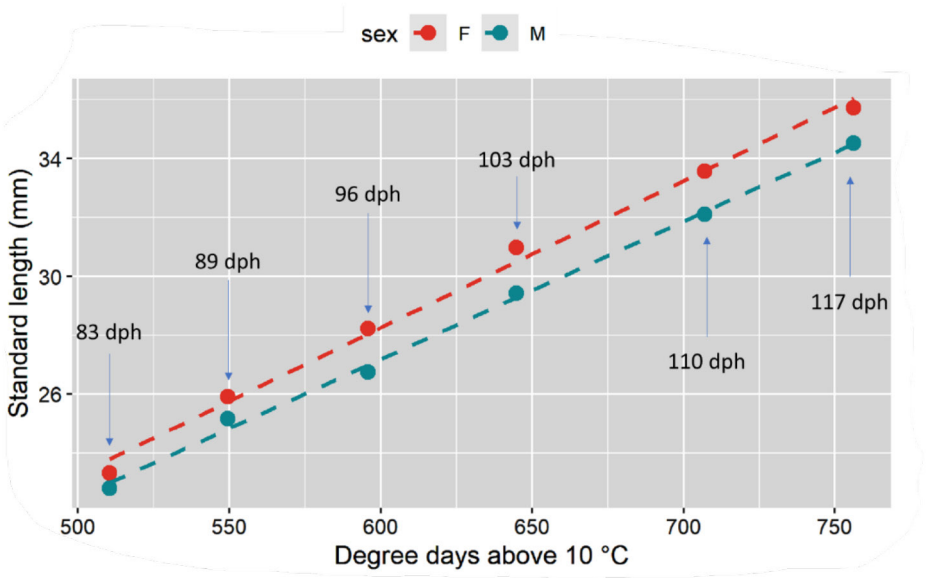

Fig. 2. Growth curve for standard length for females (F) and males (M) from 83 to 117 dph (510 to 756 degree days)

Acknowledgements

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References

Saillant, E., Fostier, A., Menu, B., Haffray, P., Chatain, B., 2001. Sexual growth dimorphism in sea bass Dicentrarchus labrax. Aquaculture, Bass SPP culture:basic and applied aspects 202, 371–387. https://doi.org/10.1016/S0044-8486(01)00786-4

Vandeputte, M., Clota, F., Sadoul, B., Blanc, M.-O., Blondeau-Bidet, E., Bégout, M.-L., Cousin, X., Geffroy, B., 2020. Low temperature has opposite effects on sex determination in a marine fish at the larval/postlarval and juvenile stages. Ecology and Evolution 10, 13825–13835. https://doi.org/10.1002/ece3.6972

THE HYDRO-MORPHOLOGY OF THE AFRICAN BONYTONGUE, *Heterotis niloticus* BREEDING POND

Seyram K. Amanie-Adjei*, Daniel Adjei-Boateng, Regina E. Edziyie

Department of Fisheries and Watershed Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
Email: Skamanieadjei@gmail.com
Telephone: +233547176057

Introduction

Nest building is an important courtship behaviour in several fish species as it is a determinant of access to a partner for reproduction, egg deposition, successful incubation and survival of offspring. It is dependent on the availability of appropriate substrate, water level and vegetation. *Heterotis niloticus,* is a highly favoured fish in East and West Africa, with high market value and highly overfished in several waters. It is known to a reproduce during the rainy season from May to September when vegetative zones of waterbodies are inundated with water of about 60cm. Attempts to artificially reproduce it have been largely unsuccessful due to high larval mortalities but are known to reproduce in ponds. This study characterized the effects of pond morphology on the breeding activity of *H. niloticus*.

Methodology

The study area is located on latitude 6.4625N and longitude 1.2611W in the Ashanti region of Ghana. The *H. niloticus* stock in the fish ponds were obtained from Volta lake at Yeji and has been kept at the Yaw Nkrumah fish farm for the past four years. The number of nests in the pond, diameter of the nest, the soil type in the nest, the vegetation found in the pond and around the nests were observed and documented. All lengths were measured with a meter-rule and nest location coordinates were taken with the Garmin GPSMAP 64sc.

Results and discussion

Forty-four nests were identified, occupying an area of 5,208.87m^2 which translates into a nest density of 0.008nests/m^2. Out of the 44 nests recorded, 54.55% were active and 45.45% were inactive. The nests were circular as shown in figure 1 and were built with a mixture of sand and decomposing vegetation. The nests were found in the littoral zones of the pond with a gentle slope and an average water level of 40cm. Nests were absent in pond areas characterized with a zero gradient, absence of vegetation, sludge and water levels above 70cm. All nest bottoms had a sandy substrate with varying particle sizes and were surrounded by different types of vegetation. The vegetation were predominantly sedges of the genus *Cyperus* i.e., *C. javanicus, C. strigosus,* and *C. odoratus*. Other vegetation types were *Impatiens balsamina, Ludwigia erecta*, and *Ammannia latifolia*. The minimum distance between two adjacent nests was 0.7m and maximum distance 131m. Figure 2 depicts the configuration of *H. niloticus* nests, pond vegetation and pond outline. The area between the yellow shape and green shape on the map is covered with vegetation, among which *H. niloticus* nests were constructed. The results show that *H. niloticus* builds nest in both dead and live vegetation inundated by water but the common characteristic of these two types of nest environment was the ability to dislodge materials from the sediment in order to create the nest. Where the nest was built in a live vegetation, the vegetation was predominantly made up of facultative hydrophytes that were characterized with highly fibrous but loose root systems that could easily be uprooted by *H. niloticus* for use in building the walls of the nests. A gentle slope in the vegetation areas of the also played a key role in the suitability of an area for nest formation. Future studies should explore the effects of deliberately manipulating water depth in fish ponds on the reproductive success of *H. niloticus* broodstock in captivity.

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Figure 1 A nest built by H. niloticus

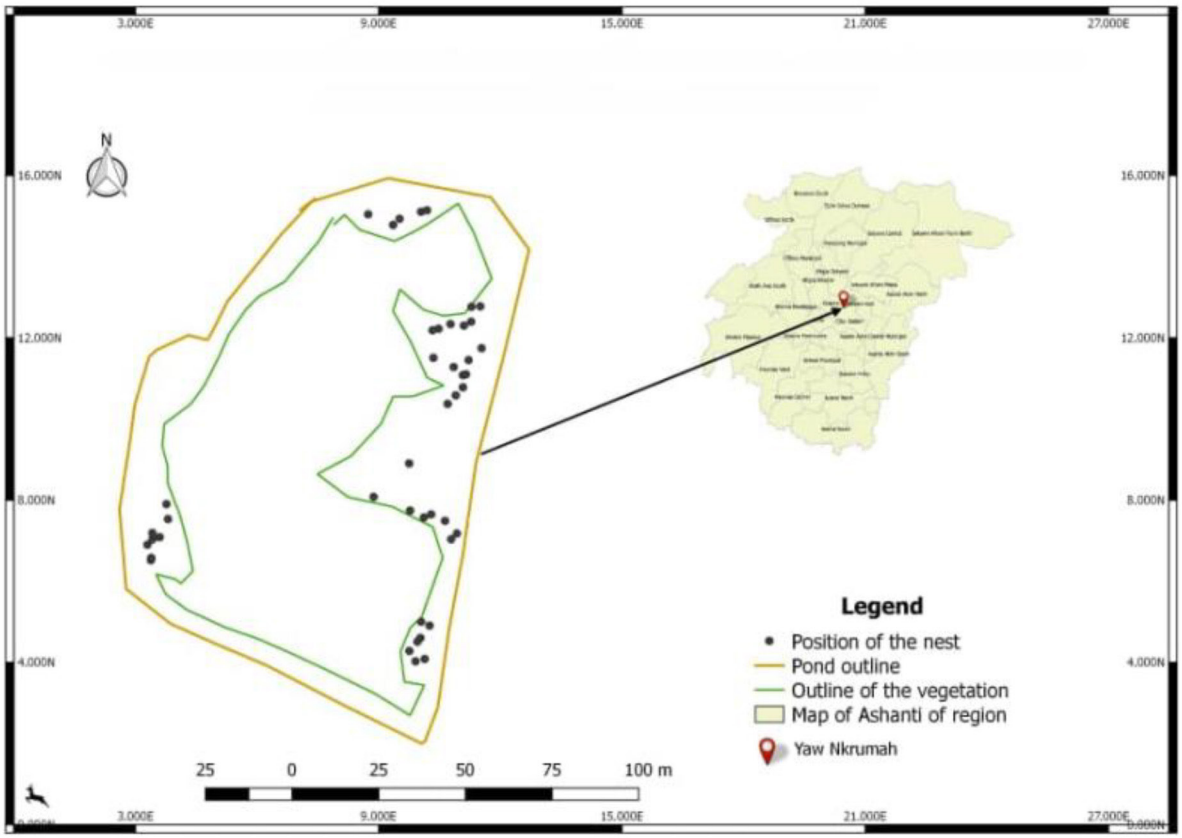

Figure 2 Map of the pond outline and position of H. niloticus nests.

OCEAN WARMING AND ACIDIFICATION IMPACTS ON NUTRITIONAL QUALITY OF SENEGALESE SOLE *Solea senegalensis*

Patrícia Anacleto*[1,2,3], Ana Luísa Maulvault[1,2,3], Sara Castanho[1], Luísa M.P. Valente[3,4], Sara Sousa[5], Cristina Soares[5], Cristina Delerue-Matos[5], Valentina F. Domingues[5], Pedro Pousão-Ferreira[1], Rui Rosa[2], António Marques[1,3]

[1]Division of Aquaculture and Seafood Upgrading (DivAV), Portuguese Institute for Sea and Atmosphere (IPMA, I.P.), Rua Alfredo Magalhães Ramalho 6, 1495-165 Lisboa, Portugal
[2]MARE – Marine and Environmental Sciences Centre, Guia Marine Laboratory, Faculty of Sciences, University of Lisbon (FCUL), Av. Nossa Senhora do Cabo, 939, 2750–374 Cascais, Portugal
[3]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
[4]ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[5]REQUIMTE/LAQV-GRAQ, Instituto Superior de Engenharia do Porto, Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal

E-mail: panacleto@ipma.pt

Introduction

Nowadays, climate change is a global environmental threat of growing concern that causes profound impacts on seafood quality and safety. Rising levels of atmospheric carbon dioxide are driving ocean warming and acidification, which may negatively impact on the nutritional proprieties of fish species used for human consumption and with high commercially value (Anacleto et al., 2014; Barbosa et al., 2017). Yet, the effects and interactions of these environmental stressors on seafood nutritional quality still requires further understanding. In this context, the main goal of this work was to study the impact of ocean warming (i.e. + 4 °C) and acidification (ΔpH=–0.3 units equivalent to ΔpCO_2~+500 μatm) on Senegalese sole (*Solea senegalensis*) condition and muscle nutritional quality after 61 days of exposure.

Materials and methods

Juvenile Senegalese sole specimens (12.8±0.8 cm total length; 25.4±2.6 g total weight) were distributed and maintained in 12 tanks (4 treatments x 3 replicate tanks) with independent recirculation aquaculture systems (n=8 per tank). After an acclimation period, fish were exposed to four scenarios, during 61 days, to understand the potential impacts to organisms under current and future expected conditions (according to IPCC projection scenario RCP8.5; IPCC, 2019): i) Control - seawater temperature set at 19 °C and pH at 8.0 (pCO_2~405 μatm; average temperature in Senegalese sole farms in Iberian Peninsula); ii) Warming – seawater temperature set at 23 °C and pH at 8.0 (pCO_2~405 μatm); iii) Acidification – seawater temperature set at 19°C and pH set at 7.7 (pCO_2~1000 μatm) and; iv) Warming + Acidification – seawater temperature set at 23 °C and pH set at 7.7 (pCO_2~1000 μatm). During the experimental period, fish were daily fed two meals a day, at a fixed ratio of 3% body weight. Six specimens per treatment were randomly collected at the end of the experiment, biometric data was registered and muscle was dissected. The samples were freeze-dried and kept at -80 °C until further analyses. Moisture, ash, total lipids, including the fatty acid profile by GC-FID, crude protein and gross energy were determined in muscle. Essential elements (potassium, K; sodium, Na; magnesium, Mg; iron, Fe; copper, Cu; zinc, Zn; manganese, Mn) were also quantified in muscle according to the procedures described by Jorhem (2000) using flame atomic absorption spectrometry (FAAS); and phosphorus (P) was determined spectrophotometrically at 430 nm.

Results and discussion

Warming acting alone induced a significant increase in fish weight, relative growth rate (RGR), moisture, ash, saturated fatty acids (SFA, mainly C16:0 and C18:0) and Zn contents. Fulton's K condition factor and hepatosomatic (HSI), atherogenicity (IA) and thrombogenicity (IT) indices were also improved. However, a significant decrease in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were observed; likewise visceral indices (VSI) and hypocholesterolemic/hypercholesterolemic (h/H) were also reduced. On the other hand, acidification acting alone contributed to a significant decrease in the content of some MUFA and PUFA, leading to an increase of RGR and SFA. The combination of the two stress factors resulted only in a significant increase in weight and RGR, and in a significant decrease in HSI, fat content and MUFA. In the three climate change scenarios, there was a significant decrease in the EPA and DHA, Σω3, Σω6, Σω3/Σω6 and h/H index, while the IA and IT indices increased.

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Conclusions

Since one of the main nutritional attributes of fish is to provide an adequate level of ω3 fatty acids (in particular EPA and DHA) for human consumption, the results obtained in this study show that warming and acidification negatively affect the fatty acid composition of sole, particularly the EPA+DHA level and the Σω3/Σω6 ratio, which may compromise the nutritional quality of this species. Additionally, the indices of fat quality, namely AI and TI significantly increased under warming alone, suggesting that this species may not represent the best choice for consumers in terms of cardio-protection. Overall, this study provides new insights to understand and foretell the climate change impacts on nutritional quality of seafood and highlights the importance to perform a risk-benefit analysis of fish consumption.

References

Anacleto, P., Maulvault, A.L., Bandarra, N., Repolho, T., Nunes, M.L., Rosa, R., Marques, A. 2014. Effect of warming on protein, glycogen and fatty acid content of native and invasive clams. Food Research International, 64: 439–445. doi.org/10.1016/j.foodres.2014.07.023.

Barbosa, V.L., Maulvault, A.L., Alves, R., Anacleto, P., Pousão, P., Carvalho, M.L., Nunes, M.L., Rosa, R., Marques, A. 2017. Will seabass (Dicentrarchus labrax) quality change in a warmer ocean?. Food Research International, 97: 27–36. doi.org/10.1016/j.foodres.2017.03.024.

IPCC. 2019. IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Pörtner, H.-O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., AlegriÏa, A., Nicolai, M., Okem, A., Petzold, J., Rama, B., Weyer, N.M. (eds.). *In press*.

Jorhem, L. 2000. Determination of metals in food by atomic absorption spectrometry after dry ashing: NMKL Collaborative study. Journal Association of Official Analytical Chemists International, 83(5), 1204-1211.

Acknowledgements: This study was supported by the project FISHBUDGET - Effects of climate change on marine fish energy budgets (PTDC/BIA-BMA/28630/2017) and benefited from the strategic program of MARE financed by FCT (MARE-UID/MAR/04292/2019). This work was also supported by UIDB/50006/2020 by the Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. The authors thank the Sparos Lda company for providing the fish feed, and the team from EPPO aquaculture pilot station (Olhão) for providing the juvenile fish for trails. The Portuguese Foundation for Science and Technology (FCT) supported the contract of PA in the framework of CEECIND/01739/2017.

EFFECT OF SLURRY ICE COOLING DURING HARVESTING AND TRANSPORTATION OF EUROPEAN SEA BASS ON FLESH MICROBIAL QUALITY

Angelakopoulos Rafael[1], Ntzimani Athina[2], Semenoglou Ioanna[2], Tsironi Theofania[2,3], Taoukis Petros[2], Moutou Katerina[1]

[1] Laboratory of Genetics, Evolutionary and Comparative Biology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
kmoutou@bio.uth.gr
[2] Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Athens, Greece
ntzimani@chemeng.ntua.gr
[3] Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
tsironi@aua.gr

Introduction

Post-harvest fish deterioration process is accelerated by increased temperatures, physical damage, and contamination. Therefore, the key to fish preservation is the immediate chilling upon catch or harvest to a temperature slightly above the freezing point and maintaining this temperature throughout the cold chain. Slurry ice is a biphasic system consisting of small spherical ice particles surrounded by seawater at subzero temperature (Cakli et al., 2006). Its reported advantages over traditional fresh-water ice include its lower temperature, faster chilling due to rapid heat exchange, and lower rate of physical damage due to its spherical microscopic particles (Kauffeld et al., 2010). The objective of the study was the evaluation of the effect of slurry ice composition during harvesting and transportation of European sea bass (*Dicentrarchus labrax*) on fish flesh and skin microbiome using conventional and novel "omics" analytical tools that have the capacity to detect non-culturable or poorly characterized microorganisms and emerging bacteria relevant to the quality level and shelf life of fish and fish products (Tsironi et al., 2019).

Materials and methods

Whole European sea bass (*Dicentrarchus labrax*) was harvested from the sea cages in Philosofish S.A. farming facilities (Greece) in slurry ice prepared from seawater, and was transported to the laboratory in polystyrene boxes within 24 h after slaughtering. Three different combinations of slurry ice and conventional flake ice were tested and coded as C: slaughtered and transported in flake ice, SC: slaughtered in slurry ice and transported in flake ice, S: slaughtered and transported in slurry ice. The ratio of ice (slurry or flake) to fish (w/w) was 1:1 and the temperature of the slurry ice was -3.2°C. Upon receipt at the laboratory, all fish samples were stored isothermally at 0±0.2°C. Microbial growth in fish flesh (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria yeasts/molds and *Enterobacteriaceae* spp.) was monitored using conventional culture-based techniques and the experimental data were fitted to the Baranyi growth model. Fish skin microbiome was characterized, analyzing the 16S rRNA gene bacterial diversity from each individual sample, targeting the V3-V4 region. Samples were collected on slaughter day and four days post-slaughter, scraping the skin with different sterile scalpel for each sample. DNA extraction was performed using the PureLink™ Genomic DNA Mini Kit (Thermofisher) with minor modifications. Sequencing of samples was performed by BGI Genomic solution and the operational taxonomic units (OTUs) were filtered and classified using Ribosomal Database Project classifier. Relative abundances of these groups were compared to highlight the differences between sampling days.

Results

Microbial counts increased with storage time, in contrast to the counts of *Brochothrix thermosphacta*, yeasts/molds (<2.0 log CFU/g) and *Enterobacteriaceae* (<1.0 log CFU/g), which remained below the detection limit during the 33-day storage period. TVC, *Pseudomonas* spp. and H_2S-producing bacteria had a similar growth pattern, with the two latter being the dominant bacteria at the end of the storage period, responsible for quality deterioration of whole sea bass. Initial counts of the aforementioned microorganisms were low (i.e. 2.0±0.2, 2.0±0.1 and 1.0±0.1 log CFU/g for TVC, *Pseudomonas* spp. and H_2S-producing bacteria, respectively) and comparable with those reported in the literature for fresh fish stored aerobically (Tsironi et al., 2019).

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Metabarcoding analysis revealed 170 OTUs common between slaughter methods on harvest day, rolling down to 52 OTUs common in all three groups four days post-harvest. Microbial composition revealed *Proteobacteria* (74-96%), and *Bacteroidetes* (1-21%) as the most abundant phyla, in accordance with the current knowledge on the skin microbiome of European seabass (Rosado et al., 2019). On harvest day, *Pseudoalteromonas* and *Marinobacter* were the dominant genus in C and S samples, respectively. Only 19 common OTUs were identified between S4 and SC4 samples that followed different storage (S4: storage in slurry ice and SC4: storage in ice flakes), indicative of the effect of storage conditions on microbiome composition. Storage in slurry ice established *Pseudoalteromonas* as the dominant genus (65%), as opposed to *Psychobacter* (39%) following storage in ice flakes. *Pseudomonas* represented just around 2% of relative abundance on day 4 post-harvest. Significant differentiation in genus composition were observed among the sample groups between the two time points, which was the combined result of slaughter and storage method followed.

Discussion and conclusion

The use of slurry ice as an alternative slaughtering method for farmed European sea bass resulted in a significantly different microbiome composition at slaughter and during storage. The comparison with conventional slaughter in ice flakes indicated that ice flake microbiome may reflect on the start microbiome of the fish and storage can dictate different trajectories in microbiome composition. Microbiome characterization of fish may provide promising new markers for fresh fish quality assessment and optimizing the slaughter and storage methods.

Acknowledgment

This research was funded by 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) website: slurryfish.chemeng.ntua.gr

References

Cakli, S., Kilinc, B., Cadun, A., Tolasa, S. (2006). Effects of using slurry ice on the microbiological, chemical and sensory assessments of aquacultured sea bass (*Dicentrarchus labrax*) stored at 4°C. European Food Research and Technology, 222, 130-38.

Kauffeld, M., Wang, M.J., Goldstein, V., Kasza, K.E. (2010). Ice slurry applications. International Journal of Refrigeration, 33, 1491-1505.

Rosado D., Xavier R., Severino R., Tavares F., Cable J., Pérez-Losada M. (2018) Effects of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed seabass (Dicentrarchus labrax). Scientific Reports 9, 18946

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. Journal of Food Engineering, 262, 83-91.

ARE SENEGALESE SOLE STRESS AND IMMUNE RESPONSES AFFECTED BY DIETARY PROTEIN SOURCE?

C. Aragão[1,2*], M. Cabano[1], R. Colen[1], J. Dias[3], B. Costas[4]

[1]Centre of Marine Sciences (CCMAR), Faro, Portugal
[2]Universidade do Algarve, Faro, Portugal
[3]SPAROS Lda., Olhão, Portugal
[4]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR) Porto, Portugal

*Presenting author: caragao@ualg.pt

Introduction
A continuous increase in aquaculture production depends on finding alternative proteins that allow a sustainable growth of the activity. Sustainability principles of zero waste and circular economy are being increasingly implemented by the aquaculture industry. Current knowledge indicates that the importance of protein goes beyond growth, and aspects like fish health and welfare must be taken into consideration in the development of novel feeds (Aragão et al., 2020; Machado et al., 2020). Therefore, it is of utmost importance to understand if new dietary protein sources, even if not affecting fish growth, may impair fish stress and immune responses.

Senegalese sole (*Solea senegalensis*) is considered a carnivorous fish species, but it has been shown to tolerate relatively-well plant-based diets (Valente et al., 2011). Rendered animal proteins are increasingly being used by the aquaculture industry and is important to understand the impacts of these protein sources on Senegalese sole stress and immune responses. Therefore, the objective of this study is to understand if processed animal proteins affect the stress and immune response of Senegalese sole juveniles when exposed to a temperature challenge.

Materials and Methods
Two isonitrogenous and isolipidic diets (55% crude protein and 7% crude fat) were formulated with practical ingredients. One of the diets contained marine ingredients (especially fishmeal) as the main protein sources (FM diet), while in the other diet marine ingredients were reduced to a minimum and processed animal proteins, such as poultry meal, porcine blood meal and feather meal, were included as protein sources. Diets were manufactured at SPAROS Lda. (Olhão, Portugal).

For each dietary treatment groups of 14 juvenile Senegalese sole (mean initial body weight: 40.6 ± 0.8 g) were distributed into 12 flat-bottomed tanks in a recirculating aquaculture system at a density of 2.7 kg of fish/m^2. Tanks were supplied with filtered and heated (19.7 ± 0.5 ºC) seawater (salinity: 33.4 ± 1.7 ‰). Each diet was randomly distributed to six replicates and automatic feeders delivered feeds in 7 meals/day. Three replicates per dietary treatment were sampled after 29 days of feeding. Fish growth and feeding efficiency was assessed during this period and plasma and liver samples were collected. The remaining three tanks per dietary treatment were left undisturbed and the water temperature was increased to 25.5 ± 0.4 ºC and maintained at this temperature for additional two weeks. After this period, plasma samples were collected for analysis of selected indicators associated with stress and immune responses. Liver samples were also collected to analyse methionine cycle metabolites.

Results and Discussion
During the period of conditioning to the dietary treatments, fish growth and feed efficiency was similar in both treatments. Furthermore, indicators associated with stress and immune responses were unaffected by the dietary treatments. Hepatic concentrations of methionine cycle metabolites were similar in both treatments, except for methionine and taurine, which is probably linked with differences in dietary amino acid composition. This indicates that fish were in identical physiological conditions before the temperature stress.

After the temperature stress challenge, all the hepatic methionine cycle metabolites but S-adenosyl methionine (SAM) were affected by the dietary treatment. Post-stress samples of indicators associated with stress and immune responses are still under analysis. Information on how alternative proteins modulate stress and immune responses in fish will contribute to a better management of fish welfare and are paramount to guarantee a sustainable aquaculture production.

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References

Aragão C., Cabano M., Colen R. Fuentes J. and Dias J., 2020. Alternative formulations for gilthead seabream diets: Towards a more sustainable production. Aquaculture Nutrition 26: 444-455.

Machado M., Engrola S., Colen R., Conceição L.E.C, Dias J. and Costas B., 2020. Dietary methionine supplementation improves the European seabass (*Dicentrarchus labrax*) immune status following long-term feeding on fishmeal-free diets. British Journal of Nutrition 124: 890-902.

Valente L., Linares F., Villanueva J., Silva J. Espe M. Escórcio C., Pires M. Saavedra M., Médale F., Alvárez-Blázquez amd Peleteiro J., 2011. Dietary protein source or energy levels have no major impact on growth performance, nutrient utilisation or flesh fatty acids composition of market-sized Senegalese sole. Aquaculture 318: 128-137.

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BIOGEARS: DEVELOPING BIOBASED ROPES FOR USE IN MUSSEL AND SEAWEED AQUACULTURE AND CREATION OF BIOBASED VALUE CHAINS AND CIRCULAR ECONOMY

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

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

Introduction

Aquaculture is an industry that creates economic value, employment, and economic support to rural and coastal areas and is called to address growing food demands, thus the European Union is keen on its large-scale expansion. Although offshore mussel aquaculture has been identified as a promising sector for aquaculture expansion, this sector makes use of ropes that are 100% petrol-based (non-biodegradable). Thus, a growth in this industry will require more rope production, which could in turn contribute to plastic waste and pollution in the marine environment. BIOGEARS addresses the challenge of minimizing the use of plastics in the sea by developing prototypes of biobased and biodegradable ropes and will examine their potential use in Integrated Multi-Trophic Aquaculture (IMTA) systems, integrating mussels and seaweed culture. The biopolymers used in the development of the biogears will allow the manufacturing of aquaculture ropes with adequate durability for offshore productions, The biopolymers biodegradability allows them to enter in-land organic recycling circuits at the end of use, hence greatly reducing carbon footprint along the whole value chain. The aim of this study is to assess the **biobased value chains** in the **circular business models** framework that can be generated by filling the gap to develop durable, fit-for-purpose and marketable biobased ropes, to boost eco-friendly aquaculture and circular blue bioeconomy.

Material and methods

To assess this, a 3 step-methodology will be used, consisting of: a **value chain description** (Step 1), a **market study** (Step 2) and a **Circular Business Model** (**CBM**). As a suitable framework, CBM is used to assess the rationale of how BIOGEARS creates, delivers and captures value with and within closed material "loops" or a business model in which the conceptual logic for value creation is based on utilizing the economic value retained in products after use in the production of new offerings, following the methods used by Lewandowski (2016). In all three steps of the methodology proposed, the current policy framework and developing strategies in plastic use at the sea, bioeconomy and circular biobased economy will be applied. For the validation of the biogears, tests at sea are being designed in different marine environments and using different culture technologies, including IMTA systems with cultivation of seaweed and mussels.

Results and discussion

As preliminary results of this study, value chain analysis (Figure 1) and Circular Business Model (CBM) (Figure 2) of biogears value chain have been outlined. In line with the current regulations on plastic, biogears are alternative solutions to non-degradable plastic. Furthermore, generating more sustainable aquaculture products and circular economy for the aquaculture sector, from the use of biopolymers to rope manufacturing, mussel and seaweed culture and to solutions for the end of life in their value chain. Besides, IMTA systems permit the circular use of nutrients among trophic levels resulting on biomass generation, being eco-friendly, and thus producing added value marketable sustainable aquaculture products in a circular way.

References

Lewandowski M. 2016. Designing the Business Models for Circular Economy—Towards the Conceptual Framework. https://doi.org/10.3390/su8010043

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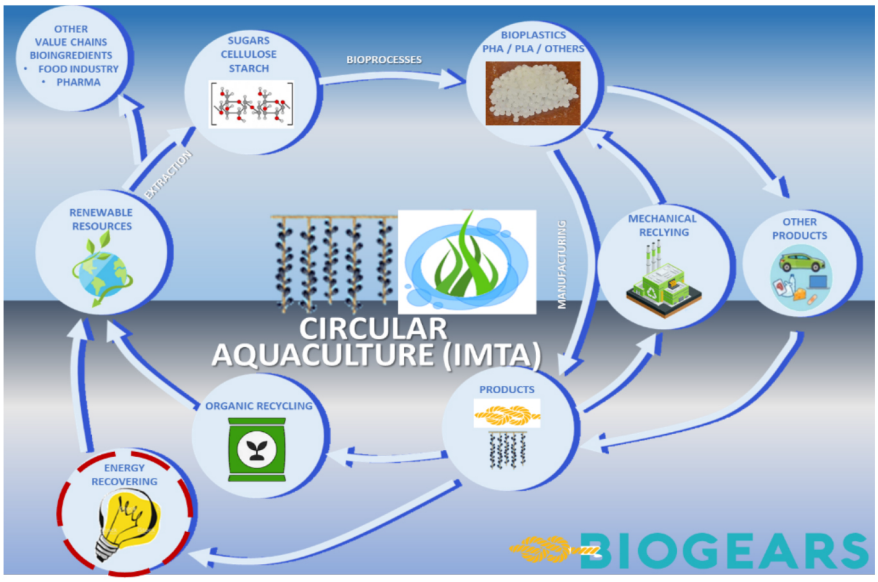

Fig. 1. Generation of **circular value chains** based on the development of biogears for IMTA; from the selection of biopolymers to rope manufacturing, mussels and seaweed culture and solutions for the end of life of ropes (biogears).

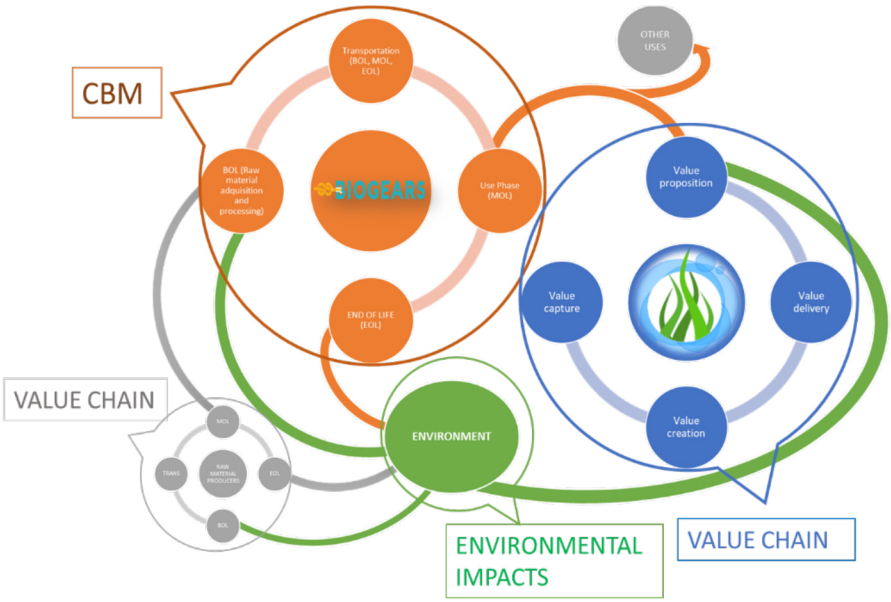

Fig. 2. Circular economy analysis approach through the analysis of the value generated by the development of the products of the BIOGEARS project, thus, biogears and mussels and seaweed cultured using IMTA production systems, in three dimensions, such as environmental, social and economic applied to the aquaculture sector.

MICROENCAPSULATED DIETS WITH MACROALGAE BY-PRODUCTS TO IMPROVE MUSSEL MYTILUS GALLOPROVINCIALIS SPAT IN-HATCHERY PERFORMANCE

Leire Arantzamendi[b*], Camilla Campanati[a], Izaskun Zorita[b], Ainhoa Juez[b c], David C. Aldridge[a]

*Presenting author: larantzamendi@azti.es
a Department of Zoology, The David Attenborough Building, University of Cambridge, Pembroke Street, Cambridge, CB23QZ, UK
b AZTI Fundazioa, Herrera Kaia, Portualdea z/g 20110 Pasaia-Gipuzkoa, Spain
c Present address: Instituto Español de Oceanografia (IEO), Centro Oceanográfico de Vigo, Subida a Radio Faro 50, 36390 Vigo, Spain

Introduction
Hatchery cultures can support marine bivalve farming, supplying spats to extensively farmed populations and improving conditions for delicate life stages throughout production. However, up to half of the production costs of inland bivalve hatcheries and nursery facilities, are used for algal provisioning. The aim of this study was to assess the impact of different diets, alternatives to conventional hatchery algal feeds, on the survival and growth of *Mytilus galloprovincialis* spat.

Material and methods
Mussel spats of 6.82 ± 1.14 mm average initial size were acclimated to lab conditions in 9L rectangular tanks supplied with abundant seawater and aeration at a constant room temperature of 18 ºC for 2 weeks. Experimental diets were formulated using *Schizochytrium* sp and *Undaria pinnatifida* macroalgae. A premix slurry was prepared containing the encapsulant and active ingredient under conditions of controlled shear, as described in Aldridge et al. (2006). Four experimental feeding treatments were tested in 8 replicated tanks of 15 mussel spats: 1) NC: Negative control (No food); 2) A: Algae (Reed Shellfish diet 1800); 3) BB: Blended microencapsulated (BioBullets: *Schizochytrium* sp. + *U. pinnatifida*; Ratio 1:1); 4) ABB: Algae + Blended microencapsulated (Ratio 1:1:1). Survival, growth, and condition index (CI) were weekly recorded and also confronted with results of mussel spats reared in natural field conditions. All statistical analyses and graphics were performed in the R environment for statistical computation (R Core Team 2015). One-way ANOVA followed by Tukey's post hoc test was applied to determine differences in growth among different diets (alpha = 0.05).

Results
Mussels from all treatments sustained high survival (> 85 %) throughout the experiment, with no differences found among laboratory treatments (Figure 1). Mussel spats fed microcapsules grew at comparable rates (i.e, Shell growth rates: 8.51 ± 3.67 μm day^{-1}; ΔCI: 6.11 ± 1.09 %) to those fed commercial microalgal diets (i.e. 8.46 ± 5.72 μm day^{-1}; ΔCI: 3.31 ± 0.80 %), and ~33 % inclusion of microalgae in the diet did not significantly improve mussels' growth performances (i.e. 9.30 ± 2.32 μm day^{-1}; ΔCI: 4.74 ± 1.41 %) from mussels fed microcapsules alone (Figure 2).

Discussion and conclusion
The growth enhancement of mussel spats observed after 6 weeks of feeding with microencapsulated feeds indicated that a mixture of inert *Schizochytrium* sp. and *U. pinnatifida* (1:1), can substitute 100 % of commercial microalgae in mussel spats diet, which can significantly reduce the hatchery costs compared to conventional feeds. Furthermore, BioBullets microencapsulation also represent a more environmentally sustainable option, by reducing the use of natural resources, such as energy and water, needed for live microalgae production. Likewise, being produced from food industry by-products (i.e., Wakame), microencapsulated feeds promote circular economy and an eco-friendlier mussel aquaculture sector.

References
Aldridge D. C., Elliot P., and Moggridge G.D. (2006). Microencapsulated BioBullets for the control of biofouling zebra mussels. Environ. Sci. Technol. 40, 975-979.

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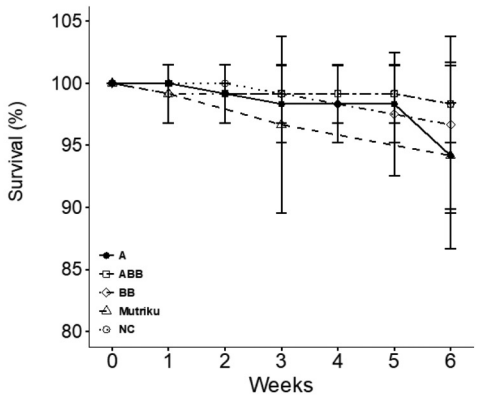

Fig. 1. Mean (± SD, n=8) weekly mussel survival (%) over the experimental time of mussels fed different diets: NC=No food, A=Microalgae, BB=BioBullets, ABB= Microalgae+BioBullets, Mutriku=Field-grown.

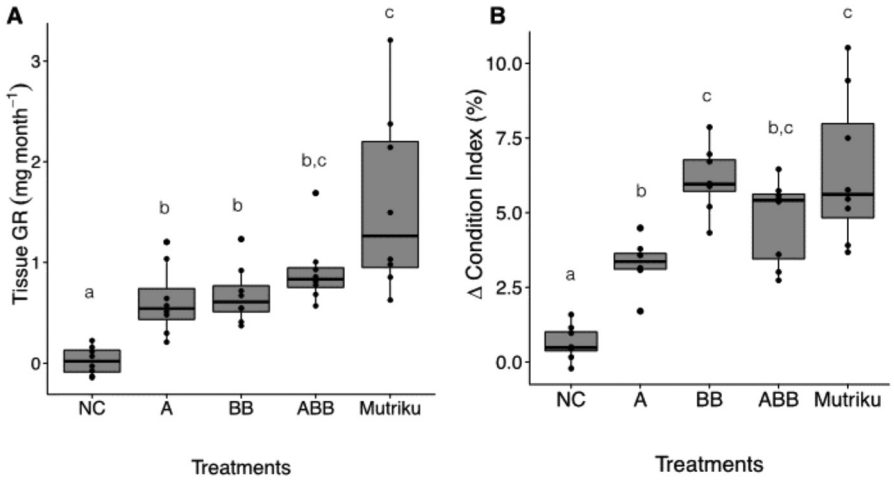

Fig. 2. Median and ± 95% confidence intervals A) Tissue growth (mg month^{-1}) and B) difference in condition index (Tissue AFDW/Shell DW x100) of mussels fed with different diets (NC= Non-fed; A=Microalgae; BB= BioBullets; ABB= Microalgae and BioBullets; Mutriku= field-grown) over six-weeks experiment.

UPSCALE NURSERY CULTURE OF *Mytilus galloprovincialis* SPAT WITH MICROENCAPSULATED DIETS

Leire Arantzamendi[b*], Camilla Campanati[a], Izaskun Zorita[b], Urzti Izagirre[c], David C. Aldridge[a]

*Presenting author: larantzamendi@azti.es
a Department of Zoology, The David Attenborough Building, University of Cambridge, Pembroke Street, Cambridge, CB23QZ, UK
b AZTI Fundazioa, Herrera Kaia, Portualdea z/g 20110 Pasaia-Gipuzkoa, Spain
c Plentzia Marine, Station (PiE-UPV/EHU), Areatza Hiribidea, 47, 48620 Plentzia, Bizkaia, Spain

Introduction

The extensive production of the Mediterranean mussel *Mytilus galloprovincialis* largely depends on environmental conditions for a stable production of spats, which are collected from the shore or rope collectors. Auxiliary inland hatchery and nursery facilities can support the production, by ensuring known quantity and quality of spats each year, regardless of uncertain coastal environmental conditions. Inland systems, however, are still not economically feasible, due to elevated costs in maintaining live microalgae as principal component of feeds. In this study we conducted a nutritional feeding trial with microencapsulated feeds (BioBullets) containing *Schizochytrium sp*. designed to identify the optimum level of commercial microalgal diet substitution (partial or complete: 0 %; 60 %; 80 %; 100 %) to support high growth of *M. galloprovincialis* spats at commercial scale and eventually aim at uplifting mussel production through a reduction of costs.

Material and methods

Mytilus galloprovincialis spats (shell length 0.5-0.8 cm) were obtained, from natural settlement on ropes, from ACUIMAR SERVICIOS MARÍTIMOS (Benalmádena, Malaga, Spain) and acclimated in the laboratory in Plentzia Marine Station (Basque Country, Spain) for 8 weeks to different feeding conditions: 1) NC (Negative Control): supplied with no food; 2) A (Algae; Conventional singular diet: ShellfishReed): supplied with 100 % commercial microalgae; 3) B (BioBullets as alternative singular diet): supplied with 100 % *Schyzochytrium* BioBullets; 4) AB_L (Mixed diet: Algae + BioBullets; with low percentages of BioBullet inclusion): supplied with 60 % BioBullets and 40 % commercial microalgae; 5) AB_M (Mixed diet: Algae + BioBullets; with medium percentages of BioBullet inclusion): supplied with 80 % BioBullets and 20 % commercial microalgae. Spat survival and growth were evaluated throughout and at the end of the 8-weeks feeding conditioning, when also levels of digestive gland atrophy, adipogranular tissue index and fatty acids composition of spat tissues were assessed.

All statistical analyses and graphics were performed in the R environment for statistical computation (R Core Team 2015). One-way ANOVA followed by Tukey's post hoc test was applied to determine differences in growth among mussel fed on different dietary treatments (alpha = 0.05).

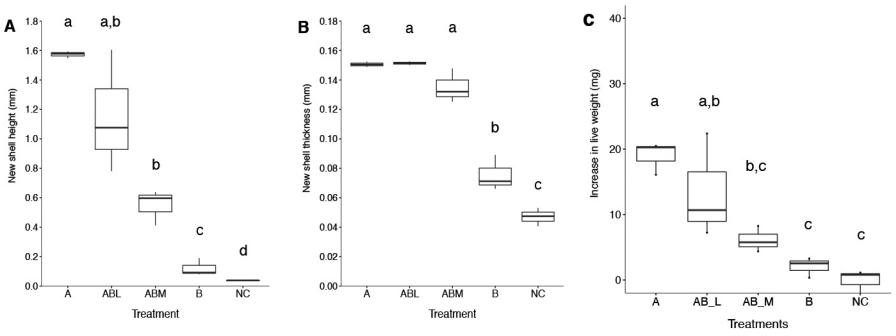

Fig. 1. Growth of A) shell height (mm) and B) shell thickness (mm) and C) increase in individual live weight (final-initial; mg) of mussels fed different diets (NC= Non-fed; A=Algae; ABL= Algae + BioBullets at low concentration; ABM= Algae + BioBullets at medium concentration; B= BioBullets) for 8 weeks. Median (± 95 % confidence intervals) with n=3 replicate tanks are reported for all treatment groups at each time point. Different letters represent significant differences ($p<0.05$) found through Tukey's post hoc pairwise comparisons following ANOVA results.

(Continued on next page)

Results

Mussels from all dietary treatments sustained high survival (>90 %) throughout the experiment with no differences among treatments found at the end of the 8 weeks experiment. Mussels fed with algae (A) and algae and BioBullets at low inclusion level (ABL) showed a significantly higher increase in live weight and shell increment from the beginning of the experiment compared to mussels fed other diets (Figure 1). The levels of digestive gland atrophy after 8 weeks of diet conditioning were consistent with mussel tissue growth: NC, or non-fed mussels presented the highest atrophy level of the digestive gland whereas mussels fed microalgae (A) showed the lowest level of atrophy, followed by mussels fed on ABL. The diets used in this study showed different FA profiles. BioBullets presented the highest level of stearic acid (18:0), ∑PUFA, ∑n-3, ∑n-6, DHA and DHA+EPA, whereas commercial microalgae (A) presented the highest levels of myristic (14:0), stearidonic (18:4n3), linoleic (18:3n-3), palmitoleic (16:1n7), ∑MUFA and EPA. Analyses revealed a close correlation between dietary and mussel tissue FA profiles. Preliminary results suggest that the spat growth performance in the feeding trial correlates well with the dietary EPA and protein contents.

Discussion and conclusion

Mussels fed only with microalgae (A) and with microalgae combined with BioBullets at low inclusion level in the diet (ABL) showed the best growth performance. Similar trends were, in fact, found among the increase in live weight, tissue and shell weight, with comparable growth rates observed between these two groups. Therefore, substitution of microalgae up to 60 % with BioBullets (*Schizochytrium* sp.) in a hatchery diet can be considered for more cost-efficient upscale nursery culture of the spat *M. galloprovincialis*. Expanding existing knowledge of the nutritional physiology of this key life stage is essential to further develop mussel aquaculture production and for improved understanding of the hatchery performance of this important commercial species.

COMPARISON OF SAMPLE PREPARATION METHODS FOR PROTEOMIC STUDIES WITH AQUACULTURE SPECIES

Mário J. Araújo[1], Lígia Sousa[1], Maria V. Turkina[2], Alexandre Campos[1]

1 Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal
2 Department of Biomedical and Clinical Sciences, Faculty of Medicine and Clinical Sciences, Linköping University, 581 83 Linköping, Sweden
E-mail: mario.araujo@ciimar.up.pt

Omic approaches are becoming increasingly used for addressing a variety of challenges in aquaculture industry related with animal welfare, nutrition, health, quality, and safety (Rodrigues *et al*., 2017). The study of the full set of the organisms' proteins – Proteomics, is one of these valuable research disciplines. The existence of imperceptive effects of changes in water quality of farming facilities and chronic exposure to stressors below detectable levels, might lead to simultaneous alterations to the set of proteins of organisms, with all the implications on their complex networks and pathways. This can be associated with effects on normal organism development processes or even the appearance of chronic diseases.

Usual methods of sample preparation for proteomic analysis include protein extraction from biological materials (tissues, fluids, etc.), protein reduction and alkylation, digestion of protein samples into peptides, desalting and concentration of samples. For high-throughput studies, samples are then analysed by high-resolution mass spectrometry. Recent developments regarding sample preparation include more precise protocols with less steps, tools, less time consuming and more affordable reagents, materials or equipment. However, reproducibility, reliability and efficiency of these sample preparation methods are still needed.

In this work we studied three distinct sample preparation methods for high-throughput proteomics in aquaculture species: Filter-Aided Sample Preparation (FASP); single-pot, solid-phase-enhanced sample preparation procedure (SP3) and S-Trap technology. Liver tissues of farmed Turbot *Psetta maxima* and hepatopancreas of wild Mediterranean mussel *Mytilus galloprovincialis* were used. The methods were compared regarding the amount and diversity of proteins identified, their functions and processes in which are involved (based on gene ontology analysis).

Our results showed that the type of organism tissue affected the evaluation of each method in regard to the amount/number of peptides, since the overall number of clusters of proteins was lower in mussel samples using SP3 and FASP (651 and 999, respectively) when comparing to turbot samples (1318 and 1570, respectively). The overall number of clusters of proteins was similar for both tissues using S-TRAP (1198 for turbot and 1242 for mussel).

The number of gene ontology (GO) terms associated with molecular function, biological processes and cellular components was generally similar using any of the three methods for turbot tissues (overall GO terms between 373 for S-TRAP and 377 for FASP). In the case of the mussel, the number of GO terms associated with molecular function and biological processes was slightly lower when using SP3, contributing for an overall lower number of GO terms (148) when comparing to FASP (195) or S-TRAP (206) methods.
The ranks (and significance) of the GO terms were not affected when comparing the full set of peptides of all sample preparation methods within each tissue. This means that the main terms related with molecular function, biological processes and cellular components were broadly represented using any of the three methods for both tissues studied (Table 1.). Additionally, we observed that the use of complex species / genera with correspondent high genetic variability, results in a considerable lack of reproducibility in protein identification between samples; and therefore in that case the use of higher number of organisms is suggested.

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Table 1. Most represented gene ontology terms of turbot, *Psetta maxima* liver and Mediterranean mussel, *Mytilus galloprovincialis* hepatopancreas, using three sample preparation methods: FASP, SP3 and S-TRAP. Homologous GO terms were based on *Danio rerio* and *Crassostrea gigas* databases for turbot and mussel, respectively.

Source	Rank	Term name	[-log10 adjusted p-value]		
			FASP	SP3	S-TRAP
Turbot, *Psetta maxima*					
Molecular Function	1	Catalytic activity	52.97	50.18	50.98
	2	Oxidoreductase activity	43.66	43.2	44.69
Biological Processes	1	Small molecule metabolic process	79.61	77.96	78.81
	2	Carboxylic acid metabolic process	54.21	51.82	54.92
Cellular components	1	Cytoplasm	105.87	102.93	103.28
	2	Cytosol	52.3	52.03	51.05
Mediterranean mussel *Mytilus galloprovincialis*					
Molecular Function	1	Structural molecule activity	15.98	22.66	26.78
	2	Catalytic activity	14.48	7.44	18.15
Biological Processes	1	Cellular amide metabolic process	13.1	13.85	22.47
	2	Small molecule metabolic process	18.81	15.57	21.12
Cellular components	1	Cytoplasm	32.68	26.63	38.13
	2	Intracellular	27.83	21.06	25.57

Acknowledgments

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References

Rodrigues, P.M.; Campos, A.; Kuruvilla, J.; Schrama, D.; Cristobal, S. Proteomics in aquaculture: Quality and safety. In Proteomics in Food Science; Colgrave, M.L., Ed.; Academic Press: Cambridge, MA, USA, 2017; pp. 279–296, doi:10.1016/B9780-12-804007-2.00017-5.

PROTEOMIC ALTERATIONS IN TURBOT (*Psetta maxima*) TISSUES AFTER EXPOSURE TO COMMERCIAL TITANIUM DIOXIDE AND SILVER NANOPARTICLES

Mário J. Araújo[1,*], Maria L. Sousa[1], María Vázquez[2], Laura Rodriguez-Lorenzo[3], Mónica Quarato[3], Maria V. Turkina[4], Santiago Cabaleiro[2], Begoña Espiña[3], Alexandre Campos[1]

1 Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal
2 Centro Tecnológico del Cluster de la Acuicultura (CETGA), A Coruña, Spain
3 Life Sciences Department, INL-International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal
4 Department of Biomedical and Clinical Sciences, Faculty of Medicine and Clinical Sciences, Linköping University, 581 83 Linköping, Sweden
E-mail: mario.araujo@ciimar.up.pt

Nanotechnology is becoming widely used in several industrial activities including aquaculture. The exposure of farming organisms such as the flatfish turbot *Psetta maxima* to nanoparticles can result from trivial practices in aquaculture.

Proteomic tools can be used for environmental monitoring and aquaculture, namely for studying fish health and development. Large scale protein identification and quantification, using high-resolution mass spectrometry approaches, is indeed allowing to attain a more comprehensive understanding of alterations caused by specific stressors, and therefore to infer the risks of using specific chemicals on aquaculture operations.

Since current information on the effects of nanoparticles on aquaculture species, at the proteomic level is still scarce, this work aimed to study the effects of commercial titanium dioxide (TiO_2) and silver (Ag) nanoparticles (NPs), two of the most widely spread and used nanoparticles, in cultured turbot.

Fish were exposed to nanoparticles in one m^3 tanks (50 fish per tank, n=3) with pre-filtered (constantly running) seawater. Nanoparticles were incorporated in the commercial dry pellet diet in a concentration of 0, 0.75 and 1.5 mg/kg TiO_2 or Ag NPs and fish were fed daily. Liver and kidney samples were collected at day 14 of exposure and further analyzed for proteomics. Protein samples were prepared following Single-pot, solid-phase-enhanced sample preparation for proteomics experiments method (SP3), using the protease trypsin, and then analyzed by Orbitrap mass spectrometry. Differences in protein expression were detected employing the statistical method Differential Enrichment analysis of Proteomics data (DEP). Functional profiling was carried out using the web tool g:profiler (https://biit.cs.ut.ee/gprofiler/gost).

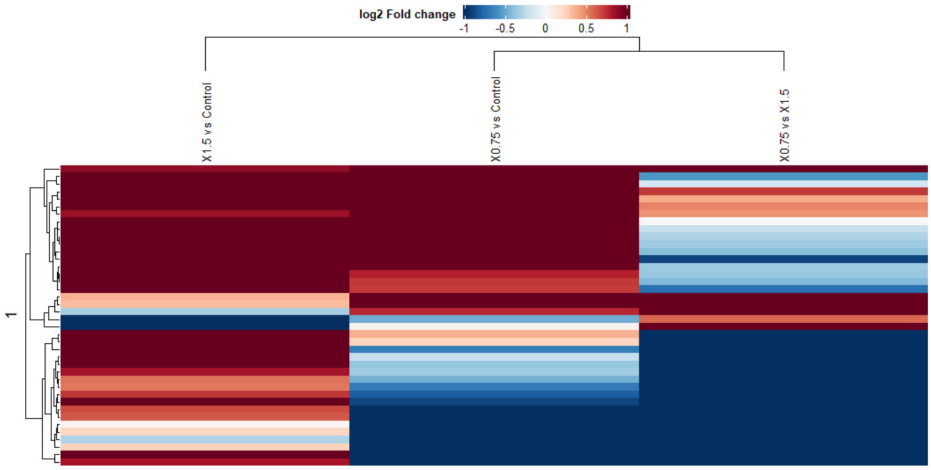

Fig. 1. Heatmap of kidney samples at the Ag NPs bioassay with the DEP, representing the 39 significant proteins variations among the treatments (control – 0 mg/kg, 0.75 mg/kg and 1.5 mg/kg Ag NPs) and respective replicates.

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The total number of proteins was not affected by the NPs, which ranged between 750 and 1000 in almost all samples except for kidney samples of Ag NPs bioassay (including the control group) with over 1500 proteins identified. Within the total number of proteins in each bioassay, the number of significantly expressed proteins between treatments was higher in both tissues at the Ag NPs bioassay when comparing to TiO_2 NPs bioassay (33 in the liver and 39 in the kidney for Ag NPs, and 13 in the liver and 12 in the kidney for TiO_2 NPs). When comparing samples from different treatments in both bioassays, the majority of the significant proteins were overexpressed in NPs exposed samples (e.g.: fig. 1).

Significantly altered proteins in response to Ag and TiO_2 NPs exposure retrieved, after a gene set enrichment analysis, a highly diverse gene ontology (GO) terms. The exposure to TiO_2 NPs seems to be related with alterations of several biological processes in tissues of turbot (overall 163 significant GO terms in kidney and 55 in liver), including positive regulation of mast cells activation or regulation of B cells differentiation in kidney and also lipid transport in liver. Ag NPs exposure seem to be associated with alterations in cellular components of kidney, such as ribonucleoprotein complexes or protein-containing complexes, with 29 GO terms associated with significant proteins from this tissue. Further discussion of these processes will be presented.

This work contributes to understanding the potential of omic approaches as a relevant tool for studying the organism mechanisms and responses at subcellular level, and therefore, for the development of aquaculture industry and improving the quality of its products.

Acknowledgments
This work is funded by NANOCULTURE Project – INTERREG Atlantic Area Program (European Regional Development Fund, ERDF) – EAPA 590/2018.

STRUCTURAL ENRICHMENT IN TANKS ENHANCES SPATIAL COGNITION OF JUVENILE GILTHEAD SEABREAM

P. Arechavala-Lopez[1,2,*], J.C. Caballero Froilán[3], D. Moranta[3], J. L. Saraiva[1]

1. Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal
2. Fish Ecology Group, Instituto Mediterráneo de Estudios Avanzados (IMEDEA-CSIC/UIB), Mallorca, Spain
3. Laboratory of Neurophisiology, Universitat de les Illes Balears (UIB), Palma de Mallorca, Spain
*E-mail: pablo@fishethogroup.net

Introduction

Environmental enrichment (EE) 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. Previous studies shown that structural EE modified the spatial distribution of fish, reducing fins erosions and aggressiveness of juvenile seabream (Arechavala-Lopez et al. 2019). However, in the present study, we wanted to assess the effects of structural EE on spatial cognition (exploratory behaviour and spatial learning abilities) of juvenile sea bream (*Sparus aurata*) under experimental conditions.

Material and Methods

A total of 90 sea bream juveniles (mean SL ±SE = 9.3 ± 0.1 cm) were randomly distributed to six 150 L rearing tanks (initial densities 5 kg m^{-3}; 15 fish $tank^{-1}$). Three tanks were enriched with five plant-fibre ropes hanging from the top, equally-distant to each other. Fish were maintained in both structural enriched (EE) and non-enriched (NE) tanks for 60 days. At the beginning (t0) and at the end (t60) of the experimental period, all fish were length measured (SL) and weighed (TW). In order to assess potential effects of EE on fish behaviour, a maze experiment was carried out on every juvenile seabream group during four trials by the end of the experiment (days 51, 54, 56 and 58). The maze consisted of four floating cylindrical cages (A: initial, E: enriched, B: bare, F: food), made of plastic net and foam rings, connected among them (see Arechavala-Lopez et al. 2020). The maze allows the assessment of spatial cognition through examining the exploratory behaviour of fish in a novel area and, as well as the spatial learning process of fish throughout the four-day trials of the experiment. Fish were recorded for 1 h, all recorded videos were visually analysed and the following behavioural parameters registered to compare between treatments and among tanks: latency of first individual to appear in a new area, latency of first individual to bite the food in area F, and frequency of fish movements between two areas.

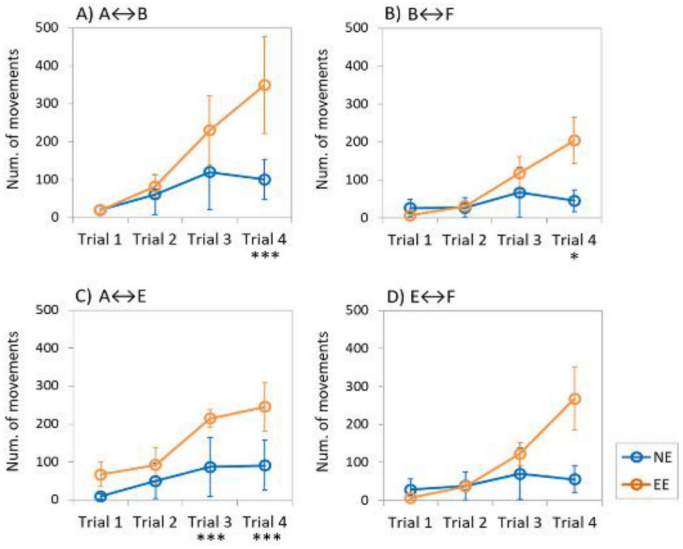

Figure 1. Mean number of movements (± SE) between areas (both ways) throughout the experimental trials. NE: non-enriched environmental conditions (blue); EE: environmental enriched conditions (orange). P-value: * < 0.05, *** < 0.001.

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Results

Morphological variables at the beginning (t0) and at the end of the experiment (t60), as well as their increments (Δt), did not show any significant difference between treatments and tanks. Fish reared with EE showed an overall exponential decrease in the latency to move to new areas, being significantly more evident when moving through the enriched area (area E) or to the area with the food (area F). Regarding the latency of the first individual to bite the food in area F, EE-reared fish showed a tendency to spend less time to bite in subsequent trials compared to NE-reared fish that showed no variation over time, although no significant differences were detected. The total amount of fish movements recorded between area A and B ($N_{A\leftrightarrow B}$), and between area B and F ($N_{B\leftrightarrow F}$), were significantly higher in EE fish during last trial compared to NE fish in both cases (Figure 1a,b). Similarly, the number of movements observed between areas A and E ($N_{A\leftrightarrow E}$) was significantly higher on EE-reared fish compared to NE-reared fish during the last trials (Figure 1c). However, no significant differences were observed between treatments on the number of movements between areas E and F ($N_{E\leftrightarrow F}$) (Figure 1d).

Discussion

This study evidenced for the first time that structural environmental enrichment influences positively on cognitive processes and welfare of juvenile seabream, enhancing their spatial cognition and exploratory behaviour. Cognition is not a single process but rather consists of three interacting aspects: perception, learning, and memory. Fish reared under enrichment are exposed to environmental challenges, higher visual complexity and new sensory stimulations. Results from the maze trials showed that EE-reared fish had better learning skills than the NE group but also that the experience is retained and consolidated through memory processes. Our results also showed that EE-reared fish presented higher behavioural flexibility and lower latency to reach the food chamber compared to NE-reared fish. Although physical structures might be a feasible, passive and non-invasive tool to improve welfare of captive fish, further works are needed in relation to structural design, husbandry conditions and fish developmental stage, as well as in other species, to be assessed under the production conditions in aquaculture.

USING ACOUSTIC TELEMETRY TO MONITOR SWIMMING BEHAVIOR OF GILTHEAD SEABREAM IN SEA CAGES

P. Arechavala-Lopez[1,2,*], L. Muñoz[2], E. Aspillaga[2], M. Palmer[2], J.L. Saraiva[1]

1. Fish Ethology and Welfare Group, Centro de Ciências do Mar (CCMAR), Faro, Portugal.
2. Fish Ecology Group, Instituto Mediterráneo de Estudios Avanzados (IMEDEA-CSIC/UIB), Mallorca, Spain
*E-mail: pablo@fishethogroup.net

Introduction

Acoustic telemetry techniques are very useful tools to monitor in detail the swimming behavior and spatial use of fish in the rearing environment at both individual and group levels. Previous studies reported the use of acoustic telemetry techniques to assess the swimming behavior within cages on diverse fish species of aquaculture interest, but there are no studies on gilthead seabream (*Sparus aurata*). Knowledge on the complexity of the swimming trajectory, the spatial distribution, the activity rhythms, is key to assess the biological requirements and welfare status of farmed fish species and, therefore, to improve both production and management in aquaculture. It is therefore necessary to develop and introduce new technological systems or tools that allow the correct observation of fish welfare at a commercial scale. The aim of this study was to evaluate the feasibility of using passive acoustic telemetry techniques in commercial conditions as a potential tool to monitor fish welfare in sea-cage aquaculture. We monitored gilthead seabream juveniles implanted with acoustic transmitters in experimental sea-cages to characterize for the first time the diel swimming and distribution patterns at a fine-scale.

Material and Methods

A total of 360 seabreams were used for the experiment, of which 10 seabreams (mean length ±SE: 20.17 ±1.09 cm; mean weight 217.58 ±55.96 g) were tagged with "accel-tag" acoustic transmitters (Thelma Biotel Ltd., model ADP-LP7), equipped with a pressure sensor and a triaxial accelerometer which provided measurements of the swimming depth (in m) and activity (m s^{-2}) respectively. Fish were then kept for one month in a experimental sea-cage , structurally similar than those used in commercial fish production, although smaller in size (12.5 m ø, 6 m depth). The swimming activity (acceleration) and spatial distribution (positioning) of each fish were monitored by an acoustic receiver array, composed by three receivers (Thelma Biotel Ltd.; model TBR 700) positioned around the sea-cage, suspended with anchored ropes attached to the floating rings of the cage structure at 5 m deep, forming a triangle. In order to assess the position accuracy of the system, a stationary reference tag (Sync-tag; Thelma Biotel Ltd., model R-MP13) was anchored at 3 m deep above one of the receivers. Data from the receivers was downloaded after completion ofthe experiment and first inspected using the software ComPort® (Thelma Biotel Ltd.), and then imported to R software for all the subsequent analysis (see further details in Muñoz et al. 2020)

Results

A total amount of 413,858 (depth and acceleration) valid receptions (99.94% of total detections) were recorded by the receiver array set in the experimental sea-cage during the whole monitoring period. Tagged fish showed similar patterns among individuals and a clear diurnal variation in group vertical movements, swimming closer to the surface during day-time compared to night time (Figure 1A). Acceleration values were similar between day and night periods, although a daily W-shaped pattern can be observed, with maximum mean values at night and during the afternoon (Figure 1B). Regarding 3D positioning, a total of 17,127 fish locations where successfully triangulated from the detection data. The volumetric space use of individuals showed differences between day and night times, where tagged fish showed greater vertical distribution during the night compared to the day (Figure 2).

Discussion

The combination of a receiver array and acoustic tags has provided for the first time detailed information on the swimming behavior and distribution of gilthead seabream directly in an experimental sea-cage. There was a clear variation in spatial use and swimming activity of farmed seabream, which seems to be influenced by circadian rhythms, synchronizing their locomotor activity to both light and feeding phases. The results demonstrated the potential feasibility of using acoustic telemetry for monitoring fish in commercial aquaculture and therefore it might be suggested as a tool for fish farmers to know in detail the specific behavior and distribution of fish in their facilities. With this knowledge, farmers may optimize the routine activities, feeding strategies and space in the farming-cages to improve both the performance and the welfare of their fish.

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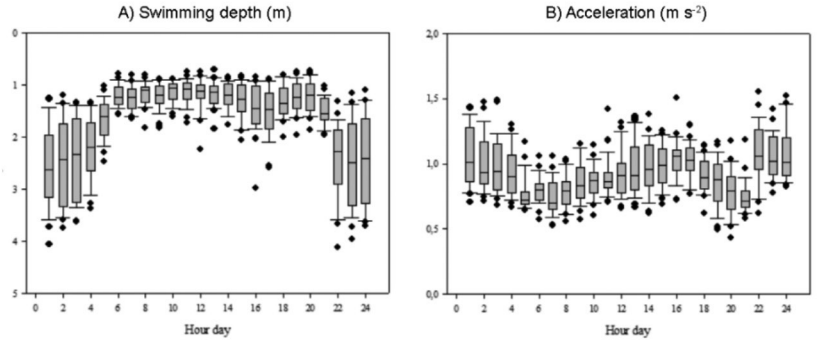

Figure 1. Daily patterns of swimming depth (A) and accelerations (B) of tagged gilthead seabream monitored in a floating sea cage.

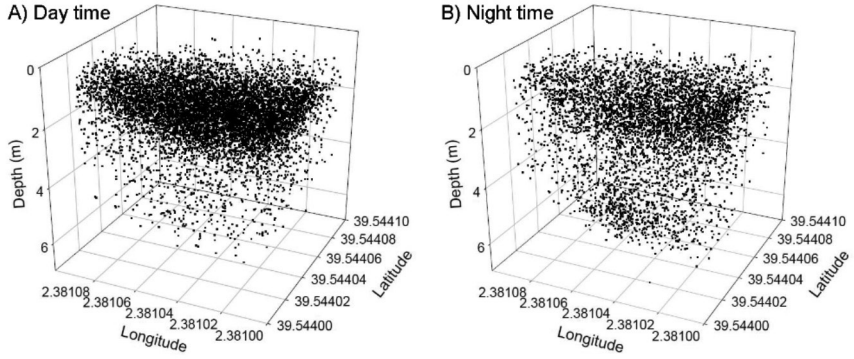

Figure 2. Spatial use (positioning) of tagged gilthead seabream during day (A) and night time (B) in a floating sea cage.

References

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

EFFECTS OF FISHMEAL REPLACEMENT BY DEFATTED *Zophobas morio* LARVAE MEAL ON GROWTH AND FEED EFICIENCY OF GILTHEAD SEABREAM (*Sparus aurata*)

Asimaki A.[1]*, Psofakis P.[1], Neofytou M.C.[1], Gkalogianni E.Z., Mente E.[1], Rumbos C.I.[2], Athanassiou C.G.[2], Fountoulaki E.[3], Henry M.[3], Karapanagiotidis I.T.[1]

[1] Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, University of Thessaly, Volos, Greece
[2] Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Volos, Greece
[3] Hellenic Centre for Marine Research, Athens, Greece
*mantwasim@gmail.com

Introduction

Aquaculture is still searching for suitable and sustainable alternative protein sources to replace fishmeal in aquafeeds. The use of insect meals for fishmeal replacement has recently attracted massive scientific interest, especially after their recent approval in the European aquafeed chain. So far, most research has focused on insect species which have already been approved for fish nutrition, such as *Tenebrio molitor and Hermetia illucens,* with very promising outputs (Henry et al. 2015). *However, other insect species that have not yet been studied extensively could also be proved suitable as fishmeal replacers. For instance, the giant mealworm, Zophobas morio*, is a large tenebrionid beetle species, with high nutritive value (Finke et al. 2002). The aim of the present study was to evaluate the use of different % inclusion of defatted mealworm in diets for sea bream juveniles; an important species of the Mediterranean Aquaculture.

Materials and Methods

Late-instar larvae of *Z. morio* coming from a colony raised in our laboratory were dried, milled and defatted under petroleum ether extraction in order to produce a *Z. morio* larvae meal containing 4% crude lipid and 69% crude protein. A total number of 360 *S. aurata* juveniles of 3.4g initial mean weight were obtained from a commercial fish hatchery, transferred to our Departmental facilities and then distributed after an acclimatization period of 10 days in triplicate to 12 closed seawater circulation system tanks (125L). Each of the four dietary group was fed isoenergetic (20 MJ/Kg) and isonitrogenous (52% CP) diets, in which fishmeal protein of the control diet (FM) was replaced by low-fat *Z. morio* at 10% (ZLF10), 20% (ZLF20) and 30% (ZLF30). Fish were fed to satiation twice a day, 6 days per week for 100 days in total.

Results and Discussion

Survival rates higher than 95% were recorded in all dietary groups without statistical difference among them (Table 1). Feed intake was similar among the groups suggesting that *Z. morio* is a highly palatable feed ingredient for *S. aurata*. In addition, all dietary groups had similar (P>0.05) final weight, specific growth rate, FCR, PER, protein and lipid retention. Up to date, studies with *Z. morio* in fish nutrition are scarce. In a previous study, we used a full fat, instead of a defatted, *Z. morio* meal as FM replacer in seabream's diet and found that a 10% replacement is possible without affecting growth performance and feed efficiency (Asimaki et al. 2020).

Table 1. Growth performance and feed utilization of *S.aurata* fed the experimental diets

Parameters / dietary groups	FM	ZLF10	ZLF20	ZLF30
Survival (%)	96.6 ± 3.3	97.7 ± 3.8	96.6 ± 3.3	95.5 ± 1.92
Feed intake (%/day)	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.6 ± 0.1
IBW (g/fish)	3.4 ± 0.0	3.4 ± 0.0	3.4 ± 0.0	3.4 ± 0.0
FBW (g/fish)	38.4 ± 2.1	42.2 ± 2.4	38.8 ± 1.8	39.2 ± 2.3
WG (g/fish)	35.0 ± 2.1	38.8 ± 2.4	35.4 ± 1.8	35.8 ± 2.3
SGR (%/day)	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	2.4 ± 0.1
FCR	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1
PER	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.5 ± 0.1
Protein retention (%)	27.3 ± 1.8	28.2 ± 1.6	28.3 ± 1.1	26.9 ± 0.7
Lipid retention (%)	63.1 ± 7.8	74.8 ± 0.6	69.8 ± 3.2	74.9 ± 4.5

Note: Values represent means ± standard deviation of triplicates. No significant differences (P> 0.05) were noted among dietary groups for any of the parameters tested.

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Alves et al. (2021) testing *Z. morio* in Nile tilapia diet reported that even 30% fishmeal replacement had no adverse effects on growth performance and feed utilization, but changed the body proximal composition and modulated the innate immune response. Jabir et al. (2012) reported that even 100% FM replacement did not reduce significantly the growth of Nile tilapia. Interestingly, when *Z. morio* was used in combination with house cricket (*Acheta domesticus*) to replace FM at 25% in the diet of perch (*Perca fluviatilis*) the growth of fish and feed efficiency decreased (Tilami et al. 2020). Studies with other insect species in seabream's diet showed that up to 25-30% FM replacement is possible by *T. molitor* (Piccolo et al. 2017) and *H. illucens* (Karapanagiotidis et al. 2015). Overall, the findings of the present study suggest that the defatted *Z. morio* is an attractive feedstuff that could successfully replace fishmeal protein in seabream's diet up to 30%

Acknowledgements
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References

Alves, A.P., Paulino, R.R., Pereira, R.T., da Costa, D.V., Rosa, P.V. (2021). Aquaculture Research 52, 529-540.
Asimaki A., Psofakis P., Ekonomou G., Mente E., Rumbos C.I., Athanassiou C.G., Fountoulaki E., Henry M., Karapanagiotidis I.T. (2020). Journal of Insects as Food and Feed, 2020; 6 (Supplement 1), pp. S24.
Finke, M.D. (2002). Zoo Biology 21, 269-285.
Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E. (2015). Animal Feed Science and Technology 203, 1-22.
Jabir, M.D.A., Razak, S.A., Vikineswary, S. (2012). African Journal of Biotechnology 11, 6592-6598.
Karapanagiotidis I.T., Daskalopoulou E., Vogiatzis I., Rumbos C., Mente E., Athanassiou C.G. (2015). European Aquaculture Society, Rotterdam, The Netherlands, 20-23 October, pp. 410-411.
Piccolo, G., Iaconisi, V., Marono, S., Gasco, L., Loponte, R., Nizza, S., Bovera, F., Parisi, G. (2017). Animal Feed Science and Technology 226, 12–20.
Tilami, S. K., Turek, J., Červený, G., Lepič, P., Kozák, P., Burkina, V., Sakalli, S., Tomčala, A., Sampels, S., Mráz, J. (2020). Turkish Journal of Fisheries and Aquatic Sciences 20, 867-878.

THE SEAFOOD 2040 PROGRAMME IN ENGLAND: WORK IN SUPPORT OF FISHERIES, AQUACULTURE AND SEAFOOD DEVELOPMENT

N. A. Auchterlonie

Chair Seafood 2040

Email: neilauchterlonie@btinternet.com

Seafood 2040 is a programme established from the work of a Task Force in 2015 set up at Ministerial request to "to explore the challenges and opportunities facing the English industry and to shape a long-term ambition that could realise the full potential of the industry by 2040". The programme is almost unique in taking a whole supply chain approach that encompasses both capture fisheries and aquaculture, as well as the processing sector, distribution, retail, and the consumer. The programme is based on the principles of collaboration, science, best practice and communication, and provides a platform for industry, government and public bodies to work together towards a thriving and sustainable seafood sector in England.

Seafood 2040 managed the delivery of the English Aquaculture Strategy (EAS) in late 2020. This presentation covers the SF2040 Programme and the detail of the EAS actions and the work in support of a developing aquaculture industry in England.

NEUROENDOCRINE AND OPIOID RECEPTOR GENES IN A TELEOST BRAIN – RESPONSE TO ACUTE INFLAMMATION

R. Azeredo[1*], M. Machado[1], P. Pereiro[2], A. Barany[3], J. M. Mancera[3], B.Costas[1,4]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Matosinhos, Portugal
E-mail: mleme@ciimar.up.pt
[2] Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain
[3] Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina, University of Cádiz, Cádiz, Spain
[4] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Porto, Portugal

Introduction

In teleost fish, similar to mammals, the hypothalamus-pituitary-interrenal (HPI)-axis response to inflammatory stimuli is defined and orchestrated by a complex network of immune and neuroendocrine mediators as well as their receptors (1). The presence/absence, abundance and distribution (either regional or at cellular level) of these effector molecules are important factors regulating communication, stimulatory and suppressive mechanisms. More in-depth knowledge of such mechanisms is very relevant when optimizing strategies that reduce stress during the fish rearing process. Aiming at disclosing both the distribution profile and behaviour of neuroendocrine and opioid mediators of European seabass (*Dicentrarchus labrax*), a study on the brain mRNA levels of these genes was conducted in an acute inflammation setting.

Material and methods

Juvenile European seabass (87.3 g ± 16.5) were acclimatized in a flow-through seawater system (temperature 18 ºC; Salinity: 40; Photoperiod: natural summer time; n = 12) for 30 days being fed a commercial diet throughout the whole experiment. At the end of this period, 8 fish were sacrificed by overdose of 2-phenoxyethanol and brains were collected and dissected into telencephalon (TLC), optic tectum (OT), hypothalamus (HYP) and pituitary (PIT). Samples were kept in RNA*later* (SIGMA) at 4 ºC during 24 h and finally stored at -80 ºC until further processing. These initial sampling was designated as time 0 h (t0h). The remaining fish were either subjected to an intraperitoneal (i.p) injection of 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 4, 24, 48 and 72 h following i.p injection (n = 4 per tank). Fish were then sampled as previously described. The expression of genes related to neuroendocrine mediators and receptors, serotonergic activity and opioid system was evaluated in the four brain regions by real-time quantitative PCR.

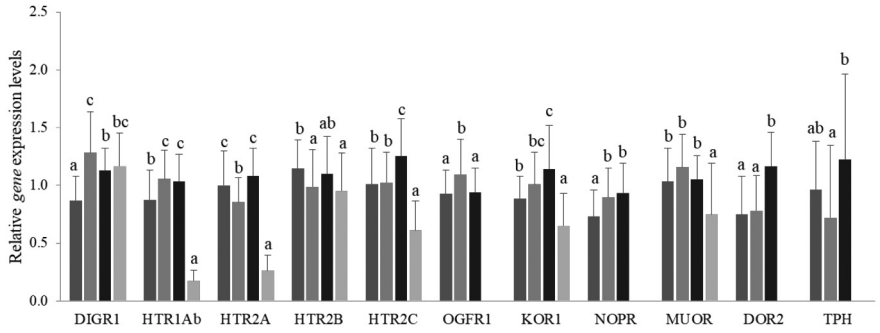

Fig. 1. Glucocorticoid receptor 1 (*gr1*), serotonin receptor 1A (*htr1a*), 2A (*htr2a*), 2B (*htr2b*), 2C (*htr2c*), opioid growth factor receptor 1 (*ogfr1*), opioid growth factor receptor 2 (*ogfr2*), kappa opioid receptor 1 (*kor1*), kappa opioid receptor 2 (*kor2*), nociception receptor (*nopr*), mu opioid receptor (*muor*), delta opioid receptor 2 (*dor2*) and tryptophan hydroxylase (*tph*) expression levels in the TLC (■), HYP (■), OT (■) and PIT (■) of European seabass intraperitoneally injected with either FIA or HBSS and sampled 4, 24, 48 and 72 h post-injection (mean ± SD, n = 64). Different letters indicate significant differences between regions for each gene. One-way ANOVA; Tukey *post hoc* test ($p \leq 0.05$)

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Results

A total of 19 genes (including 2 housekeeping genes) were evaluated in the four brain regions of the European seabass. Therefore, the present results are only part of the complete analysis. Results regarding the expression levels of the "stress" glucocorticoid receptor 1 (*gr1*) showed upregulation of this receptor in both the OT and the HYP from 4 to 72h post-injection and from 0h to 72h, respectively, regardless the nature of the stimulation. With respect to serotonin receptors, *htr2b* and *htr2c* seemed to be the most abundant receptors in the four studied regions, based on the mean expression value for each tissue, irrespective of sampling time and injection nature. Acute stress (i.p. injection itself, regardless stimulation nature) inhibited *htr2a* and *htr2b* transcripts in the TLC over time, enhanced *htr2c* over time in the OT as well as in the HYP, where *htr2b* was also gradually upregulated over time post-injection. Inflammation (FIA-injected fish) increased TLC expression of *htr2b* and *htr2c* at 4h post-injection compared to t0h. Contrarily, it downregulated *htr2c* in the PIT. Among the evaluated opioid receptors, *ogfr2* presented the highest mRNA levels in the TLC, OT and HYP. Acute stress inhibited *nopr* in the TLC and *muor* in both the HYP and the PIT. On the other hand, it induced expression of *ogfr2* and *muor* in the OT as well as *ogfr1*, *ogfr2*, and *kor2* in the HYP. Only *dor2* was observed to be downregulated by inflammation in the HYP. Tryptophan hydroxylase (*tph*) was downregulated in the TLC and the HYP by acute stress. Both corticotropin releasing factor (*crf*) and corticotropin releasing hormone-binding protein (*crhbp*) expression was induced by acute stress at 72h in the HYP. Expression values distribution across brain regions can be observed in Fig. 1.

Discussion and conclusion

This study takes a first look into key genes distribution in the European seabass and how their expression values fluctuate under inflammatory conditions. Transcription of several of these genes was sensitive to the acute stress caused by injection, as it was frequently seen modulated not only in FIA-injected fish (known to cause a mild but chronic inflammatory response) but also in fish injected with innocuous salt solution such as HBSS. From hypothalamic *crf* that was induced in both groups, to serotonin receptors that propagate this monoamine neurotransmission and thereby induce secretion of stress-related hormones, or that instead are involved in feedback mechanisms and thus regulate serotonin release. Moreover, the evaluated opioid receptors, which expression is known to be induced by inflammation in carp (3), and from which there is still scarce information on their function in teleost brain compared to mammals, responded differently to FIA and HBSS, and was also dependent on the brain region. These preliminary results are a first step unveiling the complexity in distribution patterns and responsiveness to different stimuli that not only differ from that of mammals but might also be species-specific.

Acknowledgements

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References

1. Verburg-Van Kemenade et al. 2009 DOI: 10.1016/S1546-5098(09)28007-1
2. Nadocci et al. 2014 DOI: 10.1016/j.fsi.2014.08.001
3. Chadzinska et al. 2009 DOI: 10.1016/j.bbi.2008.10.003

OFFSHORE PRODUCTION OF *Saccharina latissima* IN THE FAROE ISLANDS AND THE CHALLENGE OF SCALING UP IN THE ATLANTIC OCEAN

U. G. Bak*[1], J. Kristmundsson[2], D. Resnick, F. Marsman[1], Ó. Gregersen[1], G. Norði[2]

[1] Ocean Rainforest Sp/F, Mjólkargøta 20, FO-180 Kaldbak, the Faroe Islands
[2] Fiskaaling P/F við Áir, FO-430 Hvalvík, the Faroe Islands

Introduction
There is a present need to increase aquaculture production in the Atlantic Ocean by developing new and sustainably aquaculture value chains for food and feed production. Brown macroalgal species (kelps) are among the fastest-growing crops on the planet. To grow, they only need sunlight, CO_2, nutrients that are naturally occurring in the ocean and no fertilizer is needed. They need a substrate to attach to, e.g., a cultivation line. When this productivity becomes commercially exploited and transferred to the large surface of the open-ocean large quantities of biomass can be produced.

The global macroalgae market was €5 billion in 2014. This is expected to double by 2024 (FAO 2016). Currently, Asia is accounting for more than 99% of the production mainly from cultivated macroalgae grown in shallow seawaters (water column <30 meters). The shallow water cultivation is facing spatial and environmental problems.

The SME Ocean Rainforest has developed a MacroAlgal Cultivation Rig (MACR) suitable for offshore environments (Bak et al. 2018). This environment has previously been a major challenge for macroalgal commercialization due to harsh open conditions (Bak et al. 2020). The use of multiple partial harvesting of kelp species (up to 6 harvests without re-seeding) has allowed making the highest known harvesting yield in Europe and has the potential to reduce the production cost by 75% (Bak et al. 2018).

The H2020-project AquaVitae will demonstrate new processes that enable more cost-efficient macroalgal production and upscaling of this underexploited and sustainable biomass source in open-ocean.

Method
Establish and prove large scale offshore sustainable macroalgal cultivation in the Faroe Islands (>300 tonnes) by using optimised logistics, re-use of aquaculture equipment and site selection, to reduce the cost of production for the brown macroalgae *Saccharina latissima*.

Results
First in 2020, the Faroese legislation has allowed seaweed cultivation licenses in Faroese waters. In the past macroalgal farmers had to borrow licenses of the major salmon producers. Having macroalgal licenses is particularly important to scale up the business and to attract investors.

With the new legislation, sites selection was needed for further upscaling in the Faroese waters. As a result of the project AquaVitae, Fiskaaling pinpointed potential sites for seaweed production (Figure 1). To run the model, a set of parameters was made to describe how the conditions should be to sustain optimal growth. The main parameters were wave height, current speed, and bottom depth. Also, remaining parameters like distance to fish-farming sites, cities and harbours were included.

After the successful site selection, four attractive sites were selected, and 40 km growth lines were deployed during autumn 2020 (Figure 2), thus doubling the capacity at sea. The yield capacity in 2021 will thus be 300 tonnes wet weight. The company Ocean Rainforest are aiming for another 120 km growth lines deployed in 2021.

Beside upscaling, the project has identified new harvesting methods that enable the maximum yield. Yet there is a need to reduce operating costs even further and additional technological development to upscale the operation is needed. The upscaling in seeding material for the 40 km new 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 challenging parts of future upscaling.

Discarded anchors and buoys were re-used in the deployments of the 40 km growth lines, which reduced the expenses of capital cost significantly.

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Perspectivation

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. Through a review, we will select commercial interesting local macroalgal species for cultivation for each region and optimise the design of cultivation rig based on the principles of the MacroAlgal Cultivation Rig (MACR). Thus, does the Faroese seaweed production add crucial results for future expansion of this underexploited aquaculture industry.

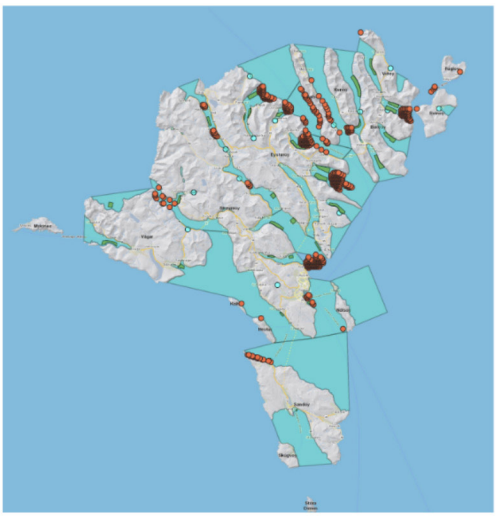

Figure 1 *Example of the overview result showing suitable sites for offshore macroalgal production from the Faroe Islands (Fiskaaling P/F 2019).*

Figure 2 *The newly deployed growth lines seeded with Saccharina latissima in the Faroe Islands (Ocean Rainforest Sp/f 2020).*

EFFECT OF DIFFERENT DIETS CONTAINING ROTIFERS AND COPEPODS AS FIRST FEEDING ON *Sander lucioperca* (LINNAEUS, 1758) LARVAE

Laura Ballesteros-Redondo[a,*], Harry W. Palm[a], Hanno Bährs[b] and Adrian A. Bischoff[a]

[a]University of Rostock, Faculty of Agricultural and Environmental Sciences, Department of Aquaculture and Sea-Ranching, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany
[b]Aquacopa GmbH, Hoher Damm 25, 17194 Jabel, Germany
*Correspondence: laura.redondo@uni-rostock.de

Introduction

Sander lucioperca production has grown in recent years (FAO 2020). The interest on its sustainable production requires more effort in areas such as the nutritional requirements for a successful larval rearing (Policar et al. 2019). Many factors should be taken into account to find accurate organisms for the first feeding of fish larvae like its swimming behaviour, its size, and its nutritional value or biochemical composition. In this context, copepods are gaining more and more attention (Ajiboye et al. 2010).

Material and methods

Two independent experiments took place in 2020 in order to investigate the effect on the larval survival and growth rates until 10dph applying several diets, which contain different concentrations and combinations of rotifers and copepods. Two diets (B100-200 and B50+A50-200) were studied during the first experiment and six diets in the second experiment (B100-400, B100-600, B85+A15-400, B85+A15-600, B70+A30-400 and B70+A30-600) (Table 1). Water parameters as well as dead larvae were monitored on a daily basis and larval samples were collected at 10dph to measure total body length. Consequently, survival rates and specific growth rates (SGR) were calculated.

Results

The highest survival rate and SGR were found in diet B100-200 during the first experiment and in the second experiment in diet B85+C15-400 (Table 1). No effect of diets in the survival was detected. There were significant differences concerning the growth rates for both experiments.

Discussion

Our study does not show a direct benefit of the introduction of copepods in the diet during the first 7 feeding days (dph3 – dph10). However, the results indicate an optimal nutritional status of pikeperch larvae in relation to a threshold in *B. plicatilis* quantity per larvae per day directly related to the stocking density. The food intake efficiency can only be reached at a certain food concentration (Imentai et al. 2019, Molnar et al 2004, Ramos et al. 2016, Szkudlarek et al. 2007, Żarski et al. 2011). Therefore, *Sander lucioperca* larvae should be reared until dph10 with at least 340 individuals of *B. plicatilis* per larvae per day at a concentration of 16 *B. plicatilis**mL^{-1} or in other words, at a stocking density of 47 larvae*L^{-1} in order to maximize survival and growth. Further studies should investigate the use of copepods for later stages of pikeperch larval rearing.

Table 1. Diet composition containing different amounts and densities of *B. plicatilis* (Rotifera) and *A. panamensis* (Copepoda) (zooplankton specimens=ZS) used in the experiments (E) and the obtained survival rate and SGR

Diet	E	Composition	ZS*$fish^{-1}$ *day^{-1}	ZS*mL^{-1}	Survival (%)	SGR (%*d^{-1})
B100-200	1	*B. plicatilis* 100%	200	6.98	71.8	1.24
B50+A50-200	1	*B. plicatilis* 50% *A. panamensis* 50%	200	6.98	69	0.8
B100-400	2	*B. plicatilis* 100%	400	18.95	88.9	2.3
B100-600	2	*B. plicatilis* 100%	600	28.42	86.7	2.2
B85+A15-400	2	*B. plicatilis* 85% *A. panamensis* 15%	400	18.95	93.3	2.7
B85+A15-600	2	*B. plicatilis* 85% *A. panamensis* 15%	600	28.42	94.4	2.1
B70+A30-400	2	*B. plicatilis* 70% *A. panamensis* 30%	400	18.95	63.3	0.9
B70+A30-600	2	*B. plicatilis* 70% *A. panamensis* 30%	600	28.42	93.3	2.0

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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.

FAO Fish Stat (http://www.fao.org/fishery/culturedspecies/Sander_lucioperca/en) (2020).

Imentai, A, Yanes-Roca, C, Malinovskyi, O, Policar, T. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding. J Appl Ichthyol. 35: 1292– 1294 (2019). https://doi.org/10.1111/jai.13963

Molnár, T., Hancz, C., Bódis, M., Müller, T., Bercsényi, M., Horn, P. The effect of initial stocking density on growth and survival of pikeperch fingerlings reared under intensive conditions. Aquaculture International 12, 181–189 (2004). https://doi.org/10.1023/B:AQUI.0000032079.62056.8c

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

Ramos, F.M., Abe, H.A. and Fujimoto, R.Y. Survival and growth of early life stages of leaf fish (*Monocirrhus polyacanthus*, Heckel 1840) cultured under different stocking densities and live food densities. J. Appl. Ichthyol., 32: 954-959 (2016). doi:10.1111/jai.13092

Szkudlarek, M., Zakęś, Z. Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. Aquacult Int 15, 67–81 (2007). https://doi.org/10.1007/s10499-006-9069-7

Żarski, D., Kupren, K., Targońska, K., Krejszeff, S., Furgała-Selezniow, G. and Kucharczyk, D. The effect of initial larval stocking density on growth and survival in common barbel *Barbus barbus* (L.). J Appl Ichthyol. 27: 1155-1158 (2011). https://doi.org/10.1111/j.1439-0426.2011.01769.x

EFFECT OF DIFFERENT MICROALGAE DIETS ON *Apocyclops panamensis* (MARSH, 1913) AS A LIVE FOOD FOR FISH LARVAL REARING

Laura Ballesteros-Redondo[a,*], Harry W. Palm[a], Hanno Bährs[b] and Adrian A. Bischoff[a]

[a]University of Rostock, Faculty of Agricultural and Environmental Sciences, Department of Aquaculture and Sea-Ranching, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany
[b]Aquacopa GmbH, Hoher Damm 25, 17194 Jabel, Germany.
*Correspondence: laura.redondo@uni-rostock.de

Introduction

In the recent years, more effort has been done to find an appropriate food to rear fish larvae in order to decrease the cost of production in aquaculture and also to increase the fish welfare. One of the main problems is to define what an appropriate food for the first days of the fish larvae feeding is. *Artemia* sp. and *Brachionus* sp. are most commonly used species for fish larvae rearing but there is still a lack of knowledge in the nutritional requirements of the fish larvae, which appear to be species specific and not to be fulfilled by these diets. In this context, copepods are gaining more and more attention (Ajiboye et al. 2010). *Apocyclops panamensis* seems to be a promising candidate for intensive culture (Phelps et al. 2005).

Material and methods

Apocyclops panamensis, a cyclopoid copepod, was provided by Aquacopa GmbH (Jabel, Germany) and cultured at the facilities of Rostock University in a 200 L zooplankton reactor at 23ºC with a light cycle of 18:6 (L:D) and salinity of 30 g/L. We fed them each 4-5 days with 0.5-1.0*10^6 cells of *Isochrysis galbana* per mL. Three experiments took place between 2020 and 2021. The experiments consisted in the analysis of the population structure (nauplie, copepodites, males and females), size and density. In order to start the experiments, original cultures were filtered several times through a net of 100 μm to remove adults and copepodites and through a net of 50 μm to take nauplii (size approx. 80 μm). We collected and concentrated the nauplie in new saltwater at a density of 4, 8 and 34 nauplie*mL, respectively to each experiment. For each experiment, we studied the same diets: NANO100% (*Nannochloropsis* sp. at 200.000 cells*mL^{-1}*day^{-1}), NANO+ISO (*Nannochloropsis* sp. at 100.000 cells*mL^{-1}*day^{-1} + *I. galbana* at 50.000 cells*mL^{-1}*day^{-1}) and ISO100% (*I. galbana* at 100.000 cells*mL^{-1}*day^{-1}), each with 3 replicates. We fed the copepods once a day (at 12 a.m.) for a period of 20 days. Several serial algae cultures of each species were used to ensure the feeding at the exponential phase. Samples were collected each second day for the population, density and size measurements and fixed with Lugol.

Results

During the first and second experiments (with an initial stocking density of 4 and 8 nauplie*mL^{-1}, respectively), survival was high and reproduction occurred around 11th day after the beginning of the experiments, indicated by the increase in density. However, during the last experiment (34 nauplie*mL^{-1}), the high initial density produced high mortality, probably due to the lack of enough food. There were no significant differences in density, in the proportion of nauplii, copepods, males and females, or in the size of each of these groups in relation to the three diets in any of the three experiments.

Discussion

Our study shows no different effect of *Nannochloropsis* sp. and *I. galbana* on the population growth, survival, reproduction and size of *A. panamensis* indicating a stable candidate for aquaculture. The differences in fatty acids of both microalgae species during the exponential phase (Roncarati et al. 2004) do not affect the *A. panamensis* population. The choice of the diet for *A. panamensis* will depend on the fatty acid profile of the copepods that is needed for each fish species. Our study reveals that *A. panamensis* is a good candidate for rearing fish larvae as each group maintains its size without depending on diet, and therefore can be sorted depending on the mouth opening of the fish. The advantages of *A. panamensis* have already been previously pointed out (Cruz-Rosado et al. 2020, Phelps et al. 2005) and consequently, more research should be carried out in order to find out whether *A. panamensis* is suitable for different fish species.

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References

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

Cruz-Rosado L, Contreras-Sánchez WM, Hernández Vidal U, Perez-Urbiola JC, Contreras García MJ (2020) Population growth of a generational cohort of the copepod *Apocyclops panamensis* (Marsh, 1913) under different temperatures and salinities. Ecosistemas y Recursos Agropecuarios 7(2): e2505. DOI: 10.19136/era.a7n2.2505

Phelps, R.P., Sumiarsa, G.S., Lipman, E.E., Lan, H.-P., Moss, K.K., Davis, A.D. (2005) Intensive and extensive produciton techniques to provide copepod nauplii for feeding larval red snapper *Lutjanus campechanus*. In: Copepods in Aquaculture (Ed. by C.-S. Lee, P.J. O'Bryen, N.H. Marcus), pp. 151-168. Blackwell Publishing Ltd, Oxford, UK

Roncarati, A., Meluzzi, A., Acciarri, S., Tallarico, N. and Meloti, P. (2004), Fatty Acid Composition of Different Microalgae Strains (*Nannochloropsis* sp., *Nannochloropsis oculata* (Droop) Hibberd, *Nannochloris atomus* Butcher and *Isochrysis* sp.) According to the Culture Phase and the Carbon Dioxide Concentration. Journal of the World Aquaculture Society, 35: 401-411. https://doi.org/10.1111/j.1749-7345.2004.tb00104.x

A NOVEL SYSTEM FOR WILD FISH MONITORING AT AQUACULTURE SITES - ARTIFICIAL INTELLIGENCE IN WILD FISH ABUNDANCE ANALYSIS

K. Banno*, H. Kaland, A. M. Crescitelli, S. A. Tuene, G. H. Aas, and L. C. Gansel

Department of Biological Sciences Ålesund, Norwegian University of Science and Technology, Postbox 1517, N-6025 Ålesund, Norway
Email: kana.banno@ntnu.no

Introduction

Aquaculture net cages in coastal areas attract wild fish (e.g. Dempster et al., 2009)1200 salmon farms operate in coastal Norway, yet their capacity to aggregate and sub sequently modify wild fish distributions is poorly known. Aggregations of wild fish at 9 farms and 9 control locations were counted on 3 separate days in June to August 2007. On each sampling occasion, 6 counts were made at 5 distinct depth-strata at each farm and control location. Wild fish were 1 to 3 orders of magnitude more abundant at farms than at control sites, depending on the location. Gadoid fish (Pollachius virens, Gadus morhua and Me-lanogrammus aeglefinus. Multiple causes for this have been discussed, amongst others that fish farms structures can work as a shelter for wild species and that uneaten feed and other potential food items are attractants for wild fish. Aquaculture sites thereby can affect availability and quality of some major species in fisheries (e.g. Uglem et al., 2020), and there is concern about biosecurity by disease and parasite transmission between farmed and wild individuals. The presence of wild fish might also stress farmed fish inside sea cages, and its impact on health and welfare of farmed fish is not fully described. Despite the importance of understanding patterns of amounts, composition and distribution of wild fish aggregating around aquaculture sites, autonomous systems for monitoring wild fish have not been fully deployed yet. In recent years, the use of artificial intelligence in the automatic recognition of fish has seen major advancements, and this technology can be applied in wild fish monitoring at fish farms. A system based on machine learning techniques to detect fish and determine their species from underwater videos is currently under development at the department of biological sciences Ålesund, NTNU in Norway. We aim to create and publish large datasets of fish species commonly seen in Norwegian waters, by performing object recognition. First results are described in Crescitelli et al. (2020) where the methodology is described. Such datasets are very well suited for training artificial neural networks to monitor fish in farming areas. This study aims to propose a complete monitoring system with multiple cameras that can stream videos to a computer for continuous analysis of wild fish distribution. As a case study, we tested our methods at coastal sea cages for Atlantic salmon (*Salmo salar*) located along the west coast of Norway. The work we present is part of an ongoing research effort.

Materials and methods

This study was conducted at a commercial Atlantic salmon aquaculture farm located in Møre and Romsdal, Norway. Long term monitoring of wild fish, mainly saithe (*Pollachius virens*), and Atlantic mackerel (*Scomber scombrus*), was performed using underwater cameras (GoPro) on multiple days from July to October 2020. Video recordings were made around two sea cages relatively close to each other, and at two anchoring buoys outside the cages that acted as short distance reference stations. At the two sea cages, the cameras were let to sink straight down into the water from six different points, equally spread around a circular cage, and videos were taken at six different depths (2m, 7m, 13m, 20m, 30m, 40m) at each location (6 positions per cage, and 1 per buoy) on 5 sampling days. Images were extracted at specific time intervals from all videos, to evaluate three-dimensional distribution patterns of wild fish. Wild fish in each image was counted, with an automated recognition system based on the neural network architecture YOLOv4. The system counted a fish only if its confidence of the detected object being a fish was above 30%. Manual count of wild fish in the same images are used to assess the accuracy of the automated recognition system.

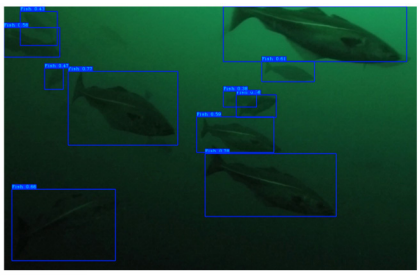

Figure 1: Example of the output of the fish recognition system.

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Results

Preliminary results show that it is possible to gain good quality of images from simple cameras with small sensors. Obtaining good images at deeper depth (below 30m) was a challenge due to limited light intensity but giving a slight upwards angle to cameras helped to improve the image quality. In addition, image processing improved images to the point that automated detection was functional. The accuracy of automated analysis was mainly dependent on water quality and distance to fish, as features that the model has learnt need to be visible. Some challenges arose to detect every individual when the fish density was high and they were highly overlapped. However, the automated recognition system precisely identified wild fish in many of the images (see an example in figure 1). The system identified few thousands of wild fish within a few meters from a cage. The distribution of wild fish was uneven both horizontally and vertically. In general, saithe tended to aggregate in deeper water (below 30m), while mackerel preferred shallower water (above 20m), and it was not always the case that wild fish was aggregating close to feed spreaders.

Discussion

This study reveals a great potential for continuous and autonomous analysis of wild fish distribution. It is likely that the cutoff distance for successful fish detection is dependent on species. Quality and amount of images used for training neural networks will affect the water quality and distance to fish that the recognition system can cope with. Using several images in short temporal succession may remedy the problem where not all fish are recognized when they are overlapping or in situations with high fish density. Uneven distribution of the wild fish is likely to be related to specie's own depth preference and availability of feed, due to transport of feed by changing water current. Based on the preliminary results, autonomous monitoring presents some advantages. One clear advantage is fast recognition, species determination and counting of fish. The system can process an image in milliseconds, which makes it suitable for real-time applications. It can also be used to analyze long video footage, reducing the human workload necessary for manual analysis drastically. Another advantage is the precision and repeatability of the data, compared to the methods where divers are manually counting wild fish. Some false positive and false negative detection were found in the results of the automated counting. However, using proper images in the learning process of the model can vastly improve the results, and results can easily be quality assured and improved by removing false positives manually with little effort. In our future work, neural networks will be trained to recognize different fish species. The system's hyperparameters will be fine-tuned and tracking individual fish will be added to further increase accuracy of automatic fish counts. Our ultimate goal is to enable an automated, continuous quantification of wild fish of relevant species. This can be used for various purposes such as investigation of wild fish migration patterns, as well as wild fish monitoring around sea cages.

References

Crescitelli, A. M., Gansel, L. C., & Zhang, H. (2020). Semi-automatic Approach to Create Fish Image Datasets for Aquaculture Applications. *2020 15th IEEE Conference on Industrial Electronics and Applications (ICIEA)*, 1450-1455.

Dempster, T., Uglem, I., Sanchez-Jerez, P., et al. (2009). Coastal salmon farms attract large and persistent aggregations of wild fish: An ecosystem effect. *Mar Ecol Prog Ser*, *385*, 1–14.

Uglem, I., Toledo-Guedes, K., Sanchez-Jerez, P., et al. (2020). Does waste feed from salmon farming affect the quality of saithe (*Pollachius virens* L.) attracted to fish farms? *Aquac Res*, *51*, 1720–1730.

ECO-INNOVATIVE BIOFORTIFIED FARMED FISH: TAILORING GILTHEAD SEABREAM AND COMMON CARP NUTRITIONAL VALUE WITH IODINE AND SELENIUM NATURALLY ENRICHED FEEDS

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

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

Introduction

The increased demand for healthier and sustainable fish products is gaining relevance not only to improve human health, but also to prevent nutritional deficiencies of iodine (I) and selenium (Se), which result in neurophysiological and immunological disorders. Different strategies have been developed to improve the nutritional quality and safety of seafood, including the fortification of fish (FAO, 2018). In this context, the development of fortified farmed fish through aquaculture feeds modulation with sustainable marine ingredients (e.g. iodine-rich macroalgae and Se-rich yeast) can be an excellent tool to increase the value of aquaculture fish products (Ramalho Ribeiro et al., 2017). The present study aimed to assess the effects of biofortified feeds, using I-rich macroalgae and Se-rich yeast, to modulate fish fillets elemental composition of two of the most commonly farmed fish species in Europe, namely gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*).

Material and methods

A control commercial diet and three experimental diets incorporating various blends of macroalgae and Se-rich yeast were formulated for the two fish species. Fish were feed for three months, mimicking the end of the production stage (i.e. just before reaching market size). Each diet was tested in triplicate tanks (n = 150 fish/diet for seabream and n = 300 fish/diet for carp) and fish were slaughtered 48 h following the last meal, by immersion in chilled seawater (seabream) or in chilled freshwater (carp) following the commercial procedure in fish farms. Fish skinless fillets were collected (n = 3 pools of 5 fish each) and I, Se and arsenic (As) contents were determined by inductively coupled plasma mass spectrometer, mercury (Hg) by atomic absorption spectrometry, Cd and Pb by flame atomic absorption spectrometry, and Cl, K, Ca, Fe, Cu, Zn and Br by micro-Energy Dispersive X-Ray Fluorescence (Barbosa et al., 2020).

Results and Conclusions

The incorporation of I-rich macroalgae and Se-rich yeast in gilthead seabream and common carp feeds resulted in increased I (37% in seabream and over 100% in carp), Se (98% in seabream and 41% in carp) and Fe (over 100% in seabream and 50% in carp) contents in fillets. Moreover, biofortified seabream and carp revealed lower Cu (<LOD in seabream and 78 % decrease in carp) and Br (<LOD in seabream and 61% decrease in carp) levels. The reduction of fishmeal and fish oil in biofortified diets resulted in lower Hg in seabream muscle (23% decrease). In contrast, biofortified diets increased As and Hg in carp fillets (over 100%). Iodine biofortification was more efficient in carp (over 100% increase), whereas Se biofortification was more pronounced in seabream (98% increase). In addition, both biofortified feeds and fillets had toxic element contents (As, Hg, Cd and Pb) below the maximum permissible levels (MPLs), demonstrating that

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the biofortification strategies used improved the nutritional quality without compromising safety. However, comparing the percentages of element deposition in fish fillet from each diet, I accumulation was higher in non-biofortified seabream (5%) compared to biofortified (1%), whereas Se and Fe were higher in biofortified fillets (28% and 2%, respectively) compared to non-biofortified fillets (25% and 1%, respectively). On the other hand, biofortified carp fillets presented lower Se and Fe (9% and 1%, respectively) accumulation compared to non-biofortified fillets (23% and 5%, respectively). Yet, both biofortified seabream and carp revealed lower percentages of toxic elements, with As accumulation ranging from 79% (biofortified) to 89% (non-biofortified) in seabream and 11% (biofortified) to 16% (non-biofortified) in carp, and Hg deposition ranging from 86% (biofortified) to over 100% (non-biofortified) in carp fillets. Concerning fish growth performance, gilthead seabream fed with higher levels of I-rich seaweed and Se-rich yeast presented lower final body weight and higher feed conversion rate. Contrarily, the different dietary strategies showed no adverse effects on common carp growth performance. The present study clearly shows the importance of developing eco-innovative and cost-effective biofortified fish products and their potential to achieve sustainable, safe and high-quality production of farmed fish in Europe.

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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

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Food and Agriculture Organization of the United Nations, Rome, pp. 227

Ramalho Ribeiro, A., Gonçalves, A., Bandarra, N., et al., 2017. Natural fortification of trout with dietary macroalgae and selenised yeast increases the nutritional contribution in iodine and selenium. Food Res. Int. 99 (3), 1103–1109. doi:10.1016/j.foodres.2016.10.030

HEALTH PROMOTING ADDITIVES SUPPLEMENTED IN INERT MICRODIETS FOR WHITELEG SHRIMP (*Penaeus vannamei*) POST-LARVAE

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

[1]Riasearch Unipessoal Lda, Murtosa (Portugal)
[2]CIIMAR, Matosinhos (Portugal)
[3]SPAROS, Lda. Olhão (Portugal)
[4]Departamento de Biologia & CESAM, Universidade de Aveiro (Portugal)
Email: andrebarreto@riasearch.pt

Introduction

Whiteleg shrimp (*Penaeus vannamei*) produced in aquaculture is a highly valued commercial product whose demand has substantially increased in recent years. Larvae and post-larvae yields in hatcheries increased intensively, currently originating around 5 million tonnes of adult shrimp per year globally. Nevertheless, initial developmental stages are critical and frequently associated with sub-optimal growth and low survivals, which, in some cases, can be related with nutritional deficiencies or infections by opportunistic pathogens. Therefore, there is room for the creation of innovative microdiet solutions that can enhance development during the initial stages and consequently improve the shrimp quality in the posterior phases, increasing their resistance to stress and pathogenic factors. In fact, industrial shrimp farming is extremely susceptible to pathogenic episodes resulting in disastrous consequences to production (Flegel, 2012; Zou et al., 2020). The use of antibiotics in the aquaculture industry is limited due to inherent environmental issues and the increased antibiotic resistance of microorganisms. Furthermore, shrimp depend uniquely on their innate immune system and cannot be vaccinated, which makes immune stimulation an extremely important strategy. Since the modulation of the immune system through nutrition is currently possible, the potential of innovative nutritional solutions that improve the health condition of shrimp is foreseen as tremendous. This study aimed to evaluate the effects of several health promoting nutrients and additives (i.e. vitamins C and E, beta-glucans, taurine and methionine) supplemented in microdiets on the growth performance, oxidative status and immune condition of *P. vannamei* post-larvae.

Methods

Four experimental microdiets were tested in triplicates. A commercial like diet was used as positive control (PC), whereas a negative control diet (NC) was considered with vitamin C and E supplementation levels below those used in PC. The two remaining diets consisted of the commercial like diet, one supplemented with taurine plus methionine (T+M) and the other with beta-glucans (BG). Whiteleg shrimp post-larvae (mean wet weight 9 mg) were kept at around 28 ºC and fed *ad libitum* for 18 days. At the end of the trial, shrimp were weighted for growth performance determination and samples were collected to determine oxidative stress parameters and assess immune conditions.

Results and discussion

Despite the apparent slightly higher RGR values with the T+M diet, no statistically significant differences in growth performances and survival were observed among treatments (Table 1), suggesting that Beta-glucans and amino acid supplementation or lower dietary vitamin C and E levels tested did not compromise the adequacy of the diets. Regarding the oxidative stress parameters measured: catalase (CAT) activity was similar across all treatments; lipid peroxidation (LPO) levels were significantly lower in the BG treatment than in the PC, with no significant differences between the remaining treatments; total glutathione (tGSH) content was significantly higher in the BG treatment than in the NC, with no significant differences between the remaining treatments. These results suggest that beta-glucans used in the BG diet may prevent oxidative damage (lipid peroxidation), which can be relevant during production cycle. Additionally, the higher tGSH levels observed in the BG treatment when compared to the NC may signal an improved antioxidant response capacity, due to the inclusion of beta-glucans in the BG diet and the decrease of vitamin C and E levels in the NC diet. As for the immune status, no significant differences between treatments were observed regarding the parameters measured (Table 2). The results obtained in this study demonstrate that the nutrients/additives tested can have modulatory effects on the steady state values of important parameters of the antioxidant defense. Nevertheless, these effects were not observed in the immune parameters assessed.

Results expressed as mean ± standard deviation. For initial weight n = 60 observational units; for final weight, FCR, RGR and survival n = 3 experimental units.

Results expressed as mean ± standard deviation (n = 3 experimental units). Different superscript letters indicate statistical differences ($P<0.05$) between treatments in a One-way ANOVA.

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Table 1 Initial and final weight, relative growth rate (RGR), feed conversation ratio (FCR) and survival of whiteleg shrimp post-larvae fed the experimental diets for 18 days.

	PC	NC	T+M	BG
Initial weight (mg)		8.8 ± 0.0		
Final weight (mg)	110.8 ± 18.4	110.8 ± 19.3	114.0 ± 9.5	94.4 ± 9.2
RGR (% day^{-1})	15.0 ± 1.1	14.1 ± 1.3	15.3 ± 0.5	14.0 ± 0.6
FCR	0.9 ± 0.2	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.2
Survival (%)	86.2 ± 7.6	87.0 ± 6.6	85.5 ± 6.1	87.5 ± 5.0

Table 2 Catalase (CAT), lipid peroxidation (LPO), total glutathione (tGSH), lysozyme, pro-phenoloxidase and bactericidal activity levels in whiteleg shrimp post-larvae fed the experimental diets for 18 days.

	PC	NC	T+M	BG
CAT (mg ml^{-1})	22.4 ± 6.3	22.9 ± 8.0	28.9 ± 19.3	21.4 ± 7.2
LPO (nmol g wt^{-1})	15.6 ± 3.1 ^{a}	14.0 ± 2.2 ^{ab}	14.6 ± 2.6 ^{ab}	12.7 ± 2.1 ^{b}
tGSH (nmol mg protein^{-1})	5.0 ± 0.7 ^{ab}	4.7 ± 0.9 ^{a}	5.0 ± 0.8 ^{ab}	5.7 ± 1.1 ^{b}
Lysozyme (μg mg protein^{-1})	1.5 ± 0.6	1.2 ± 0.5	1.1 ± 0.3	1.2 ± 0.4
Pro-phenoloxidase (U ml^{-1})	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
Bactericidal activity (%)	12.9 ± 8.1	12.6 ± 6.9	14.6 ± 11.9	14.5 ± 7.8

References

Flegel, T.W., 2012. Historic emergence, impact and current status of shrimp pathogens in Asia. J Invertebr Pathol 110, 166–73.

Zou, Y., Xie, G., Jia, T., Xu, T., Wang, C., Wan, X., Li, Y., Luo, K., Bian, X., Wang, X., Kong, J., Zhang, Q., 2020. Determination of the Infectious Agent of Translucent Post-Larva Disease (TPD) in *Penaeus vannamei*. Pathogens 9, 741. https://doi.org/10.3390/pathogens9090741

PISCIDINS IN THE EUROPEAN SEA BASS *Dicentrarchus labrax*: DIFFERENT ANTIMICROBIAL ACTIVITIES FOR DIFFERENT PEPTIDES

C. Barroso[1,2,3*], P. Carvalho[4], C. Carvalho[1,5], N. Santarém[1,5], 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] Parasite Disease Group, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[6] CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal
E-mail: carolina.barroso@i3s.up.pt

Introduction

Antimicrobial peptides (AMPs) are one of the host's first line of defenses against a wide range of infectious agents, with broad antimicrobial and immunomodulatory activities (Katzenback, 2015). Fish present a specific group of AMPs, the piscidins. These peptides have been characterized in several fish species, being altered when fish are subjected to an infection, and showing antimicrobial activity against multiple pathogens. Furthermore, several studies have shown the potential of using synthetic peptides to promote fish survival upon infection (Katzenback, 2015). Previous studies show that species from the Moronidae family present a diverse group of piscidins, with different antimicrobial activities (Salger et al, 2011; 2016). However, in the European sea bass (*Dicentrarchus labrax,* Moronidae family), a commercially important fish produced in aquaculture, the identification of the several piscidin types and their biological roles remains poorly explored. Here, we characterize the piscidin family in sea bass. The expression of piscidin genes is evaluated after infection, as well as the antimicrobial activity of piscidin peptides against several fish and mammalian pathogens.

Material and Methods

Healthy European sea bass (*D. labrax*), with an average weight of 50 g, were used for piscidin characterization and infection. Fish were i.p. infected with 1.0×10^5 CFU of *Photobacterium damselae* spp. *piscicida* (*Phdp*) and samples collected at 24, 48, 72 and 96 hours post infection. To determine the antibacterial activity of piscidins, several bacterial strains were incubated for 24 hours with serial dilutions of the synthetic peptides. The following bacteria were used: *P. damselae* spp. *piscicida, P. damselae* spp. *damselae, Vibrio anguillarum, V. alginolyticus, Aeromonas salmonicida, A. hydrophila, Edwardsiella tarda* and *Yersinia ruckeri*. To determine the anti-parasitic activity of these peptides, two mammalian parasites, *Leishmania infantum* and *Trypanosoma brucei brucei*, were incubated for 72h with the different piscidins.

Results

We identified six piscidins in sea bass, divided into three different sub-groups, each group showing similarities in the amino acid sequences and size of the mature peptides: Piscidins 1/4; Piscidins 2/5; and Piscidins 6/7. Although similar, piscidins from each group show a diverse antimicrobial activity, with piscidins 1 and 5 being the most active peptides. On the contrary, piscidins 6 and 7 present a weak activity. Most of piscidin genes present the highest basal expression in the intestine, with the exception of piscidin 2, that is highly expressed in the gills and spleen. Our data show an up-regulation of piscidin genes after infection with *Phdp*, with many of these synthetic peptides being highly active against this particular bacterial strain.

Discussion and conclusions

Our findings indicate that, *in vitro*, piscidin peptides have a direct effect, not only on several bacteria that cause mortalities in fish species, but also on some mammalian pathogens. We show that piscidins constitute an important component of fish immune defenses, being highly expressed during infection, and showing a high diversity in terms of amino acid sequences and antimicrobial activities. Further studies are necessary to understand the roles of these peptides, particularly piscidins 6 and 7. Due to their limited antimicrobial activity, these piscidins may be involved in other yet unknown mechanisms. Furthermore, peptide administration in models of infection will be studied in detail, to understand the potential of these peptides as novel prophylactic or therapeutic compounds.

References

Katzenback B., 2015. Antimicrobial Peptides as Mediators of Innate Immunity in Teleosts. Biology (Basel) 4, 607–39.

Salger, S.A., Reading, B.J., Baltzegar, D.A., Sullivan, C.V., Noga, E.J., 2011. Molecular characterization of two isoforms of piscidin 4 from the hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Fish Shellfish Immunol 30, 420–424.

Salger, S.A., Cassady, K.R., Reading, B.J., Noga, E.J., 2016. A diverse family of host-defense peptides (piscidins) exhibit specialized anti-bacterial and anti-protozoal activities in fishes. PLoS ONE 11, e0159423.

LONG TERM TESTING OF A VLP-BASED VACCINE AGAINST VIRAL NERVOUS NECROSIS IN EUROPEAN SEA BASS *Dicentrarchus labrax*

Sofie Barsøe[1]*, Ansgar Straatman[2], Dagoberto Sepúlveda, Niccolò Vendramin[1], Niels Lorenzen[1]

[1] DTU Aqua, Technical University of Denmark, Lyngby, Denmark
[2] W42 Industrial Biotechnology GmbH, Dortmund, Germany
e-mail: sofhan@aqua.dtu.dk

Introduction
Viral nervous necrosis (VNN) is a devastating disease of European sea bass hatcheries and fish farms in the Mediterranean [1]. Outbreaks often occur in the warm summer months when water temperatures reach ≥25C°, which is the optimal temperature for the etiological agent, the red-spotted grouper nervous necrosis virus (RGNNV). RGNNV belongs to the genus *betanodavirus* (also called Nervous Necrosis Virus (NNV)), which is a group of small ichosahedral RNA(+) viruses with a bi-segmented genome of RNA1 and RNA2. The RNA1 encodes the polymerase while the RNA2 encodes the capsid proteins (CP) of the surface structure. NNV can be found globally and a reservoir exist in the wild fish stocks, with virus detected in up to 120 different species, which could potentially be impacted by the disease [2]. The rising water temperatures of the oceans result in migrations and movement of fish to new habitats which will most likely further increase the prevalence, distribution and disease of NNV [2,3].

Vaccination of farmed fish is a potential way of reducing the VNN outbreaks. For this, safe and efficacious vaccines are needed. During the EU project Targetfish (GA 311993) an innovative virus-like particle (VLP) vaccine against viral nervous necrosis in European sea bass was developed. The CP encoded by the RNA2 of RGNNV was expressed in an eukaryotic expression system (*Pichia pastoris*). After disruption of cells, CP auto-assembled into VLPs could be obtained. Similar vaccines have previously been shown to induce protection against challenge with NNV a few months post vaccination [4]. However, in relation to use under farming conditions, long-term protection is required and we here tested whether immunity to VNN could still be demonstrated 3 and 7 months post vaccination.

Materials and Methods
1044 European sea bass (average size: 5g) from a commercial breeder with NNV free status were randomly vaccinated by intraperitoneal injection with 50ul of either VLP (40ug/fish), a commercial vaccine or phosphate buffered saline (PBS) (n =348 fish/group). Each group was subcutaneously tagged with colored elastomer to allow mixing of the vaccinated groups. The fish were kept in two 180 L tanks, with an equal number of all groups (174 fish/group/tank) in aereated artificicial saltwater (10‰ salinity) and individual heaters keeping the water temperature at 19C° (+/- 1C°). Monitoring of immunity included examination of the antibody response in ELISA and serum neutralization, as well as survival in experimental NNV challenge (Figure 1).

Challenge with RGNNV (283.2009) grown in SSN-1 cells was performed by immersion or by injection (IM) of mixed fish groups kept in replicate aquaria at 22C°, whereafter the temperature was gradually increased to 25C° during the first few days, and kept there for the rest of the experiment (28-32 days). The fish were monitored several times daily and euthanized if clinical signs of VNN were detected, such as spiraling swimming pattern and loss of buoyancy control. Brain was sampled from a number of euthanized/dead fish for re-isolation of virus.

Results and conclusion
A VLP induced RGNNV specific antibody response was detectable in ELISA at least 8 months post vaccination. Neutralizing antibodies were detected in all analyzed samples from the VLP vaccinated fish in serum neutralization at 3 (n=10/10), 4 (n=9/9) and 6 (n=5/5) months post vaccination (later samples not analyzed at the time of writing) with titers from 1:160 – ≥1:640, the majority (18/24) being ≥1:640.

Survival at 3 and 7 months post vaccination, showed superior survival of the VLP vaccinated fish following both challenge routes at both times (Table 1), supporting the potential of the VLP as an efficacious vaccine against VNN disease in terms of applied perspectives.

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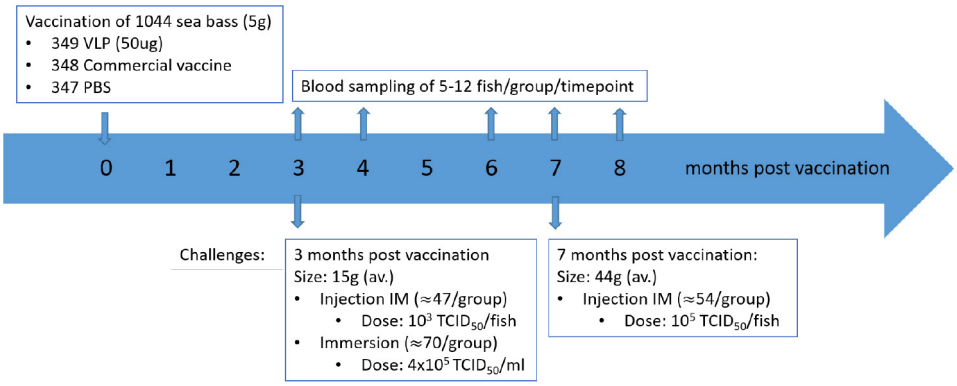

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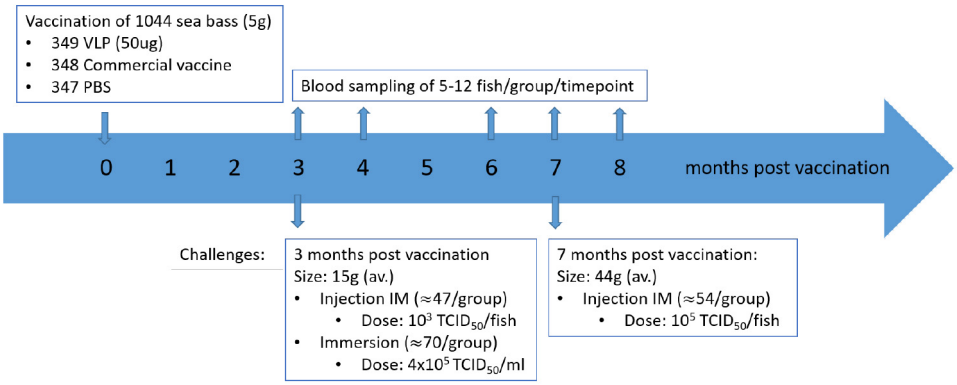

Figure 1: Experimental timeline

Table 1: Survival% after experimental challenge with RGNNV.

Time post vaccincation	**Challenge route**	**Survival %**			**RPS (VLP)**
		VLP	Commercial	PBS	
3 months	Bath challenge	94.1	70.8	80.9	69.1
3 months	Injection IM	92.7	67.6	54.1	84.1
7 months	Injection IM	90.6	29.1	26.7	87.2

Acknowledgements
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References

1. Muniesa, A.; Basurco, B.; Aguilera, C.; Furones, D.; Reverté, C.; Sanjuan-Vilaplana, A.; Jansen, M.D.; Brun, E.; Tavornpanich, S. Mapping the knowledge of the main diseases affecting sea bass and sea bream in Mediterranean. *Transbound. Emerg. Dis.* **2020**, 1–12, doi:10.1111/tbed.13482.
2. Bandín, I.; Souto, S. Betanodavirus and VER disease: A 30-year research review. *Pathogens* 2020, *9*.
3. Cheng, L.; Abraham, J.; Zhu, J.; Trenberth, K.E.; Fasullo, J.; Boyer, T.; Locarnini, R.; Zhang, B.; Yu, F.; Wan, L.; et al. Record-Setting Ocean Warmth Continued in 2019. *Adv. Atmos. Sci.* **2020**, *37*, 137–142, doi:10.1007/s00376-020-9283-7.
4. Thiéry, R.; Cozien, J.; Cabon, J.; Lamour, F.; Baud, M.; Schneemann, A. Induction of a protective immune response against viral nervous necrosis in the European sea bass Dicentrarchus labrax by using betanodavirus virus-like particles. *J. Virol.* **2006**, *80*, 10201–10207, doi:10.1128/JVI.01098-06.

EFFECTS OF DIFFERENT PROTEIN SOURCES ON THE NUTRIENT PROFILE AND GROWTH OF WHITHELEG SHRIMP *(Litopenaeus vannamei)*

Annalena Barth *, Martin Tschirner, Thomas Wilke

*Department of Animal Ecology & Systematics, Justus Liebig University Giessen
Heinrich-Buff-Ring 26-32 (IFZ), D-35392 Giessen, Germany
E-mail: Annalena.Barth@bio.uni-giessen.de

Shrimps are globally one of the most important aquaculture species with an estimated annual production volume of about 6 million tonnes, dominated by the whiteleg shrimp *Litopenaeus vannamei*. However, even when produced in sustainably managed aquaculture systems, the utilized feed is often highly unsustainable. To address the physiological nutritional requirements of shrimp, it is necessary to include a large amount of high-quality protein in the feed. The most widespread protein source used is fishmeal, mainly obtain by reducing large amounts of small pelagic fish into fishmeal and fish oil. With many fish stocks being fished at or even over maximum capacity, alternative protein sources will have to be exploited to sustain expected future growth rates of aquaculture. Additionally, the protein sources used should meet some essential requirements, such as high protein content, adequate amino acid profile, high digestibility, and good palatability. In order to precisely determine these requirements for *L. vannamei*, we are currently conducting a feeding trial with three different feeds. The three trial groups are fed with: 1) natural fresh feed (squid, mussels, krill and polychaets), 2) standard dry feed, commonly used in the shrimp industry, and 3) dry feed complemented with fresh black soldier fly larvae (*Hermetia illucens*). The trial is performed in a closed recirculating system, the shrimps are sampled for size, mass and antenna length as well as photographed at the start and the end of the experiment. From experiences of preliminary trials, increases in size and mass are expected to be higher in natural diet group than with the standard dry diet, and comparable effects are expected with the dry diet complemented with insect larvae. In addition, an amino and fatty acid profile is generated after the trial to determine nutrient efficiency of the different feeds. The trial is expected to provide deeper insights into the metabolization of nutrients by whiteleg shrimps, enabling further work on sustainable insect-based diets to conserve the natural resources of the oceans in the future.

ADVANCING IRISH AQUACULTURE THROUGH THE IMPLEMENTATION OF A KNOWLEDGE TRANSFER METHODOLOGY AND STAKEHOLDER ENGAGEMENT

Cliona Ní Challacháin[1]*, Georgia Bayliss-Brown[1] and Lucy Watson[2]

[1] ERINN Innovation Ltd, Unit 3, Olympic House, Pleasant Street, Dublin 8, Co. Ireland
[2] Bord Iascaigh Mhara, 12 Crofton Road, Dún Laoghaire, Co. Dublin, Ireland
Email: cliona@intrigo.eu

In 2014, an Irish-led consortium (BIM, AquaTT, SmartBay) bid for and won a European Commission (EC) Horizon 2020 project called COLUMBUS (www.columbusproject.eu). The €4M project, 36-month project, comprising 25 partners, was solely focused on piloting effective methods for carrying out the knowledge transfer of past marine and maritime projects that had been previously funded by the EC. The intended impact was a measurable increase in accessibility and uptake of research Knowledge Outputs (discrete units of knowledge) by end-users: policy, industry, science and wider society, contributing to the EC's Blue Growth strategy. COLUMBUS carried out an extensive, human-resource intensive process to retrospectively identify, engage and shortlist high-potential knowledge from a large, complex and diverse ecosystem of projects. COLUMBUS was recognised as a significant achievement and demonstrated that a robust, systematic process can be replicated across marine sectors (and beyond). Importantly, such a modular process can improve the likelihood of transfer, value creation and measurable impact.

Across Europe and globally, investment and activity in further research around all aspects of aquaculture is increasing. The possibilities are endless as to how science, technology and knowledge can positively impact the Irish Aquaculture Community.

Recognising this potential, Ireland's Seafood Development Agency, Bord Iascaigh Mhara (BIM), launched a request to tender in December 2018 for a contract to help advance the Irish aquaculture sector. The idea was to look for commercially-relevant knowledge (including technology and innovations) in Ireland and across Europe, and to find out if BIM could support the industry in reaching its 2030 production targets by facilitating the transfer of this knowledge. Through the same tender, BIM enabled the setting up of an Irish 'Mirror Platform' of the European Aquaculture Technology and Innovation Platform (EATiP) recognising the important role Mirror Platforms play in transferring innovation and knowledge to industry.

The COLUMBUS Knowledge Transfer methodology was refined and applied within this BIM contract to identify, appraise and transfer innovative knowledge and technology to the Irish aquaculture community, as well as highlighting its potential for fisheries and seafood processing. In parallel, the Irish Aquaculture Technology and Innovation Platform (IATiP) was launched and communication as well as knowledge transfer activities carried out with the Irish Aquaculture Community.

In this presentation, the project's aims, processes and key outcomes will be presented along with case studies of Knowledge Transfer covering seaweed and microalgae, shellfish and finfish.

REPLACEMENT OF FISHMEAL BY ANTARCTIC KRILL MEAL IN DIETS OF EUROPEAN SEA BASS *Dicentrarchus labrax*: EFFECTS ON GROWTH PERFORMANCE, FEED UTIZATION AND LIVER METABOLISM

Tibiabin Benitez-Santana*, Silvia Torrecillas, Daniel Montero and Marisol Izquierdo

Aker BioMarine Antartic AS
Oksenøyvn 10. PO Box 496NO-1327 Lysaker, Norway
tibiabin.benitez-santana@akerbiomarine.com

A sustainable growth of the aquaculture sector implies the use of sustainable novel raw materials as replacement of the traditional fish meal (FM) and fish oil (FO) ingredients. This fact will lead to the development of functional diets as part of a management strategy to reduce the effects on fish growth performance and health derived from low FM/FO dietary contents. In this sense, krill meal (*Euphausia superba*) may be a potential candidate to potentiate fish growth and health status.

European sea bass (*Dicentrarchus labrax*) were fed a commercial relevant diet with either a 15% fishmeal content (KM0) or the same diet substituted by 30% (KM5) or 50% Antarctic krill meal (KM7.5) for 12 weeks in triplicates. Diets were isoproteic (45%) and isolipidic (18%).

At the end of the feeding trial, growth performance, liver morphology, liver proximate composition and fatty acid profile, as well as liver lipid metabolism related genes were evaluated. After two months of supplementation, krill meal-supplemented fish presented increased feed intake ($p<0.05$), regardless of the dietary level. However, feed conversion ratio (FCR) was only significantly lower ($p<0.05$) in fish fed the KM7.5 diet. At the end of the feeding trial, fish fed KM-based diets presented increased ($p<0.05$) final weight, final length, relative growth, specific growth rate (SGR) and improved FCR, irrespective of the KM dietary level. Livers of European sea bass fed the experimental diets presented similar ($p>0.05$) biochemical composition and fatty acid profile. Despite the similar content of liver lipids, fish fed KM diets presented a healthier liver morphological profile. Hepatocytes of KM fed fish presented lower vacuolization levels, better alignment of the hepatocyte nuclei along the sinusoidal lines, and in general lower sings of steatosis. Liver gene expression results revealed a down regulation of the *3*-hydroxy-3-methylglutaryl-coenzyme A reductase *(hmgcr)* and delta-6-desaturase (*fads2*) expression, when fish were fed the KM7.5 diet compared to fish fed the KM0 diet. Besides, a significant negative correlation between the gene expression levels of *hmgcr, fads2* and KM dietary levels were observed. On the other side, fatty acid binding protein 7 (*fabp7*) and KM were significantly positively correlated.

Altogether profiling KM as a potential growth and health promoter in European sea bass fed low fish meal and oil diets.

A BIOENERGETIC MODEL TO ADDRESS CARBON SEQUESTRATION POTENTIAL OF SHELLFISH FARMING: EXAMPLE FROM Ruditapes philippinarum IN THE VENICE LAGOON

Camilla Bertolini [(1)*], Bernardini, I. [(2),], Brigolin, D. [(3),], Milan, M. [(2)], Matozzo, V. [(2),], Roberto Pastres [(1),(4)]

1. Università Ca'Foscari, Campus scientifico, Via Torino, 155, 30170 Mestre, Venezia (Italy)
2. Università di Padova, Polo di Agripolis, Viale dell'Universita', 16 - 35020 Legnaro (PD)
3. IUAV, Santa Croce, 191, 30135 Venezia VE
4. Bluefarm, s.r.l., Venice, Italy
E-mail: camilla.bertolini@unive.it

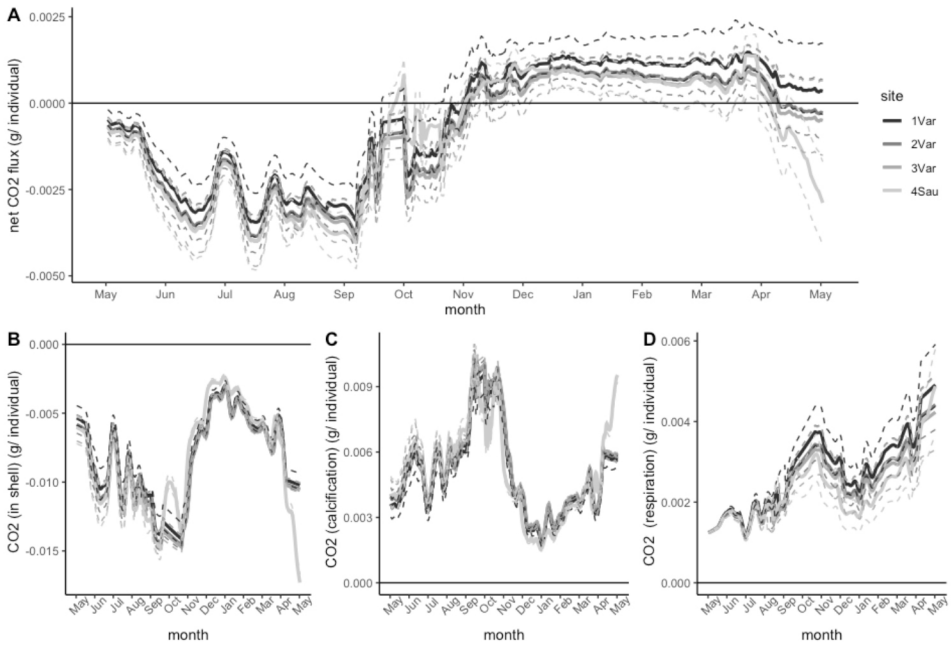
Introduction

Shellfish are increasingly been looked at as sustainable food sources that provide additional ecosystem services, such as water filtration and carbon sequestration (van der Schatte Olivier et al. 2020). However, their role as CO_2 sinks or sources is still highly debated: in order to quantify their contribution to CO_2 budgets the shell accretion dynamics need to be taken into account (Morris & Humphreys 2019)"ISSN":"00448486","abstract":"Mollusc aquaculture is a high-value industry that is increasing production rapidly in Europe and across the globe. In recent years, there has been discussion of the potential wide-ranging environmental benefits of this form of food production. One aspect of mollusc aquaculture that has received scrutiny is the production of calcareous shells (CaCO3.

Bioenergetic models that investigate bivalve growth are mostly focused on the growth of the soft tissues, with the shell component usually calculated with allometric scaling relationships. Partitioning of energy into soft tissue, reproductive tissue and shell would allow one to better quantify the dynamics of energy fluxes and calcification, thus relating growth, condition index and CO2 budget to site specific environmental conditions

Methodology

Shell accretion was added as a state variable in a bioenergetic model of the Manila clam (Ruditapes philippinarum), which was developed as part of this study. A key parameter which takes into account energy allocation into shell growth, was calibrated for four sites located in the Venice lagoon, where clams from the same cohort were monitored for one year during a transplant experiment. The model was then used to calculate CO_2 fluxes resulting from respiration and shell calcification, taking in account $CaCO_3$ stocked in the shell and CO_2 emission. The function Ψ, that allows one to calculate the amount of CO_2 released with respect to $CaCO_3$ formed, was estimated using environmental forcing functions (temperature, salinity, pH and alkalinity) observed in the proximity of each site.



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Results and discussion

The findings of the study show that the energy invested towards shell accretion varies slightly among sites and that clams play a role as a moderate sink of CO2 when the whole year is considered. The area where clams were found to sink the least amount of CO2 was also the area where clams had a higher condition index, suggesting a contrast between optimal farming areas for food and the inclusion of shellfish in the carbon markets (Filgueira et al. 2015). Moreover CO2 fluxes were characterized by a marked seasonal variability: due to respiration, clams were net sources of CO2 in wintertime, when growth slowed down. The model presented provides a useful framework for site selection in the context of balancing optimal food production and sustainability taking in account environmental variables. This can be useful towards precision shellfish farming and can help predicting shellfish ecosystem services in the context of a changing climate.

Acknowledgments

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References

Filgueira R, Byron CJ, Comeau LA, Costa-Pierce B, Cranford PJ, Ferreira JG, Grant J, Guyondet T, Jansen HM, Landry T, Mckindsey CW, Petersen JK, Reid GK, Robinson SMC, Smaal A, Sonier R, Strand Ø, Strohmeier T (2015) An integrated ecosystem approach for assessing the potential role of cultivated bivalve shells as part of the carbon trading system The Danish Shellfish Centre-DTU Aqua, 7900 Nykøbing Mors, Denmark. MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser 518:281–287

Morris JP, Humphreys MP (2019) Modelling seawater carbonate chemistry in shellfish aquaculture regions: Insights into CO2 release associated with shell formation and growth. Aquaculture 501:338–344

van der Schatte Olivier A, Jones L, Vay L Le, Christie M, Wilson J, Malham SK (2020) A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture 12:3–25

COMBINING DATA FROM SUCESSIVE COHORTS TO INCREASE THE ACCURACY OF ESTIMATED BREEDING VALUES: A CASE STUDY IN A GILTHEAD SEA BREAM (*Sparus aurata*) COMMERCIAL LINE

A. Bestin*[a], S. Cariou[b], J.-S. Bruant[b], F. Enez [a], M. Vandeputte[c,d], P. Haffray [a]

[a] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[b] FMD (Ferme Marine du Douhet), 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

Selective breeding is a cumulative process where genetic gain increases as generations go by. It is supposedly more accurate as the pedigree is well recorded with a sufficient depth and as phenotypic data cumulates. In the end, the choice of a candidate rather than another is based on the accuracy of its breeding value (EBV). However, in complex breeding schemes where cohorts within generations overlap, it is not so clear whether combining data from same generation cohorts would indeed lead to increase EBVs accuracy.

Material and Methods

This study focuses on 2 cohorts produced from the FMD sea bream line. They were created with one-year interval from the same breeding nucleus through the use of different breeders (no shared parents). The mating scheme comprised ~190 full and half-sib families per cohort. Within cohorts, families were mixed altogether since hatching and randomly split into 3 batches: one underwent an infection challenge to *Photobacterium damselae piscicida* in a reference laboratory, one was reared either in inland facility (cohort 1) or in sea cage (cohort 2), and the third batch was mass selected on growth (candidates). The pedigree was known through *a posteriori* DNA parentage assignment.

Phenotypes were recorded on the sibs of the candidates: dead-or-alive status from the disease challenge tests ("Resistance" trait in Fig. 1), body weight at commercial size, Fulton's condition coefficient, fat content, gutted yield, de-headed gutted yield and head yield. Candidates themselves had no phenotypes.

Three datasets were used: A) candidates and sibs from cohort 1, where accuracy was estimated based only on cohort 1 data ("Cohort1_alone" in Fig. 1), B) candidates and sibs from cohort 2, where accuracy was estimated based only on cohort 2 data ("Cohort2_alone"), C) candidates and sibs from both cohorts, enabling accuracy estimation for i) cohort 1 individuals based on both cohorts data ("Cohort1_knowing2"), ii) cohort 2 individuals based on both cohorts data ("Cohort2_knowing1") and iii) all individuals based on both cohorts data ("Cohorts_1_2_altogether).

The accuracy of candidates' EBV was calculated as the square root of the coefficient of determination CD, using this formulae: (Haffray et al 2018) where se is the standard error of the EBV estimated with remlf90 and the additive genetic variance estimated with remlf90 (Misztal et al 2002) with a multivariate animal model.

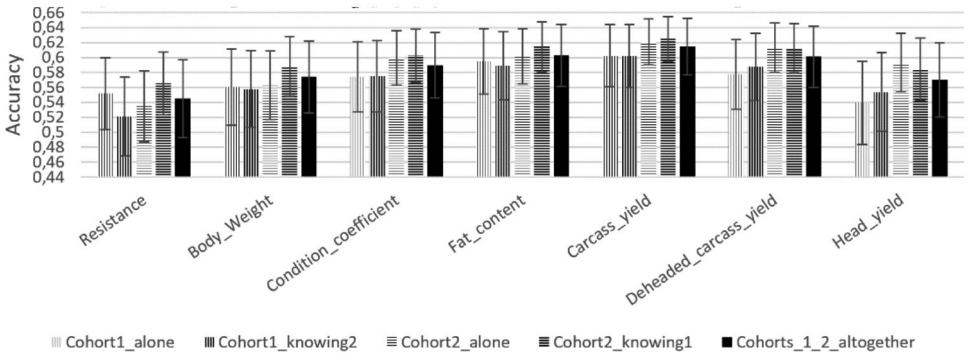

Figure 1: Mean accuracies (bars) and standard deviation (error bars) of EBVs for all traits according to different datasets

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Results and Discussion

Fig. 1 presents the mean accuracy of EBVs for candidates in cohort 1 and cohort 2. The lowest accuracy estimate was 0.52 for disease resistance of candidates from cohort 1 where data from cohort 2 was added to the dataset. The highest accuracy estimate was 0.62 for gutted yield of candidates from cohort 2 where data from cohort 1 was added to the dataset. These are rather high levels of accuracies for EBVs estimated through a pedigree BLUP evaluation on candidates without own phenotypic records.

Fig. 1 shows that adding data from cohort 2 lowered accuracy for cohort 1 candidates, except for de-headed gutted carcass yield and head yield. On the contrary, adding data from cohort 1 increased the accuracy of cohort 2 candidates (except for head yield). When data from both cohorts were pooled to estimate in a single step EBVs for candidates from both cohorts, the mean accuracy was in-between the results achieved within cohort (except for body weight and fat content).

This could be surprising knowing that cohorts 1 and 2 shared two thirds of parental origins (even if parental individuals themselves were used only once for the creation of a single cohort). Despite this, the co-ancestry coefficient (calculated from the full pedigree information i.e. ~ four generations available) between cohort 1 and 2 was 0.032 ± 0.03 and more than 80% of the individuals shared a co-ancestry coefficient of 0.05 or less (max.:0.204). In other words, the degree of kinship between these successive cohorts was low, which could explain the reduction in accuracy observed when dealing with a bigger dataset. This phenomenon was observed by Tassiello et al (2019) for photobacteriosis resistance when exploring its genomic prediction by machine learning based on two datasets (cohort 1 and another cohort from another commercial line). It appeared that the predicting ability of the datasets was too low to be useful in selective breeding. This was explained by the absence of kinship between these cohorts.

It would be worth comparing in future work intra and inter-generation data as two successive cohorts do not represent necessarily the whole population whereas generations do. Successive generations should be more related than successive cohorts, thus pooling data at the generation level might increase accuracies.

Conclusion

The more the data does not necessarily mean the higher the accuracy of EBVs. Indeed, the degree of kinship between batches may have an effect, and the accuracy could worsen compared to intra-cohort data treatment. Further research with cohorts showing various levels of between-cohort co-ancestry will be necessary to confirm this hypothesis.

Acknowledgement

Cohort 1 was analyzed in FISHBOOST project (European funding: 7th Framework Program KBBE.2013.1.2-10, grant agreement n° 613611). Cohort 2 was analyzed in the RE-SIST project (15th "FUI") managed by AQUIMER and labelled by Mer Bretagne competitiveness clusters, and funded by the French Government, BPI France and Bretagne regional council.

References

Haffray P., Enez F., Bugeon J., Chapuis H., Dupont-Nivet M., Chatain B., Vandeputte M., 2018. Accuracy of BLUP breeding values in a factorial mating design with mixed families and a marker-based parentage assignment in rainbow trout Oncorhyncus mykiss. Aquaculture, 490:350-354.

Misztal I, Tsuruta S, Strabel T, Auvray B, Druet T, Lee D H, 2002. BLUPF90 and related programs (BGF90). 7th WCGALP. Montpellier, France.

Tassiello O., Montanucci L., Babucci M., Ferraresso S., Carnier P., Bargelloni L., 2019. Exploring machine learning methods for genomic prediction of disease resistance and body length in the gilthead sea bream. Aquaculture Europe 2019, Berlin, Germany.

COASTAL COMMUNITY PERCEPTIONS OF MULTI-USE OFFSHORE INSTALLATIONS COMBINING FISH FARMING WITH RENEWABLE ENERGY SYSTEMS

Suzannah-Lynn (Suzi) Billing*[1], George Charalambides[1], Paul Tett[1], Michelle Giordano[4], Carlo Ruzzo[2], Felice Arena[2], Anita Santoro[3], Fabrizio Lagasco[4], Silvia Vela[4], Giulio Brizzi[5]

corresponding author: suzi.billing@sams.ac.uk
[1] Scottish Association for Marine Science, Oban, Scotland PA37 1QA, UK
[2]Mediterreanean University of Reggio Calabria, Natural Ocean Engineering Laboratory (NOEL), Reggio Calabria, Italy
[3]Wavenergy.it srl, Reggio Calabria, Italy
[4]RINA Consulting S.p.A., [5]Chlamys srl

Introduction

Increasing competition for use of space in coastal seas may be ameliorated by combining several uses in one offshore structure. The H2020 BlueGrowthFarm project (no. 774426, commenced on 1st June 2018) aims to combine fish-farming with wind and wave energy capture on a floating platform. Such a Multi-use Offshore Installation (MOI) has been designed, and design tools have been calibrated on results obtained with scaled prototypes. A 1:15 prototype is now under construction for deployment near Reggio Calabria in southern Italy. Another site of interest for experiments with MOI is near the island of Islay in western Scotland. Because it is becoming increasingly clear that such structures need social licence as well as developmental and environmental consents, we investigated the conditions for MOI social licence in Reggio Calabria and on Islay during September-November 2019.

Methods

A reference group of local stakeholders was formed and met in the Port Authority of Reggio Calabria in September 2019. At the same time, a survey of public opinion was carried out amongst citizens and visitors who were walking on the sea-front. Similar questions were asked in a survey on Islay in October-November 2019, which combined face-to-face interviewing with an on-line questionnaire.

Findings

A total of 127 and 108 individuals completed the Islay and Reggio surveys, respectively, with most respondents in both cases locals. Although there were some differences in detail, the broad pattern of our findings was the same in both places. People thought better of marine renewable energy generation than fish-farming, but remained moderately likely to eat fish produced in MOI. The majority distrusted regulators to control environmental impacts. The main differences were that people in Reggio Calabria perceived benefits from MOI industrial activity in the region, and they were more likely to accept development by non-local owners than were people on Islay.

Discussion

We have interpreted the data in relation to a sociological framework that combines theory for Social Licence to Operate (SLO) (Hall et al., 2015) with theory for Action Situations from the IAD/SES Framework of Ostrom and McGinniss (McGinnis & Ostrom, 2014). We characterise action situations along two dimensions: from formal (constrained by explicit, typically legally-prescribed, rules and hence largely determined by the settings of the governance system) to informal (outcomes determined largely by within-community interactions); and according to degree of completion. The latter dimension is the one that unites and distinguishes SLO from action situations, the former tending towards temporal persistence, the latter by definition terminating in an outcome that is accepted as resolving the core issue. However, our hypothesis that community's diffuse and perhaps heterogenous opinions might `crystalise' around an issue, leads to the idea that a community's granting of consent can be understood as the outcome of an action situation. We plan to investigate this further with the local community after the deployment of the scaled prototype in Reggio Calabria.

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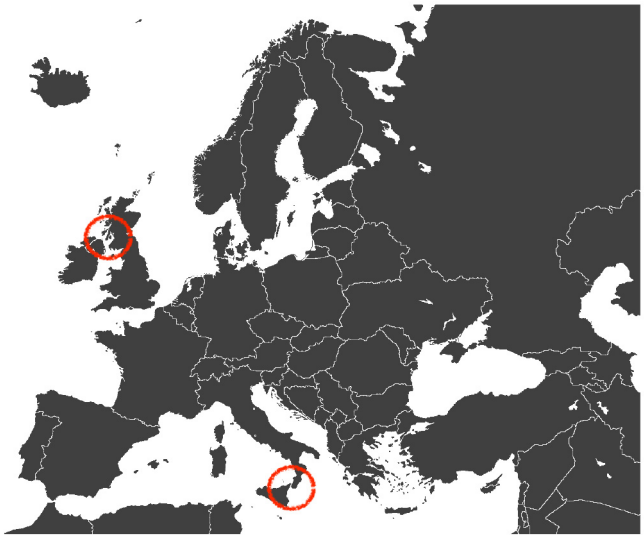

Links and references

BlueGrowthFarm website: http://www.thebluegrowthfarm.eu

N. Hall, J. Lacey, S. Carr-Cornish, and A. M. Dowd, "Social licence to operate: Understanding how a concept has been translated into practice in energy industries," J. Clean. Prod., vol. 86, pp. 301–310, 2015, doi: 10.1016/j.jclepro.2014.08.020.

M. D. McGinnis and E. Ostrom, "Social-ecological systems framework: initial changes and continuing challenges," Ecol. Soc., vol. 19(2), no. 30, p. 12, 2014, doi: 10.5751/ES-06387-190230.

VARIATION OF HIGH-VALUE BIOACTIVE COMPOUNDS FROM *Alaria esculenta* CULTIVATED ON LONG-LINES IN BANTRY BAY, IRELAND

Silvia Blanco Gonzalez[1*], Laurence Jennings[2], Olivier P. Thomas[2] Julie Maguire[1]

[1]Bantry Marine Research Station Ltd, Bantry, Gearhies, P75 AX07 (Co. Cork), Ireland
[2] Marine Biodiscovery, School of Chemistry and Ryan Institute, National University of Ireland Galway (NUI Galway), University Road, H91 TK33 Galway, Ireland
Email: sblanco@bmrs.ie

Introduction

To avoid pressure on natural populations through harvesting, seaweed aquaculture is undergoing global expansion during the last decades. Seaweed farming is widely perceived as one of the most environmentally benign types of aquaculture as it does not require additional feed or fertilisers (Cottier-Cook *et al*., 2016). Because of its cost-effectiveness as a food supply, animal feed and high-value biochemical products (bioactives), there has been increasing interest in the cultivation of seaweed in the past years (Buschmann *et al*., 2017).

Established in the south-west of Ireland, Bantry Marine Research Station Ltd (BMRS) is one of the institutions with two large-scale 6-hectare and a 16-hectare at-sea seaweed farms in Europe. BMRS aims to produce over 20 tonnes (wet weight) of *Alaria esculenta* on long-lines in West Cork. *A. esculenta* contains numerous high-value compounds such as fucoxanthin, phenolic compounds and polysaccharides, with the ability to benefit both human and animal wellbeing. However, to maximize the cultivation of *Alaria esculenta* for these high-value bioactives, optimum deployment and harvesting dates of the long-lines must be established.

Material and methods

Long-lines with *Alaria esculenta* seeded string were deployed in a staggered manner in the autumn/winter of 2018 and sampled at intervals (from March 18th to May 23rd, 2019). The ropes were sampled according to their date of deployment (Rope No. 2 -deployed on October 18th-, rope No. 5 -deployed on November 1st- and rope No. 7 -deployed on December 11th-). Morphological and biomass data were obtained along long-lines from multiple sampling points (East, Middle and West).

Three different high-value bioactives, fucoxanthin, total phenolic compounds and polysaccharides, were extracted from the freeze-dried material. Characterisation and quantification were performed using different chromatographic and colourimetric methods. Antioxidant activity of the extracts was also determined.

Results

In mid-April, biomass hit values ranging from 8 to 12 kg/m wet weight. On April 1, a higher content of fucoxanthin rich extracts (0.89± 0.09 mg/g of dry seaweed) was observed in the rope deployed in November. Conversely, total phenolic compounds increased in later samplings (from mid April to the end of May) reaching values of 5.60 ± 1.19 mg PGE/ g of dry seaweed in the rope deployed in October. The rise of phenolic compounds correlates with the presence of epiphytes and grazers on *Alaria esculenta* blades. A strong correlation was found between the total phenolic content concentration and the concentration of phlorotannins (fig. 1), suggesting that 6-11% of the total phenolic content are phlorotannins.

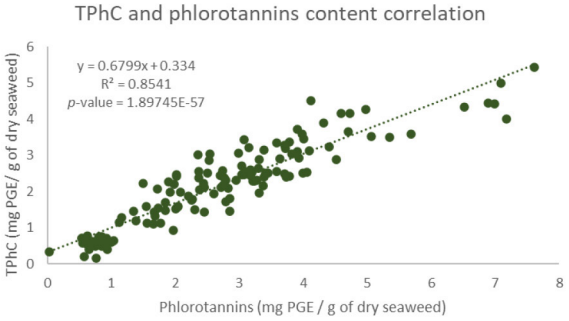

Figure 1: Graph representing the correlation between total phenolic content and phlorotannins.

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Conclusion

The study reveals that *Alaria esculenta* is a fast-growing kelp species amenable to aquaculture. It is rich in high-value bioactive compounds such as fucoxanthin and phenolic compounds. The findings suggest that a variation of the compounds' concentration occurs with time. Total phenolic compounds (and phlorotannins) increased drastically from mid-April until the end of May, while the peak of fucoxanthin occurred on the 1st of April 2019.

Acknowledgements

This work was funded under the Knowledge Gateway Scheme "Structural and Functional Characterisation of high-value bio-active compounds from *Alaria esculenta* cultivated on long-lines in Bantry Bay" and is in funding under the European Maritime Fisheries Fund (EMFF) and administered by Bord Iascaigh Mhara (BIM) on behalf of the Department of Agriculture, Food and the Marine, and supported by Bantry Marine Research Station Ltd. Part of this project (Grant-Aid Agreement No. PBA/MB/16/01) is carried out with the support of the Marine Institute and is funded under the Marine Research Program by the Irish Government to Marine Biodiscovery at NUI Galway.

References

Buschmann, A.H., Camus, C., Infante, J., Neori, A., Israel, A., Hernández-González, M.C., Pereda, S.V., Gómez-Pinchetti, J.L., Golberg, A., Tadmor-Shalev, N. & Critchley, A.T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology*, **52**(4):391-406.

Cottier-Cook, E.J., Nagabhatla, N., Badis, Y., Campbell, M.L., Chopin, T., Dai, W., Fang, J., He, P., Hewitt, C.L., Kim, G.H., Huo, Y., Jiang, Z., Kema, G., Li, X., Liu, F., Liu, H., Liu, Y., Lu, Q., Luo, Q., Mao, Y., Msuya, F.E., Rebours, C.,

EFFECT OF TEMPERATURE AND THE TIMING OF SPERMIATION ON SPERM PRODUCTION INDUCED BY RECOMBINANT GONADOTROPINS IN SENEGALESE SOLE

I. Blanquet[1*], F. Chauvigné[2], D. Rosado[1], M. Martins[1], C. Vilafranca[1], F. Silva[1], N. Duncan[3], I. Giménez[4] and J. Cerdà[2]

[1]SAFIESTELA-SUSTAINABLE AQUA FARMING INVESTMENTS, S.A., 4570-275 Póvoa de Varzim (Portugal)
Email: isidroblanquet@sea8.eu
[2]IRTA-Institute of Biotechnology and Biomedicine (IBB), Universitat Autònoma de Barcelona, 08193 Barcelona (Spain)
[3]IRTA-Sant Carles de la Ràpita, 43540 Tarragona (Spain)
[4]Rara Avis Biotec, S.L., 46002 Valencia (Spain)

The tiny volumes (microliters) of sperm produced by the flatfish Senegalese sole poses great challenges for the development of *in vitro* fertilization methods needed for the selective breeding of the species in aquaculture. Recent efforts aimed at increasing the volume of sperm produced have shown that the *in vivo* use of homologous recombinant gonadotropins, follicle-stimulating and luteinizing hormones (rFsh and rLh, respectively), can be an effective therapy. However, these methods need yet to be refined and tested at the industrial level.

In this work, we have investigated the effect of different doses of rFsh and the temperature at the time of rLh induction on the production of sperm by sole males under commercial production conditions. Approximately 100 males (~500 g in weight) were acclimated to 12ºC and a 10 h light:14 h dark photoperiod for about 2 months, and subsequently treated with saline or an intramuscular injection of two doses of rFsh (10 or 18 μg/kg) once a week during five consecutive weeks. During the week 6, the temperature was maintained at 12ºC or was increased to 17ºC, and then one dose of rLh (18 μg/kg) was injected in all groups. The sole rFsh and rLh were produced by Rara Avis Biotec in cultured mammalian cell lines using a proprietary technology. The plasma levels of the androgen 11-ketotestosterone (11-KT) and the production of sperm at 24, 48 and 72 h after rLh injection were determined.

The treatment with rFsh and rLh increased the circulating levels of 11-KT, indicating that the hormones were biologically active. Males treated with the highest dose of rFsh produced ~10 times more sperm than the controls (952± 182 vs 87± 36 x10^6 spermatozoa/kg). However, in the rFsh-treated fish, the production of sperm was 71% higher at 48 h post rLh injection than at 24 or 72 h. Interestingly, at a low dose of 10 μg/kg rFsh, the increase in the temperature to 17ºC stimulated the production of sperm at 48h after rLh treatment to approximately the same level (1.057±300 x106 spermatozoa/kg) than that observed in males treated with 18 μg/kg rFsh at 12ºC.

These results indicate that the best time for sperm collection after rLh injection of rFsh-treated sole males is 48 h. In addition, the data suggest that a low dose of rFsh of 10 μg/kg followed by an injection or 18 μg/kg rLh at 17ºC may be a suitable protocol to stimulate sperm production for commercial purposes, which will reduce the cost of the recombinant hormones.

Acknowledgements
This work was financed by the project 038433_REARLING, funded by Portugal and the European Union through FEDER/ERDF, COMPETE 2020, in the framework of Portugal 2020, coordinated by Isidro Blanquet (Sea8 Group, Povoa de Varzim, Portugal) and in collaboration with Neil Duncan and Joan Cerdá (IRTA) and Ignacio Giménez (Rara Avis Biotec S.L., Valencia, Spain).

THE EFFECT OF HUSBANDRY PRACTICES ON COMMON DISEASES OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) NURSERIES IN THE MEKONG DELTA OF VIETNAM

Annette Simone Boerlage[a]*, Tran Thi Tuyet Hoa[b], Tran Thi My Duyen[b], Dang Thuy Mai Thy[b], Nguyen Thi Thu Hang[b], Roger W. Humphry[a] and Nguyen Thanh Phuong[b]

[a] Epidemiology Research Unit, Department of Veterinary and Animal Science, Northern Faculty, Scotland's Rural College (SRUC), Inverness, UK
[b] College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 street, Can Tho City, Vietnam
Correspondence: ntphuong@ctu.edu.vn

Introduction
Striped catfish (*Pangasianodon hypophthalmus*) is one of the most important aquatic products of Vietnam. Most catfish farms are small holdings that are owned, operated, and managed by a single farmer, who farms the catfish intensively in earthen ponds. Hatchery, nursing, and grow-out ponds are rarely integrated. Husbandry practices such as emptying the pond between stocks and treating the pond bottom with lime or salt are common ingredients for farmers' health management strategies aiming to keep disease under control. There is an incomplete picture of the prevalence of common diseases in nurseries, and little evidence-based knowledge on effectiveness of husbandry practices on occurrence of these common diseases.

Material and Methods
Semi-structured questionnaires were conducted by face-to-face interviews from January to May 2018 on 63 randomly selected striped catfish nursing farms in An Giang province (n = 25), Can Tho province (n = 16), and Dong Thap province (n = 22). Occurrence of disease in the last crop was assessed for bacillary necrosis of pangasius (BNP), motile *Aeromonas* septicemia (MAS), parasitic disease, saprolegniasis, tail rot disease, swollen swim bladder disease and pale gill and pale liver syndrome. Answers to the questions on husbandry practices resulted in 60 – 86 variables. These were used to explore risk factors for presence the three most common diseases (BNP, MAS and parasitic disease), using logistic regression. Outcome was an odds ratio (OR), which is a measure of association that indicates the relative change in risk. An OR > 1 means a husbandry practice is a risk factor, an OR < 1 means it is protective. An OR = 1 means there is no effect of this husbandry practice.

Results
Prevalences of disease were BNP (75% of farmers), MAS (60%), parasitic disease (48%), saprolegniasis (19%), pale gill and liver syndrome (17%), tail rot (14%) and swollen swim bladder disease (3%). Eighty-eight percent of farmers experienced more than one disease during their last crop. Most farmers reported more than one disease occurring during their last crop, with most frequently "BNP and MAS" (22% of observations), "BNP and parasitic disease" (10%) and "parasitic disease and MAS" (10%). For BNP, both increasing the duration between stocking and start of feed training (OR = 0.85, 0.75-0.95 95% CI), and using iodine to treat fish at stocking (OR = 0.23, 0.05-0.95) were protective. For MAS, an increase in number of years of experience in catfish nursing was a risk factor (OR = 1.39, 1.06-2.02) and the duration between stocking and start of feed training was protective (OR = 0.88, 0.78-0.97). For parasitic disease, draining the sludge from the pond was protective (OR = 0.32, 0.01-0.97), and increasing the number of days between letting water back into the pond and stocking the fish was a risk factor (OR = 2.49, 1.13-6.40).

Discussion
The high prevalence of diseases occurrence is concerning and shows that current preventive and mitigation measures need to be improved. The high number of crops with multiple diseases may indicate a reduced general resilience of the fish, which indicates that mitigation strategies would benefit from a comprehensive approach, instead of focused on single diseases or pathogens. Of the many different husbandry practices assessed, only a few had an effect on disease occurrence, implying that there is scope to optimize the health management strategies of these farmers towards improved sustainability of striped catfish nursing farms.

Acknowledgements
This work was supported by Tay Nam Bo programme (grant number: 17/2017/HĐ-KHCN-TNB.ĐT/14-19/C18). The authors would like to thank fish farmers for their cooperation and students and extension workers of the sampling sites for their hard work.

NUTRIENT REMOVAL PERFORMANCE OF MACROALGAE IN SEA URCHIN INTEGRATED MULTI TROPHIC AQUACULTURE SYSTEM

A. Bóia *, M. Ressurreição, T. Verdelhos

Affiliation: University of Coimbra, Marine and Environmental Sciences Center, Department of Life Sciences, Calçada Martins de Freitas, 3000-456 Coimbra, Portugal
*Email: margaridaboia@gmail.com

Introduction:
In recent decades, aquaculture development has been governed by a growing perspective of environmental awareness along with public concern about the quality of its products (Correia, 2013). As such, great emphasis has been placed on topics such as food safety and quality, potential health impacts, animal welfare and environmental sustainability of the activity, either by producers, stakeholders, scientific community, or by the general public.

Within this scope, huge effort has been made for developing and implementing building more sustainable systems through technological innovations in order to improve water use efficiency and diminishing environmental impact. Integrated multi-trophic aquaculture (IMTA) aims at the integrated production of *sp*ecies of different trophic levels under a circular economy approach, minimizing energy losses and environmental deterioration.

On the other hand, recirculating aquaculture systems (RAS) exhibit many advantages, such as decreased environmental impacts, low water consumption, high fish yield and reduced land use, providing a controlled environment for aquaculture practice. Integrating IMTA aquaculture with RAS systems will certainly promote sustainable aquaculture production with environmental, economic, and social advantages.

On an IMTA system, the uneaten feed and wastes of one *sp*ecies are recaptured and converted into feed, fertilizers, and energy to another *sp*ecies. There are multiple possible configurations for IMTA systems, integrating the production of vertebrate and invertebrate *sp*ecies, and macroalgae (Correia, 2020). Algae use solar energy and available nutrients (particularly Carbon, N and P) to photosynthesize new biomass while assimilating the inorganic nutrients dissolved in water (Silva, 2017). In recirculating systems, it is essential to keep nutrient concentrations below certain limits, once some forms of N, such as nitrite (NO_2^-) and ammonia (NH_4^+) are toxic and, in certain concentrations, can be lethal, and the use of macroalgae to assimilate these excessive nutrients can be viewed as a viable bio-based solution to address this problem. And, furthermore, the produced macroalgae biomass can represent a valuable extra income to the aquaculture activity.

In the present study, the feasibility of IMTA system on a pilot scale, combining *P. lividus* (primary *sp*ecies) with *Ulva sp*. and *Gracillaria sp*. as extractive *sp*ecies, was assessed, evaluating the nutrient removal performance of the used macroalgae.

Material and Methods:
The experimental design consisted of a RAS system with 9 tanks with recirculating water for sea urchin production and 6 individualized tanks for macrolagae trials. The system is equipped with aeration, mechanical and biological filtration (e.g. *sp*onge filters, bio-balls), an air-cooled water chiller, and a water pump. Organisms (sea urchins and macroalgae) were collected at Buarcos (Figueira da Foz, Portugal), transported to the laboratory, processed (cleansed with distilled water, litter and epiphytes were removed) and placed in the system under the test conditions.

The assay was carried out in August/September 2020 and in January 2021 using different macroalgae *sp*ecies (*Ulva sp*. and *Gracillaria sp*.) During the assay, temperature, pH, dissolved oxygen and salinity were daily measured with a multiparameter (Hanna Instruments Inc., Rhode Island, USA), to monitor and ensure water quality. Daily water samples were collected for analysis – determination of ammonia, nitrate, nitrite and phosphate concentrations, through autoanalyser method.

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Results:

	Weight Initial	Weight Tend
	g	g
Sea urchin	1120	1235
Ulva sp	405,46	468,49
Gracillaria sp	706,87	779,21

Table I: Sea urchin and macroalgae biometric measures, weight (g) and diameter (mm).

	T°C					Sal				
	sea urchin	*Ulva sp*		*Gracillaria sp*		sea urchin	*Ulva sp*		*Gracillaria sp*	
		20% exchange water	50 % exchange water	20% exchange water	50 % exchange water		20% exchange water	50 % exchange water	20% exchange water	50 % exchange water
Initial	18,06	16,88		18,79		37,66	38,67		37,86	
Tacl	18,3	16,81	16,7	17,2	17	37,12	37,6	38,23	37,78	38,
T1	18,31	16,74	16,64	17,17	16,86	37,6	37,01	39,27	38,61	38,
T2	18,23	17,08	17	17,38	17,28	37,19	38,4	37,86	38,87	

Table II: Water quality parameters (Temperature (°C), Salinity (SAL).

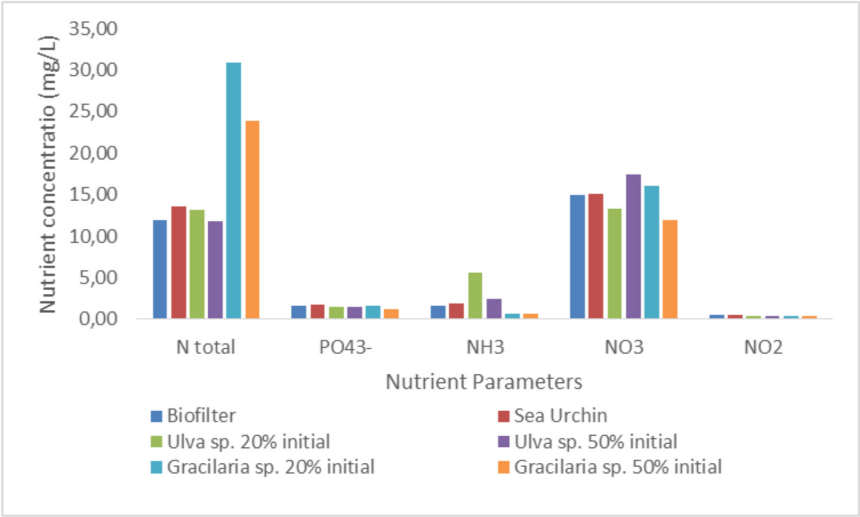
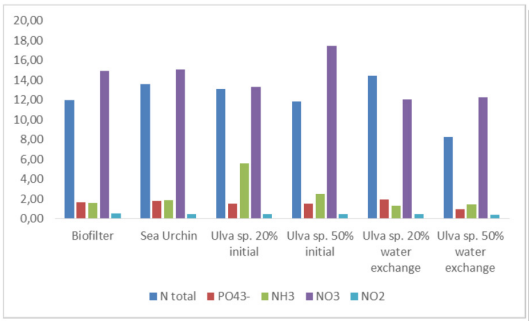


Fig 1. - Chemical parameters (ammonia, nitrites, nitrate, phosphates and n total in mg/L) in the recirculating aquaculture system at initial time for both macroalgaes.



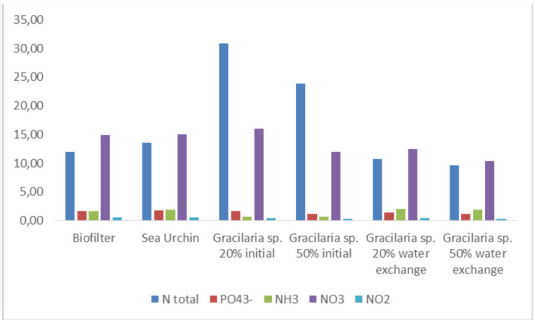


Fig 2a. – The initial values for nutrient (mg/L) in biofilter, sea urchin tank and *Ulva sp* tank for different water exchange and values obtained for exchange water day. **Fig 2b.** – The initial values for nutrient (mg/L) to *Gracillaria sp* experiment with the initial values and at exchange water day.

(Continued on next page)

Discussion:
During this test there was a growth for both *sp*ecies, the initial weights were 405.46 g for *Ulva sp*. and 706.87g for *Gracillaria sp*, and the finals for *Ulva sp*. 468.4g and 779.21g for *Gracillaria sp*.

In addition to the growth in weight of the macroalgae, we also verified their capacity to remove nutrients in the water of the recirculation system where the sea urchins were. Regarding the concentrations of nutrients, ammonia (NH3) on the first day has a concentration of 5.61 mg / L where 20% water is changed and 2.51 where the water change is 50%, on the day of water we have a reduction to 1.30 mg / L and 1.44 mg / L respectively, in the trial with *Ulva sp*. In the case of *Gracillaria sp*. we obtained as an initial value 0.67 mg / L and 0.72 mg / L for 20% and 50% of water change; however, on the day of the water change, the ammonia values were 2.06 mg / L and 1, 89 mg / L, there was no reduction in this parameter.

In the case of nitrites (NO2-) the initial values are 0.47 and 0.46 mg / L and the final values 0.46 and 0.45 mg / L, there seems to be no reduction during the days preceding the water exchange in the case of *Ulva sp*. for *Gracillaria sp*. there was no decrease or increase in the concentration of nitrites. The values for 20% water exchange are 0.40 mg / L and the end is 0.39 mg / L and for 50% water exchange the initial value was 0.33 mg / L and the end 0.35 mg / L.

The initial phosphate concentration in the *Ulva sp* tank was similar to 1.50 and 1.56 mg / L for the 20% water exchange, this final value was 1.96 mg / L but where 50% was changed, the final value was 0.97 mg / L . In the case of *Gracillaria sp*. a slight reduction was obtained, the initial concentrations were 1.61 and the final 1.41mg / L for the 20% exchange and 1.18 for 1.15 mg / L for the 50% water exchange.

Conclusion:
Through the results we can verify that this test promoted the growth of two *sp*ecies of macroagae, as intended in multi-trophic aquacultures. Although not all nutrient concentrations were reduced during the test, either by the introduction of two different macroalgae *sp*ecies or by the volume of water exchanged.

Further studies are still needed and we can conclude that each multi-trophic aquaculture system has *sp*ecificities that influence the removal of nutrients and the growth of macroalgae in these systems.

References:
Alexander, K.A, Angel, D., Freeman S., Israel, D., Johansen, J., Kletou, D., Meland, M., Pecorino, D., Rebours, C., Rousou, M., Shorten, M., Potts, T., (2015) Improving sustainability of aquaculture in Europe: Stakeholderdialogues on Integrated Multi-trophic Aquaculture (IMTA), Environmental Science & Policy 55, 96-106

Barrington K., Ridler N., Chopin T., Robinson S., Robinson B., (2010) Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquac Int 18:201-211

Basauyux, O., Mathieu, M., (1999) Inorganic nitrogen and its effect on growth of the abalone Haliotis tuberculata Linnaeus and the sea urchin Paracentrotus lividus Lamarck, Aquaculture 174, 195-107

Correia, M. Azevedo, I., Peres, H., Magalhães, R., Oliva-Teles,A., Almeida, C., Guimarães, L., (2020) Integrated Multi-Trophic Aquaculture: A Laboratory and Hands-on Experimental Activity to Promote Environmental Sustainability Awareness and Value of Aquaculture Products, Front. Mar. Sci., doi: 10.3389/fmars.2020.00156

Shpigel, M., Shauli, L., Odintsov, V., Ben-Ezra, D., Neori, A., Guttman, L., (2018) The sea urchin, Paracentrotus Lividus, in an Integrated Multi-Trophic Aquaculture (IMTA) system with fish (Sparus Aurata) and seaweed (*Ulva* lactuca): Nitrogen partitioning and proportional configurations. Aquaculture, 490, 260-269

AN INDIVIDUAL-BASED BIOENERGETIC MODEL OF RAINBOW TROUT (*Onchorhynchus mykiss*)

Matteo Bolzonella[1,*], Edouard Royer[1] and Roberto Pastres[1,2]

[1]Department of Environmental Sciences, Informatics and Statistics (DAIS), Università Ca' Foscari Venezia, Via Torino 155, 30172 Venezia Mestre, Italy.
[2] Bluefarm, s.r.l., Venice, Italy
E-mail: matteo.bolzonella@unive.it

Introduction

In Italy, there is a long tradition of rainbow trout (*Oncorhynchus mykiss*) farming, which requires high-quality and well-oxygenated water. However, this activity negatively affects quality of downstream water in terms of several parameters, e.g. BOD_5, NH_4 (Sidoruk, 2019). Royer et al. (2021) developed a novel approach for the implementation of the framework of Precision Fish Farming to efficiently control and predict short-term evolutions of concentration of dissolved oxygen in raceway and they highlighted the importance of having reliable tools concerning the temporal evolution of fish weight according to change in environmental and management conditions.

In this work, a new dynamic bioenergetic individual model of rainbow trout is presented, which enables one to simulate the evolution of fish weight in relation to water temperature, feed ration and feed quality. Furthermore, the model allows the estimation of oxygen consumption and ammonia excretion rates, which can be used to develop a population individual-based model in order to simulate short-term evolution of concentration of dissolved oxygen and of ammonia.

Material and Methods

This model belongs to the class of bioenergetic models (Dumas et al., 2010). The dynamics of weight is simulated according to the energy budget method, in which growth results as the difference between energy provided via fish feed and energy used for maintenance (Brigolin et al., 2010). Ammonia excretion rate was estimated as the difference between Nitrogen provided with fish feed and the amount of Nitrogen stocked in new tissue, according to chemical composition of trout (Dumas et al., 2007). As other bioenergetic models, this model needs several parameters in order to properly simulate fish physiology. Most of them were taken from literature review, e.g. oxygen consumption rate was set equal to the mean of the values presented in Royer et al. (2021). However, the parameter ruling the energy intake at a certain temperature was obtained using a calibration process in which model results were compared with weight observed with periodic samples. Furthermore, the model was quantitatively validated with a comprehensive dataset collected using Biomass Daily (BD), a real time non-invasive device for monitoring weight distribution. Data for calibration and validation were provided by a rainbow trout farm located in the Northern Italy.

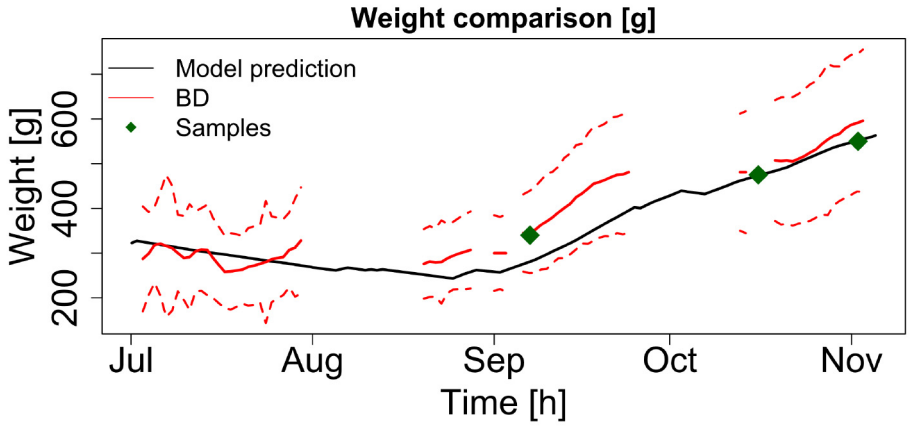


Figure 1. Comparison of weight predicted by the model (black line), observed by BD (mean weight: red solid line, standard deviation: red dashed line) and farmer samples (green points)

(Continued on next page)

Results and discussion

Figure 1 shows the comparison of weight predicted by the model (black solid line) with the mean weight estimated by direct samplings periodically made by the farmer (green points). In addition, this figure compares these data with the evolution of mean weight (red solid line) observed by BD with its daily standard deviation (red dashed lines). The visual comparison shows a good agreement between the observations and the model output. Two Goodness of Fit indexes were calculated, namely R^2 and RMSE, to evaluate the performance of model prediction against samples; R^2 is equal to 99.43% and RMSE is equal to 34g. As regards the comparison between model prediction and mean weight observed by BD, visually one can see a good agreement also for these two time series. In particular, weight predicted by the model is always located in the uncertainty interval determined by the standard deviation around the mean value. The two Goodness of Fit indexes comparing weight predictions and mean value observed by this device are: R^2= 86.34% and RMSE = 50g. The high variability of daily number of observations as well as possible bias in the detection by BD can address the decrease in terms of performance indexes between the two datasets.

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References

Brigolin, D., Pastres, R., Tomassetti, P., & Porrello, S. (2010). Modelling the biomass yield and the impact of seabream mariculture in the Adriatic and Tyrrhenian Seas (Italy). *Aquaculture International*, *18*(2), 149–163.

Dumas, A., de Lange, C. F. M., France, J., & Bureau, D. P. (2007). Quantitative description of body composition and rates of nutrient deposition in rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *273*(1), 165–181.

Dumas, A., France, J., & Bureau, D. (2010). Modelling growth and body composition in fish nutrition: Where have we been and where are we going? *Aquaculture Research*, *41*(2), 161–181.

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.

Sidoruk, M. (2019). Effect of rainbow trout production in a two-stage cascade system on water quality. *Journal of Elementology*, *24*(2), 739–753.

EFFECT OF HYDROLYZED MICROALGAE FROM BIOREFINERY ON GROWTH, FILLET COMPOSITION AND HEALTH IN SIBERIAN STURGEON (*A. baerii*) FINGERLINGS

T. Bongiorno*[1], L. Foglio[1], L. Proietti[1], M. Vasconi[2], V.M. Moretti[2], A. Lopez[2], D. Carminati[3], F. G. Acién Fernández[4], A.J. Vizcaíno[5], A. Galafat[5], F.J. Alarcón[5], K. Parati[1]

[1]Istituto Sperimentale Italiano L. Spallanzani - Loc. La Quercia, 26027 Rivolta d'Adda (CR), Italy
[2]Dipartimento di Scienze Veterinarie, Università degli Studi di Milano, Italy
[3]Centro di ricerca Zootecnia e Acquacoltura, CREA-ZA, 26900 Lodi (LO), Italy
[4]Department of Chemical Engineering, University of Almerıa, 04120 Almerıa, Spain
[5]Department of Biology and Geology, Ceimar-University of Almerıa, 04120 Almería, Spain
E-mail: t.bongiorno@tstechnologies.it

Introduction

The use of microalgae as additive in aquaculture has received a lot of attention due to their positive effect on weight gain, increased protein deposition in muscle, improved resistance to disease, improved feed digestibility, physiological activity, starvation tolerance and fillet quality (Becker *et al*., 2004). However, microalgae display some critical issues related to both high production cost and structure/composition of their cell wall, which is a protective barrier that reduces the bioavailability of the intracellular nutrients (Wu *et al*., 2017). Previously results about inclusion in aquafeed of microalgae from a biorefinery suggest that this biomass could be a valuable nutrient source able to ensure an adequate growth performance and health of gastrointestinal tract in Siberian sturgeon (Bongiorno *et al*. 2020). Moreover, recent study showed that the application of a hydrolysis treatment to microalgae biomass improves the release of low molecular weight bioactive peptides and free amino acids, increasing nutrient bioavailability and functional properties in fish (Galafat *et al*., 2020). The development of a highly digestible hydrolyzate produced from microalgal biomass cultivated on biorefinery would limit the disposal costs sustained by the companies and lower the production costs of the microalgae through recovery of some by-products and agro-industrial waste. This study was aimed to evaluate the effects of the dietary inclusion of two enzyme hydrolyzates of microalgae *Nannochloropsis gaditana* and *Scenedesmus almeriensis*, obtained by both biorefinery and conventional cultivation system, on different growth and fish heath parameters in Siberian sturgeon (*A. baerii*) fingerlings.

Materials and methods

Nannochloropsis gaditana and *Scenedesmus almeriensis* growth in both condition on conventional Synthetic Medium (SM) and diluted Pig Manure (PM) were hydrolyzed according to Galafat *et al*. (2020). Four complete diets were formulated to be grossly iso-proteic and iso-lipidic. A control diet (C) was prepared using a blend of conventional animal and vegetal protein sources, while experimental diets were prepared replacing the 10% of protein and lipid by hydrolysed *N. gaditana* grown on Synthetic Medium (H-NSM) or on pig manure (H-NPM), and hydrolysed *S. almeriensis* grown on Synthetic Medium (H-SSM) or on Pig Manure (H-SPM). All the ingredients were mixed and pelleted by a cold extrusion process (70°C). The experimental diets were manufactured at the Servicio de Dietas Experimentales of the Universidad de Almería (http://www.ual.es/stecnicos_spe). 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 240 juvenile *A. baerii* (average 12.8±0.3g each) were randomly allocated among 15 circular tanks (16 fish/tank) in a RAS system under controlled rearing conditions (temperature, 19°C, DO 9.6 mg/L, artificial day-lenght, 12h). Diets were offered in two daily meals with a fixed feed ratio (3% body weight) over 6 weeks and each group were weighted every week under moderate anesthesia. At the end of the trial, survival rate (%), Final Body Weight (FBW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Intake (FI), were evaluated. A pool of the respective skinned fillets from three fish per tank (nine fish per dietary treatment) and a pool of livers from the same fish were frozen and stored at -20°C for proximate and fatty acid analysis and superoxide dismutase (SOD) and catalase (CAT) activity evaluation. In addition, two fish per tank (six fish per dietary treatment) were sacrificed and dissected for intestinal tract sampling and subsequent enzymatic and TEM and SEM microscopy analysis. Data of growth performance, biometric indices, proximate composition, fatty acids, antioxidant enzymes were expressed as mean ± standard deviation. Prior to statistical analysis, all the data were evaluated for normality distribution. Differences between treatments were analysed 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).

Results

All the experimental diets used during the feeding trial resulted similar for their proximate and fatty acid composition as well as for their microbiological quality (data not reported). Growth performance, nutrient utilization, somatic indices and fillet proximate composition of *A. baerii* fingerlings fed the test diets over 40 days are shown in table 1. Dietary treatments significantly affected FBW SGR, FCR, PER ($P<0.05$), enzyme activities, microvilli height and absorption surface of the intestinal mucosa of *A.baerii* fingerlings but no fillet composition and liver antioxidant activity (SOD, CAT) ($P>0.05$).

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Table 1 Growth performance, nutrient utilization, somatic indices and fillet proximate composition of *A. baerii* fingerlings fed the test diets over 40 days.

	C	H-NSM	H-NPM	H-SSM	H-SPM
Growth parameters					
IBW[1]	12.8	12.8	13.4	12.3	12.6
FBW[2] (g)	44.2[b]	43.0[b]	48.2[c]	39.9[a]	40.2[a]
FI[3] (g)	346.2	319.0	354.7	322.7	329.5
SGR[4]	3.1[b]	3.03[b]	3.20[b]	2.95[a]	2.90[a]
FCR[5]	0.69[ab]	0.73[b]	0.65[a]	0.74[b]	0.75[b]
PER[6]	2.8[d]	2.7[c]	2.7[c]	2.6[b]	2.5[a]
SR[7] (%)	100	95.8	95.8	97.9	97.9
K-factor	0.30	0.28	0.29	0.29	0.29
Proximate composition (g/100g muscle on wet basis)					
Moisture	79.05	79.22	78.83	78.64	77.59
Total protein	16.55	16.86	16.94	17.33	18.11
Total lipids	3.48	2.95	3.28	3.13	3.33
Ash	0.92	0.98	0.95	0.90	0.97

[1]IBW (g): Initial fish biomass in the tan k (g)/number of fish in the tan k; [2]FBW (g): Final fish biomass in the tan k (g)/number of fish in the tan k; [3]FI (g): Daily Feed intake x days; [4]SGR:100 x [(ln final body weight - ln initial body weight) / days]; [5]FCR: feed intake /weight gain; [6]PER : body weight gain (g)/(feed intake (g) x protein level in the diet (%);[7]SR: final number of live fish/initial number of live fish x 100

Discussion and Conclusion

Few data are available on the potential inclusion of microalgae obtained from a biorefinery as functional ingredient in aquafeed, and in particular, on its application as hydrolyzed biomass (Galafat *et al*., 2020). Previous results suggest that the use of the microalgae from biorefinery are valuable nutrients source able to ensure both an adequate growth performance and healthy gastrointestinal tract in different fish species (Bongiorno *et al*., 2020; Valente *et al*., 2019; Kiron *et al*., 2012). The data observed in this study confirm the potential use of the hydrolyzed microalgae *N. gaditana* and *S. almeriensis* as functional ingredient in the Siberian sturgeon aquafeed in partial replacement of fish meal/oil, in fact all the experimental diets tested, both based on microalgae grown on SM and on PM ensure a balanced and complete level of the nutrients, suitable for the growth of sturgeon juveniles and nutritional quality of the fillet, analogous to the control group fed with a fish meal/oil-based diet. Especially the inclusion of hydrolyzed *N. gaditana* cultivated on PM achieved better results in terms of growth and nutrient utilization parameters. Moreover, the presence of hydrolyzed microalgae in the diets stimulates microvilli length and absorption surface in intestine. In particular, *S. almeriensis* stimulates proteolytic activity, microvilli length, absorption surface and apical area. Further studies on this topic are needed to making more sustainable the microalgae and aquafeed production through a circular bioeconomy approach.

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References

Becker W. (2004) Microalgae for Aquaculture: The Nutritional Value of Microalgae for Aquaculture. In: Richmond, A. (eds.): Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Oxford, 2004, 380-391

Bongiorno T., Foglio L., Proietti L., Vasconi M., Lopez A., Pizzera A., Carminati D., Tava A., Vizcaíno A.J., Alarcón F.J., Ficara E., Parati K. (2020) Microalgae from Biorefinery as Potential Protein Source for Siberian Sturgeon (*A. baerii*) Aquafeed. Sustainability 12(21):8779.

Galafat, A., Vizcaíno, A.J., Sáez, M.I. et al. (2020) Evaluation of *Arthrospira* sp. enzyme hydrolysate as dietary additive in gilthead seabream (*Sparus aurata*) juveniles. J Appl Phycol 32: 3089–3100).

Kiron V., Phromkunthong W., Huntley M., Archibald I., De Scheemaker G. (2012) Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquac Nutr 18(5):521–531.

Valente, L.M.P., Custódio, M., Batista, S.*et al*. (2019)Defatted microalgae (*Nannochloropsis sp*.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. Fish Physiol Biochem 45:1067–1081.

Wu C., Xiao Y., Lin W., Li J., Zhang S., Zhu J., Rong J. (2017) Aqueous enzymatic process for cell wall degradation and lipid extraction from *Nannochloropsis* sp. Bioresour Technol 223:312–316

COMPARISON OF BALLAN WRASSE *Labrus bergylta* AND LUMPFISH *Cyclopterus lumpus* DELOUSING PERFORMANCE UNDER SUMMER AND WINTER CONDITIONS AND THE IMPACT OF CRYPTIC SEA LICE COLOUR VARIANTS

A. Brooker*, A. Davie and H. Migaud

Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK
Email: a.j.brooker@stir.ac.uk

Introduction

Cleaner fish (ballan wrasse *Labrus begylta* and lumpfish *Cyclopterus lumpus*) are a key component of caligid sea lice management in Atlantic salmon (*Salmo salar*) aquaculture in North Atlantic countries. Delousing performance in both species is extremely variable and is likely influenced by a broad range of biological and environmental factors. Both species of cleaner fish have different environmental preferences, with ballan wrasse preferring warmer conditions than lumpfish. Furthermore, unpigmented or 'cryptic' colour variants of sea lice (*Lepeophtheirus salmonis*) have been found at commercial salmon farms, and their occurrence is thought to be a result of cleaner fish delousing as a selection pressure. In this study, the impact of Scottish summer and winter water temperatures and cryptic colour variants on delousing performance in ballan wrasse and lumpfish was tested in a series of tank-based trials.

Materials and methods

Trials were performed in a flow-through indoor tank system (12 × 750 L circular tanks) supplied with natural seawater pumped ashore and with a natural simulated photoperiod (17:7 h light:dark). Prior to the start of each trial, Atlantic salmon were subjected to a controlled sea-louse copepodid infection, and once the sea lice had developed to the late chalimus stage, the salmon were randomly distributed into the experimental tanks. When the sea lice had developed into motile adult stages (1–2 weeks), cleaner fish were introduced into treatment tanks (10% cleaner fish:salmon ratio) with control tanks containing only salmon. Ten salmon from each tank were randomly sampled before the cleaner fish were introduced and every 24–48 h afterwards for up to eight days, and attached adult sea lice were counted to estimate delousing rates. Three trials were conducted: (1) summer conditions/lumpfish size, (2) lice colour, and (3) winter conditions.

Results and conclusions

In the summer trial (mean water temperature 14.5 ºC), all treatment groups were more effective at delousing female lice than male lice with numbers of female lice less than 50% after 96 h. Male lice were deloused at a slower rate with wrasse more effective than lumpfish. In the winter trial (mean water temperature 9.0 ºC), lice numbers in all treatment groups were less than 30% after 4 days and less than 15% after 7 days. Although there were some compounding effects in the summer trial (large salmon size and high infection level), which may have limited the relative delousing rates, both lumpfish and wrasse appear to be effective delousers in both Scottish summer and winter water temperatures.
Large lumpfish (80.4 ± 11.0g) were marginally more effective at delousing than small lumpfish (40.8 ± 5.9g) although there was no clear effect of lumpfish size on delousing (female lice 23.3 ± 8.8% vs. 42.1 ± 18.4% and male lice 80.5 ± 14.0% vs. 84.7 ± 2.9% for large and small lumpfish, respectively).

Cryptic lice did not affect the delousing efficacy of ballan wrasse with all female lice removed after 48 h and all male lice removed after 96 h. Lumpfish were less effective at delousing cryptic lice than pigmented lice (female lice 9.9 ± 5.7% vs. 41.4 ± 32.8% and male lice 60.2 ± 6.2% vs. 74.3 ± 16.0% remaining after 7 days for pigmented and cryptic lice, respectively). An increased prevalence of cryptic lice in farmed salmon may reduce the delousing efficacy of lumpfish whereas ballan wrasse are effective against both pigmented and cryptic variants.

SPAT MORTALITY IN FARMED BLUE MUSSELS (*Mytilus edulis*) IN SCOTLAND

C. C. Broughton[1]*, J. Bailey[1], D. Green[1], S. Carboni[1],

[1]Institute of Aquaculture, University of Stirling, FK7 4LA Stirling, Scotland, United Kingdom
E-mail: c.c.broughton@stir.ac.uk

Introduction

The industry for blue mussels (*Mytilus edulis*, Linnaeus, 1758) has been a growing sector in Scotland for the past ten years, and relies heavily on the wild collection of seed (spat) for the on-growing product. There are several external risks that could impact mussel production, specifically unreliable spat resources and poor water quality, pollution, bio toxins (GLOBEFISH and FAO, 2019) and infectious diseases (Bower et al., 1994). In recent years, problems involving spat availability and mortality have occurred impacting the mussel industry to the extent of possible closures of some businesses. One site in particular has been experiencing a severe case of spat mortality in the winter months for the past decade. Interestingly, it is only in the winter months where the mortalities have been observed and only the spat is affected, whereas older mussels from the previous season seem to remain in a healthy state. The investigation of this mortality case involves an experimental design which observes the mortality on a fortnightly basis by deploying lantern nets from the grow-out lines in the affected site. The experiment was also set up at a control site in a different body of water which has not been experiencing any mortalities.

Materials and methods

20 lantern nets, each stocked with 1000 individuals, were deployed across the affected site and 10 nets were deployed across the control site late October of 2018. The collection and counting of dead spat occurred every fortnight after stocking, for a period of 11 weeks. Moribund animals and survivors, were collected at each time point for analyses including histopathology, bacteriology and virology. Temperature and salinity were continuously monitored by data loggers (HOBOware) in the proximity of the experimental nets at both affected and control site. Additionally, water samples for heavy metal analysis by Ion Coupled Plasma Mass Spectrometry, chlorophyll, TPM and POM were collected at each time point.

Results and discussion

Preliminary results show that the spat mortality was significantly higher in the affected site compared to the control site, reaching 68.3 percent over 4 time points within the 11-week period. Most mortalities were observed at T1 and T2 reaching 54.9 percent (30d post stocking) after which the mortality rate then subsides at T3 (62.7%), 44d post stocking, as opposed to a final cumulative mortality of 0.9 percent at the control site. These results offer actual numbers of mortalities rather than previous estimates and also support the observation of spat indeed dying in the winter months. Chlorophyll, TOM and POM samples corresponded to the seasonal primary production cycle. The gathered data will be used in a software to generate a growth prediction model, assessing current growth with food availability. The histopathology showed inflammation around the digestive glands, mantle and occasionally in the gills from both sets of samples, however, granulocytomas were predominantly present in the samples from the affected site. Further analyses will include diagnostic techniques including bacterial identification, DNA sequencing and virology of the spat collected.

References

Bower, S. M., McGladdery, S. E. and Price, I. M. 1994. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: Vibrio spp. (Larval and Juvenile Vibriosis) of Oysters., Annual review of fish diseases 4: 1–199.

GLOBEFISH and FAO 2019. The European market for mussels. Available at: http://www.fao.org/in-action/globefish/fishery-information/resource-detail/en/c/338588/ (Accessed: 25 February 2019).

COMMERCIAL SCALE EFFICIENCY AND ENVIRONMENTAL ASSESSMENT OF INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) – PROJECT INTRODUCTION

Alexandra Bulgakova*
Supervisors: Professor Trevor Telfer (IoA), Dr Stefano Carboni (IoA) & Dr Adam Hughes (SAMS)

Institute of Aquaculture, University of Stirling, FK9 4LA (Scotland, UK)
Email: a.k.bulgakova@stir.ac.uk

Introduction

The project will investigate whether commercial scale Integrated Multi-Trophic Aquaculture (IMTA) reduces environmental impact by exploiting fish farm waste using extractive species such as algae and/or bivalve molluscs and increases profitability by diversification of species produced. In doing this, the project intends to deliver guidelines for siting and management of the different aquaculture system components for full-scale IMTA sites, develop disease and potential treatment management plans for the salmonid and extractive IMTA species within IMTA systems, and produce policy recommendations on application of IMTA within the licensing and governance framework for Scotland. Systematic investigations will include chemical, ecological, socioeconomic and physical/site suitability factors to assess the IMTA process.

Research objectives and questions

To systematically investigate key factors affected by the scaling up of IMTA systems to full commercial size salmon farms, including:

- Mechanisms of nutrient transfer & uptake between trophic levels in the system;
- Growth of the extractive species;
- Implications of discharge of chemical treatments from the salmon farms;
- Physical effects of the positioning of the different systems & their interactions;
- Effects of these integrated systems on the wider environment;
- How these systems affect other users of coastal resources;
- Consequences for site selection, regulation and licensing.

Methods and materials

Measurement and modelling of oceanographic conditions and water quality parameters such as temperature, salinity, particulate matter, chlorophyll-a, soluble/particulate N & P data, contaminant concentration, current speed and direction, wave height, rainfall and windspeed will be collected using in situ measurements using CTD, ADCP and weather station data to understand how parameters may affect nutrient transfer and uptake, alongside the growth of the extracted species (Sanderson *et al*, 2008). Data will be modelled in FVCOM and MIKE3D ECOLab.

Nutrient transfer and trophic system analysis will assess the origin, flow and fate of nutrients using stable isotopes and lipid biomarkers, analysed and modelled using 'SIAR' package, 'Ecopath with Ecosim' and dynamic energy budget (DEB) modelling (Redmond *et al*, 2010; Callier *et al*, 2013; Irissari *et al*, 2015). The purpose of this is to understand if nutrients from salmonid aquaculture are being directly utilized by the extractive species.

Plankton (zoo- and phytoplankton) communities will be assessed using plankton nets and microscopy; benthic communities (invertebrates, algae, vertebrates, phanerograms, invasive species) will be assessed using a combination of video and grab samples; biofouling communities will be assessed using video and net sampling. Changes in community structure (Callier *et al*, 2013) will be analysed using GIS habitat mapping and benthic quality indices, when compared with reference locations, will give an understanding of how IMTA differs in impacts compared with monoculture aquaculture sites.

Assessment of bivalve extractive species will be undertaken for growth rates, condition indices, lysosomal membrane stability, filtration rates, absorption efficiencies, analysis of micro parasites and contamination (Cheshuk *et al*, 2003; Aguado-Gimenez *et al* 2014; Wartenberg *et al*, 2018). Assessments of algal extractive species (Neori *et al*, 2004) will be undertaken for biomass yield, morphological traits such as thallus length/weight, blade parameters, nitrogen uptake/assimilation/storage mechanisms and photosynthetic activity. Stable isotopes and fatty acids will be utilized alongside morphological measurements to understand if nutrients have been transferred to extractive species, and what effect they might have on productivity and the environmental footprint of the IMTA system.

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Using data on environment, local and national infrastructure, resource availability, local geography, water quality and socioeconomics, modelling in GIS software layers will provide a tool for assessing site suitability for each of the species, and combined species as IMTA (Nobre *et al*, 2010; Hughes & Black, 2016; Falconer *et al* 2020).

References

Aguado-Giménez, F., Hernández, M.D., Cerezo-Valverde, J., Piedecausa, M.A. and García-García, B., 2014. Does flat oyster (*Ostrea edulis*) rearing improve under open-sea integrated multi-trophic conditions? *Aquaculture international*, *22*(2), pp.447-467.

Callier, M.D., Lefebvre, S., Dunagan, M.K., Bataille, M.P., Coughlan, J. and Crowe, T.P., 2013. Shift in benthic assemblages and organisms' diet at salmon farms: community structure and stable isotope analyses. *Marine Ecology Progress Series*, *483*, pp.153-167.

Cheshuk, B.W., Purser, G.J. and Quintana, R., 2003. Integrated open-water mussel (*Mytilus planulatus*) and Atlantic salmon (*Salmo salar*) culture in Tasmania, Australia. *Aquaculture*, *218*(1-4), pp.357-378.

Falconer, L., Middelboe, A.L., Kaas, H., Ross, L.G. and Telfer, T.C., 2020. Use of geographic information systems for aquaculture and recommendations for development of spatial tools. *Reviews in Aquaculture*, *12*(2), pp.664-677.

Hughes, A.D. and Black, K.D., 2016. Going beyond the search for solutions: understanding trade-offs in European integrated multi-trophic aquaculture development. *Aquaculture Environment Interactions*, *8*, pp.191-199.

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J. and Robinson, S.M., 2015. Availability and utilization of waste fish feed by mussels *Mytilus edulis* in a commercial integrated multi-trophic aquaculture (IMTA) system: a multi-indicator assessment approach. *Ecological Indicators*, *48*, pp.673-686.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M. and Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, *231*(1-4), pp.361-391.

Nobre, A.M., Ferreira, J.G., Nunes, J.P., Yan, X., Bricker, S., Corner, R., Groom, S., Gu, H., Hawkins, A.J., Hutson, R. and 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. *Estuarine, Coastal and Shelf Science*, *87*(1), pp.43-62.

Redmond, K.J., Magnesen, T., Hansen, P.K., Strand, Ø. and Meier, S., 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). *Aquaculture*, *298*(3-4), pp.202-210.

Sanderson, J.C., Cromey, C.J., Dring, M.J. and Kelly, M.S., 2008. Distribution of nutrients for seaweed cultivation around salmon cages at farm sites in north–west Scotland. *Aquaculture*, *278*(1-4), pp.60-68.

Wartenberg, R., Limbu, K., Feng, L., Wu, J.J., Chan, L.L., Telfer, T.C. and Lam, P.K., 2018. The Feasibility of Integrating the Noble Scallop *Mimachlamys nobilis* with Existing Fish Monoculture Farms in the South China Sea: A Bioeconomic Assessment from Hong Kong. *Journal of Shellfish Research*, *37*(3), pp.635-650.

Wartenberg, R., Feng, L., Wu, J.J., Mak, Y.L., Chan, L.L., Telfer, T.C. and Lam, P.K., 2017. The impacts of suspended mariculture on coastal zones in China and the scope for Integrated Multi-Trophic Aquaculture. *Ecosystem Health and Sustainability*, *3*(6), p.1340268.

EFFECTS OF BIOACTIVE PEPTIDES ON GROWTH PERFORMANCE, PLASMA BIOCHEMISTRY, GUT HEALTH AND STRESS RESISTANCE OF GILTHEAD SEA BREAM AND EUROPEAN SEA BASS

S. Busti[1]*, L. Parma[1], S. Ciulli[1], E. Volpe[1], F. Errani[1], F. Dondi[1], M.C. Sabetti[1], E. Brini[1] L. Morsiani, M. Mazzoni[1], G. Lattanzio[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.
E-mail: serena.busti2@unibo.it

Introduction

One of the main key points for the development of sustainable aquaculture, is to define health-promoting circular feed ingredients which maximize performance and stimulate the defence mechanisms of fish. Aquaculture processing by-products are a rich source of bioactive peptides, molecules which can be used as functional feed ingredients to achieve better growth and fish health, thus promoting a sustainable aquaculture development (Siddik, M. A. B. et al., 2021). This study was undertaken in order to assess the potential effects of the bioactive peptides derived from Atlantic salmon (*Salmo salar*) processing by-products on growth, blood biochemistry, immune response and gut histology in European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) reared under normal and after suboptimal condition.

Materials and methods

One trial with European sea bass and gilthead sea bream was conducted in the same recirculation aquaculture system (RAS). Fish (initial weight: 73.6 ± 0.8g), were fed over 58 days with three experimental diets containing different levels of bioactive peptides (0% BP0, 5% BP5, and 10% BP10) in substitution to fish meal (FM). After the end of the trial fish were subjected to suboptimal rearing conditions (high water temperature, 30°C and low oxygen, 70% saturation level) for 8 days. Growth and feed efficiency parameters (specific growth rate, SGR, feed intake, FI, feed conversion rate, FCR and survival), blood biochemistry, immune response marker genes of liver (Ferritin, Hepcidin, Complement Component C3) and distal intestine (Interleukin 1 β, Interleukin 8, Interleukin 10, Interferon 1 α, Mx protein, Transforming growth factor β), and gut histology (haematoxylin-eosin) were assessed at the end of the trial and after suboptimal rearing conditions. Data were analysed by a two-way ANOVA followed by a Tukey's multiple comparison test.

Results

At the end of the trial, no significant differences ($p > 0.05$) due to the different diets were observed in terms of final body weight, SGR, FI, and FCR and survival in both species. Most plasma biochemistry parameters did not show significant differences related to diets and time (suboptimal rearing conditions) for both species. However, in sea bream, glucose and lactate showed a significant diet and time effects with higher values in BP10, especially after suboptimal rearing conditions. Creatinine increased slightly in BP10 after suboptimal rearing conditions, while aspartate aminotransferase (AST) was higher in BP10 compared to the other treatments at both time points examined. Triglycerides and cortisol values were significantly lower after suboptimal rearing conditions for all diets. In sea bass, suboptimal rearing conditions led to an increase of alanine transaminase (ALT) values in BP0 and BP5, while at the same time lactate increased in BP10 and triglycerides displayed a significant decrease in BP0 and BP5.

Discussion and Conclusion

The results showed equivalent growth performance and feed utilization regardless of the different diets indicating that the bioactive peptides derived from Atlantic salmon processing by-products could replace 5% and 10% of FM without compromising the growth and feed utilization. This is in line with other studies in which a moderate replacement of FM was tested (Kim, H. S. et al., 2014; Wei, Y. et al., 2016). At the end of the trial, most of the blood parameters were similar to those observed in European sea bass and gilthead sea bream in previous studies (Bonvini E. et al., 2018; Busti, S. et al., 2020), indicating a general optimal nutritional conditions and welfare. In conclusion, our preliminary data suggest that bioactive peptides recovered from farmed salmon by-products have a promising implication as suitable ingredients for European sea bass and Gilthead sea bream.

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References

1. Siddik, M. A. B., Howieson, J., Fotedar, R., Partridge, G. J. Enzymatic fish protein hydrolysates in finfish aquaculture: a review. Reviews in Aquaculture, 13, 406–430 (2021).
2. Kim, H.S., Jung, W.G., Myung, S.H., Cho, S.H., Kim, D.S. Substitution effects of fishmeal with tuna byproduct meal in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder (*Paralichthys olivaceus*). Aquaculture 431, 92–98 (2014).
3. Wei, Y., Liang, M., Mu, Y., Zheng, K., Xu, H. The effect of ultrafiltered fish protein hydrolysate level on growth performance, protein digestibility and mRNA expression of PepT1 in juvenile turbot (*Scophthalmus maximus L.*). Aquaculture Nutrition 22, 1006–1017 (2016).
4. Busti, S., Bonaldo, A., Dondi, F., Cavallini, D., Yúfera, M., Gilannejad, N., Moyano, F. J., Gatta, P. P., Parma, L. Effects of different feeding frequencies on growth, feed utilisation, digestive enzyme activities and plasma biochemistry of gilthead sea bream (*Sparus aurata*) fed with different fishmeal and fish oil dietary levels. Aquaculture 529 (2020).
5. Bonvini, E., Bonaldo, A., Parma, L., Mandrioli, L., Sirri, R., Grandi, M., Fontanillas, R., Viroli, C., Gatta,P. P. Feeding European sea bass with increasing dietary fibre levels: Impact on growth, blood biochemistry, gut histology, gut evacuation. Aquaculture, 494, 1-9 (2018).

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PUBLIC PERCEPTION AND ACCEPTANCE OF AQUACULTURE AND FARMED SEAFOOD IN EUROPE

S.C. Franco *[1], B. Clark [2], H. Scheulter [2] and S. Kuznesof [2]

corresponding author: sofia.franco@sams.ac.uk
[1] Scottish Association for Marine Science, Oban, Scotland PA37 1QA, UK
[2] School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, Tyne and Wear, NE1 7RU, UK

Introduction
The understanding public perception of aquaculture and farmed seafood is essential to earn acceptance of aquaculture, given its potential to inform decisions by food system businesses, third sector and policy-makers. This concerns not only evidence on perception and desirability of current operations, products and future development, but also evidence to guide the development of public engagement strategies.

With social acceptance recognised as key to unlock aquaculture development in Europe, a systematic literature review (Clark et al., under review) aimed to bring together the literatures on consumers' attitudes and perceptions, on aquaculture and farmed seafood, and social license to operate. This sought to explore the European publics' perceptions towards aquaculture and to examine the key factors which influence social acceptance of aquaculture in Europe. By focusing on this geo-political area, potential differences were analysed, used to identify the factors associated with public acceptance and explore implications for future research.

Material and Methods
Four databases (Business Source Premier, Scopus, Web of Knowledge, Google Scholar) and three sources of grey literature were searched, including the Eurobarometer and FAO websites and conference contributions to EAS and WAS, to ensure a comprehensive search for grey literature and minimise publication bias. Studies were selected on eligibility criteria and included if they examined attitudes, perceptions, knowledge, risk-benefits and links to other aspects of aquaculture production; were published between 1998 and 2018 in English or Spanish; and collected collect primary data from members of the European general public (i.e. within EU Customs Union and European Economic Area). Review articles and studies that examined public attitudes through proxy measures (e.g. news media headlines) were excluded, as were studies examining attitudes towards seafood consumption or attributes which did not include aquaculture as a method of production or where study participants were non-public stakeholders (such as industry or policy-makers).

Studies returned (1421 records identified, post-duplicate removal) were screened in two independent phases against inclusion criteria, by two researchers independently, firstly, for titles and abstracts to identify publications that did not meet the inclusion criteria (199 records after initial screening), and secondly, for included full text publications, with disagreements resolved through discussion (67 records after full text screening).

A total of 57 studies were included in the narrative analysis, coded using a thematic approach which explored the variations and relationships in the data; tested by one researcher and checked by a different team member in order to agree on a revised coding template for application throughout. Studies included in the qualitative synthesis were checked for quality (GRADE-CERQual assessment), on methodological limitations, coherence, adequacy and relevance.

Results and discussion
The studies included (see Clark et al. under review, for overview) covered a range of foci, from specific aspects of aquaculture production, through to more generic overall perceptions, with emerging themes relating to general attitudes towards, cultural differences and knowledge of aquaculture.

Studies examining general public's awareness of aquaculture identified this awareness as low, with higher knowledge associated with experiential (e.g. industry involvement) and education sources, further reported as important to pro-environmental attitudes, ethical attributes and consumption. In the absence of this knowledge, perceptions can be modelled on farmed terrestrial species. Findings indicate geographical differences in knowledge and consumption (e.g. higher in Mediterranean countries) and perceptions (e.g. lower welfare perception in Northern and Western EU countries). Some studies suggest women have higher product involvement, with welfare and the environment as important themes; with higher acceptance of farmed seafood in younger demographics.

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Positive aspects of aquaculture included consistent and affordable supply of fish, as well local economic contribution (via employment and development in areas of aquaculture production), whilst ambivalent and more variable views were noted in relation to the environment. Some sustainability themes were less clear or confusing to the public (e.g. organic aquaculture; differences between eco-labels). The few studies specifically on social licence to operate noted a similar framing of benefits of aquaculture in terms of local economic contribution, with environmental protection from impacts emerging as a strong theme for communities and visitors, with some distinctive views.

One study explored existing differences in perception between groups of species (e.g. seaweed, molluscs), though most focused on farmed fish. Comparative studies between fish species cited preferences for 'familiar' fish, a concept which also underpinned stated product-format preferences, consumption frequency and preparation. Farmed seafood was generally evaluated against wild-caught seafood, with studies reporting stated differences in perceived quality, health credentials, visual evaluation and food safety, generally in favour wild-caught fish, with exception to food safety, where consensus lacked. However, in general this failed to translate into purchase behaviour. Price was key to purchase, with farmed fish perceived as cheaper and more readily available. Some studies demonstrated lower willingness-to-pay (WTP) for the terms 'farmed' or 'aquaculture', with higher WPT for quality credence product attributes and eco-labels. Seafood purchasing evaluative criteria (e.g. sensory attributes, convenience, product format, product information and labelling), communication and information seeking are discussed in more detail in Clark et al. (under review).

More research is needed on information sources on across types of aquaculture and farmed seafood and their relative importance across contexts, as well as environmental, experiential and individual factors modulating attitudes. Given the particular relevance of the low public awareness and understanding of aquaculture, a focus on the assessment of the effectiveness of public communication strategies, product-related information, education campaigns and initiatives to engage communities, involved consumers or general public is paramount to establish a baseline of best practices and to better understand pathways to a socially acceptable and sustainable development of the sector.

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References
Clark B., S. Kuznesof, H. Scheulter, Franco S.C. under review. A systematic review of public perceptions and factors influencing social acceptance of aquaculture in Europe.

APPARENT NUTRIENT AND ENERGY DIGESTIBILITY OF COMMERCIAL FEED INGREDIENTS WITH OR WITHOUT PROTEASE (JEFO) IN RAINBOW TROUT

Brian C. Small*[a], Seunghan Lee[a], Ronald W. Hardy[a], M.A. Kabir Chowdhury[b], Vikas Kumar[a]

[a] University of Idaho, Aquaculture Research Institute, Hagerman Fish Culture Experiment Station, Hagerman, ID 83332, USA
E-mail: bcsmall@uidaho.edu
[b] Jefo Nutrition Inc., Saint-Hyacinthe, Quebec J2S 7B6, Canada
E-mail: kchowdhury@jefo.ca

Introduction

One of the most important aspects in evaluating the effectiveness of alternative feed ingredients is the determination of digestibility. Several alternative feed ingredients have been tested in aquaculture feeds to replace fishmeal for sustainable aquaculture. Nevertheless, imbalanced amino acid profiles, poor digestibility and palatability, and presence of anti-nutritional factors (ANFs) limit their use in aquafeeds (NRC, 2011). Therefore, one of the strategies to supplement enzymes for improving the nutrient digestibility. Among enzymes, proteases have potential use in reducing ANFs, such as protease inhibitors, and breaking down macromolecular proteins (Li et al., 2016). The efficacy of supplemental protease across a wide range of protein ingredients has not been previously investigated. Therefore, this study was conducted to evaluate the effects on apparent digestibility coefficients (ADCs) of dry matter, crude protein, amino acids, and gross energy when dietary protease was added to 17 different protein ingredients using rainbow trout as a model species.

Materials and Methods

In vivo digestibility was determined for 17 ingredients with and without protease supplementation (175 g kg^{-1}, Jefo Nutrition Inc., Quebec, Canada) fed to rainbow trout. The ingredients consisted of two feather meals, two poultry by-product meals, two meat and bone meals, sardine meal, menhaden meal, black soldier fly larvae meal, *Methanococcus maripaludis* single cell protein, soybean meal, canola meal, distiller's dried grains with solubles (DDGS), cottonseed meal, peanut meal, sunflower meal, and algae (*Spirulina* sp.) meal. A batch of test diet containing 30% test ingredient and 70% reference diet mash (combined on a dry-matter basis) was prepared and analyzed. Trout (average weight, 250 g) was used in the digestibility trial. Each of the experimental diets (reference and 34 test diets) was fed to two replicate tanks of fish in a completely randomized design to apparent satiation. Feces were expelled from each fish using gentle pressure on the lower abdomen of fish. ADC of diets and ingredients, for dry matter, protein, amino acids and energy were calculated using the following formula described by Bureau et al. (1999). Apparent digestibility was calculated using fecal material pooled from 30 fish/tank, and all data are expressed as the mean ± standard error of the mean (SE). Data were subjected to a Student's t-test to test for protease effect using SPSS Version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

ADC of dry matter for rainbow trout ranged from 51.0–86.6% for animal products and single cell protein and 33.1–70.1% for plant products without protease supplementation. ADC (without protease supplementation) of protein and energy ranged from 55.4–84.5% and 58.1–90.2%, respectively, for animal products and 70.0–83.8% and 32.9–76.0%, respectively, for plant products. Supplementation with the commercial protease (175 mg protease complex/kg of diet) resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and most individual amino acids.

Conclusion

Supplementation with the protease complex resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and the majority of individual amino acids. Overall, this research demonstrates the benefit of the evaluated protease supplementation on the digestibility of feed ingredients commonly used in rainbow trout and other commercially cultured fish feeds, although the degree of improvement in digestibility varied among ingredients.

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References

Bureau, D.P., Harris, A.M., Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345–358.

NRC (National Research Council), 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington, D.C.

Li, X.Q., Chai, X.Q., Liu, D.Y., Chowdhury, M.A.K, Leng, X.J., 2016. Effects of temperature and feed processing on protease activity and dietary protease on growths of white shrimp, *Litopenaeus vannamei*, and tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture Nutrition 22, 1283– 1292.

A PARENTAL LOW PROTEIN DIET DID NOT AFFECT GROWTH BUT MODULATE LIPID METABOLISM, IN RAINBOW TROUT

T.Callet[1], H.Li[1,2,3], C.Héraud[1], L.Larroquet[1], A.Lanuque[1], F.Sandres[1], F.Terrier[1], A.Surget[1], S.Panserat[1] and L.Marandel[1]

1. INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, NUMEA, Saint-Pée-sur-Nivelle
2. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China
3. University of Chinese Academy of Sciences, Beijing, 100049, China
therese.callet@inrae.fr

Introduction

Toward a sustainable aquaculture, it is essential to continue to reduce the use of marine ingredients. Plant-derived carbohydrates are considered a promising substitute for protein contained in fishmeal because of their inexpensive price and most readily available amounts (Prabhu *et al*., 2017). Even though carbohydrates are considered to be poorly utilized by fish of high trophic levels such as rainbow trout (*Oncorhynchus mykiss*), recent studies have suggested that rainbow trout broodstock are able to grow and reproduce with a low protein/high carbohydrates diet (Callet *et al*., 2020).

Besides, it is now recognized that nutritional insult received during the prenatal period could have a long-term effect on individual phenotype and metabolism. In mammals, both a paternal and a maternal low protein (LP) diets affect their offspring metabolism, growth and survival (Guo *et al*., 2020). However, such effects have not been widely studied in teleost fish. Before increasing the proportion of plant-derived carbohydrates in broodstock diet in farming condition, it is necessary to test the effects of such a parental diet on their offspring. As the effects of nutritional programming could be revealed during period of stress, we investigate the effects of such diet when their offspring were fed with a complete plant-based diet.

Material and methods

Two-year old male and female trout were fed either a control diet with a high protein content (NC, 63.89% protein and 0% carbohydrate) or a diet containing a lower proportion of protein but a higher carbohydrate content (LP-HC, 42,96% protein and 35% carbohydrate) for an entire reproductive cycle for females and 5 months for males. Crossed-fertilizations were carried out in order to obtain 4 groups of fish: NN fish from both males and females fed the control diet, HN fish from only females fed the LP-HC diet and males fed the control NC diet; NH fish from only males fed the LP-HC diet and females fed the NC diet; and HH fish from both parents were fed the LP-HC diet. After 6 month, fish were challenged during a 3 months trial with a complete plant-based diet. Growth parameters were monitored to reveal any effect on offspring phenotypes. Comparisons of hepatic global methylation and hepatic transcriptomes were performed to investigate the effect of a LP diet on offspring metabolism. Then, qPCR analyses were carried out to investigate specific pathways revealed by the transcriptomic analyses.

Results and discussion

At the end of the trial, no significant differences were observed on the final body weight (with the weight of NN 283.29 ± 6.25 g, NH 323.37 ± 20.05 g, HN 319.33 ± 4.91g, and HH 303.99 ± 11.28 g), regardless of the parental nutritional history. Even though fish phenotype were not affected, the parental LP-HC diet strongly altered their offspring hepatic metabolism. First, the global DNA methylation in liver was modified in HN and HH fish, revealing a strong effect of the maternal LP-HC diet; effect which were increased by the male LP-HC diet. Transcriptomic analyses did not detect differences between control fish (NN) and HN or NH fish. The paternal and the maternal LP-HC diet only did not highly alter hepatic metabolism. Hepatic transcriptomes of HH fish were however highly affected, suggesting the existence of the synergistic effect of the maternal and the paternal LP-HC diet.

Of particular interest, expression of some genes related to lipid and cholesterol metabolism were affected by the paternal LP-HC diet. These results are consistent with result obtained in mammals (Carone *et al*., 2010). Interestingly, the cholesterol biosynthesis pathway known to be up-regulated when fish are fed with a complete plant-based diet (Zhu et al., 2020), was further enhanced by the paternal LP-HC diet. The *de novo* lipid lipogenesis pathways were also affected by the parental LP-HC diet (either maternal or paternal or both) and lead to an increased in triglycerides levels in plasma of HN and HH fish and an increased in whole body lipid content in NH and HH fish. Finally and more importantly, the biogenesis of polyunsaturated fatty acids (PUFA), pathway known to be enhanced when rainbow trout are fed a complete plant-based diet, were also increased by the parental LP-HC diet. Interestingly, similar effects of nutritional programming through broodstock have already been described in gilthead sea bream (Izquierdo *et al*., 2015).

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Together, the results suggest that plant-derived carbohydrates could replace fishmeal in trout broodstock diet as no strong adverse effects on their offspring phenotype were detected, in contrast to results typically observed in mammals. In aquaculture species, it has been suggested that nutritional programming could be used as a strategy to improve fish performances. Even though, a LP diet did not significantly improve growth in rainbow trout, such nutritional programming could modulate PUFA biogenesis which could be of particular interest to improve fish ability to use diets devoid of marine ingredients.

References

Callet, T.; Hu, H.; Larroquet, L.; Surget, A.; Liu, J.; Plagnes-Juan, E.; Maunas, P.; Turonnet, N.; Mennigen, J. A.; Bobe, J. & others. Exploring the impact of a low-protein high-carbohydrate diet in mature broodstock of a glucose-intolerant teleost, the rainbow trout. Frontiers in physiology, Frontiers, 2020, 11, 303

Carone, B. R.; Fauquier, L.; Habib, N.; Shea, J. M.; Hart, C. E.; Li, R.; Bock, C.; Li, C.; Gu, H.; Zamore, P. D. & others. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell, Elsevier, 2010, 143, 1084-1096

Guo, T.; Luo, F. & Lin, Q. You are affected by what your parents eat: Diet, epigenetics, transgeneration and intergeneration. Trends in Food Science & Technology, Elsevier, 2020, 100, 248-261

Izquierdo, M.; Turkmen, S.; Montero, D.; Zamorano, M.; Afonso, J.; Karalazos, V. & Fernández-Palacios, H. Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. Aquaculture, Elsevier, 2015, 449, 18-26

Prabu, E.; Felix, S.; Felix, N.; Ahilan, B. & Ruby, P. An overview on significance of fish nutrition in aquaculture industry. International Journal of Fisheries and Aquatic Studies, 2017, 5, 349-355

Zhu, T.; Corraze, G.; Plagnes-Juan, E. & Skiba-Cassy, S. Cholesterol metabolism regulation mediated by SREBP-2, LXRα and miR-33a in rainbow trout (*Oncorhynchus mykiss*) both in vivo and in vitro. Plos one, Public Library of Science San Francisco, CA USA, 2020, 15, e0223813

MUCOSAL INMUNE RESPONSE AND MICROBIOTA MODULATION DURING A NATURAL INFECTION OF *Vibrio harveyi* IN EUROPEAN SEA BASS *(Dicentrarchus Labrax)*

M. Cámara-Ruiz[1], I. Cerezo Ortega[2], F.A. Guardiola[1], J.M. García-Beltrán[1], M.C. Balebona[2], M.Á. Moriñigo[2] and M. Á. 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
[2]Group of Prophylaxis and Biocontrol of Fish Diseases, Department of Microbiology, Campus de Teatinos s/n, University of Malaga, 29071, Málaga, Spain*

Introduction

Disease outbreaks remain to be one of the main bottlenecks for the sustainable development of the aquaculture industry (Opiyo et al., 2018)096 MT. However, production reduced drastically in the past 3 years, with 14,952 metric tonnes (MT. In marine aquaculture, many species from the *Vibrio* genus are serious opportunistic pathogens responsible of significant losses for aquaculture producers (Ina-Salwany et al., 2019). Over the last years, researchers have studied how the microbiota influences the innate immune system, which is of pivotal importance for fish disease resistance (Kelly and Salinas, 2017). The present study aims to characterize the modulation of mucocal immune response and microbial composition during a course of a natural episode of infection by *V. harveyi* in a very important Mediterranean farmed fish species, European sea bass (*Dicentrarchus labrax*).

Material and methods

A total of 18 specimens of European sea bass without external skin macroscopic wounds, non-infected (NI), and with external macroscopic wounds, infected (I), were sampled. Firstly, in order to demonstrate the causal agent of the macroscopic lesions observed, sterile cotton swabs were gently rubbed against the macroscopic wounds (I) and against skin (NI), and spread on a plates of Tryptic Soy Agar (TSA, Difco Laboratories) supplemented with NaCl. Skin mucus samples were taken to determine the local immune response to the infection. The following parameters were analysed: total protein levels, total immunoglobulin levels, total IgM levels, peroxidase activity, lysozyme activity and protease activity. Regarding skin mucus microbiota, 16S rDNA next generation sequencing (NGS) Illumina platform was used to obtain the results.

Results

The bacteria isolated from the skin wounds of injured European sea bass were molecularly identified as *V. harveyi*. However, these bacteria were not isolated from any samples of fish without external lesions (did not grow on the agar plates).

Regarding the mucosal immune response, no statistically significant differences were observed in total protein, immunoglobulin or IgM levels between non-infected and infected fish. However, a decrease in protease activity was observed in skin mucus from infected fish in comparison to non-infected fish.

Regarding skin mucus microbiota, microbial richness (chao1) showed no significant differences between the two experimental groups meanwhile infected fish (I) showed significantly higher microbial diversity than the non-infected group (NI). With respect to beta diversity (NMDS), results indicated that there were significant differences across microbial communities among the two experimental groups ($p < 0.05$). Regarding taxonomy at phylum level, the 4 most dominant phyla in the infected group were *Proteobacteria*, *Verrumicrobia*, *Patescibacteria* and *Bacteroidetes*. In the non-infected group, the most dominant phyla were *Verrucomicrobia*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. The species sorted by LDA score were mainly *Proteobacteria* (*V. harveyi*) for the infected group while *Verrucomicrobia* was mainly associated with the non-infected group.

Discussion

Most infections caused by microorganisms start at or affect the mucosal epithelia of fish. Mucosal surfaces face many antigens while living in harmony with commensal and opportunistic microorganisms (Kelly and Salinas, 2017). Comparing the microbiota of healthy and diseased fish from the same phylogeny, belong to the same species, which are in the same developmental stage and kept under the same conditions is one of the approaches to identify changes in microbiota and mechanisms which can be involved in natural diseases outbreaks (Legrand et al., 2020). In this study, the significant

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decrease in protease activity indicates the importance of protease activity in the local immune response. Our results demonstrated that European sea bass infected with *V. harveyi* suffered a microbial composition shift in the skin mucus. *Proteobacteria* were the dominant phyla colonizing the skin mucus and out-competed other phylum in agreement with other studies (Llewellyn et al., 2017; Reid et al., 2017)host and host-associated microbiota are increasingly understood as important determinants of disease progression and morbidity. Salmon lice, including the parasitic copepod Lepeophtheirus salmonis and related species, are perhaps the most important problem facing Atlantic Salmon aquaculture after feed sustainability. Salmon lice parasitize the surface of the fish, feeding off mucus, scales and underlying tissue. Secondary bacterial infections are a major source of associated morbidity. In this study we tracked the diversity and composition of Salmo salar skin surface microbiota throughout a complete L. salmonis infection cycle among 800 post-smolts as compared to healthy controls. Among infected fish we observed a significant reduction in microbial richness (Chao1, $P = 0.0136$. Moreover, LEfSe analysis, pointed *V. harveyi* as a biomarker for the infected group while *Verrucommicrobia* for the non-infected group. Identified biomarkers of the immune responses and microbiota detected in the present study can contribute to the early-detection system of this disease in aquaculture and avoid significant losses.

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References

Ina-Salwany, M.Y., Al-saari, N., Mohamad, A., Mursidi, F.A., Mohd-Aris, A., Amal, M.N.A., Kasai, H., Mino, S., Sawabe, T., Zamri-Saad, M., 2019. Vibriosis in Fish: A Review on Disease Development and Prevention. J. Aquat. Anim. Health 31, 3–22. https://doi.org/10.1002/aah.10045

Kelly, C., Salinas, I., 2017. Under pressure: Interactions between commensal microbiota and the teleost immune system. Front. Immunol. 8, 1–9. https://doi.org/10.3389/fimmu.2017.00559

Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., Oxley, A.P.A., 2020. A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. Rev. Aquac. 12, 1101–1134. https://doi.org/10.1111/raq.12375

Llewellyn, M.S., Leadbeater, S., Garcia, C., Sylvain, F.E., Custodio, M., Ang, K.P., Powell, F., Carvalho, G.R., Creer, S., Elliot, J., Derome, N., 2017. Parasitism perturbs the mucosal microbiome of Atlantic Salmon. Sci. Rep. 7, 1–10. https://doi.org/10.1038/srep43465

Opiyo, M.A., Marijani, E., Muendo, P., Odede, R., Leschen, W., Charo-Karisa, H., 2018. A review of aquaculture production and health management practices of farmed fish in Kenya. Int. J. Vet. Sci. Med. 6, 141–148. https://doi.org/10.1016/j.ijvsm.2018.07.001

Reid, K.M., Patel, S., Robinson, A.J., Bu, L., Jarungsriapisit, J., Moore, L.J., Salinas, I., 2017. Salmonid alphavirus infection causes skin dysbiosis in Atlantic salmon (*Salmo salar* L.) post-smolts. PLoS One 12, 1–17. https://doi.org/10.1371/journal.pone.0172856

MID−INFRARED SPECTROSCOPIC SCREENING OF METABOLIC ALTERATIONS IN STRESS−EXPOSED GILTHEAD SEABREAM (*Sparus aurata*)

Raquel Carrilho*, Cláudia Raposo de Magalhães, Denise Schrama, Marco Cerqueira, Ana M. Rosa da Costa and Pedro M. Rodrigues

Centre of Marine Sciences, CCMAR, Universidade Do Algarve, Campus de Gambelas, Edifício 7, 8005−139 Faro, Portugal
Email: rvcarrilho@ualg.pt

Introduction
In aquaculture, fish are exposed to production routines that can be stressful, causing harmful effects and welfare issues. To cope with this situation, physiological responses occur in fish, including the activation of metabolic pathways involved in energy production. To access these metabolic alterations, Fourier transform-infrared (FTIR) spectroscopy, a form of vibrational spectroscopy, commonly used in metabolic fingerprinting, can be applied to differentiate functional biochemical groups in the liver of fish exposed to different rearing conditions.

Material and methods
Gilthead seabream (*Sparus aurata*) adults were submitted to three different stressful rearing conditions, namely overcrowding (OC), net handling (NET) and hypoxia (HYP).

Spectra, obtain through FTIR analyses of liver samples, were preprocessed before multivariate statistical analysis. Principal components analysis (PCA) was used for identification of the most important wavenumbers and pattern recognition. Key spectral features were selected and used for classification using the k-nearest neighbour (KNN) algorithm to evaluate if the spectral changes allowed for an accurate discrimination between experimental groups. Furthermore, liver glycogen was assessed using a commercial kit.

Results and conclusions
The total spectrum obtained was characterized by 15 bands which were assigned to specific vibrational modes, functional groups and biochemical compounds.

PCA analyses of the samples suggests that the separation between control and OC30 (OC trial) and NET4 (NET trial) groups occurred along the PC1 axis, while the differences between control group and HYP15 (HYP trial) are observed along the PC2 axis. Loading plots showed positive loading values in the OC30 and NET4 plots, indicating a higher concentration of the biomolecules corresponding to the indicated spectral ranges, while the inverse occurs for the HYP trial plot. PCA loadings suggested that main variations in the spectra responsible for the distinction between the experimental groups were due to differences in the intensity of absorption bands associated with proteins, lipids and carbohydrates. KNN algorithm obtained the highest accuracy values for the classification analysis of the OC groups, with 10 spectral features, what demonstrates that the use of these wavenumbers could discriminate between control and crowded fish with reasonable classification accuracy.

Statistical differences were found, in glycogen analysis, exclusively for the NET trial, between control and stressed fish, with significantly lower levels in NET4. This suggests that these fish were using glycogen stores for energy production, since stressed fish use carbohydrates as rapid energy source and a decrease in hepatic carbohydrates content in NET4 fish was observed.

Overall, FTIR spectroscopy and chemometric analyses of spectra can be useful in an initial approach before more laborious high-throughput techniques such as omics-related analyses.

CO-CULTURE OF ATLANTIC SALMON (*Salmo salar*), SCALLOP (*Pecten maximus*) AND KELP (*Alaria esculenta*) AT A PILOT SCALE MULTI SPECIES RESEARCH SITE – IMPAQT PROJECT

Joanne Casserly*, & Frank Kane

Marine Institute, Rinville, Oranmore, Co. Galway, Ireland
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. 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. As yet, IMTA is not widely adapted at a commercial level. It has been only tested at a very small scale in Europe and the management of large-scale areas remains challenging. 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).

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. As part of this several IMTA pilot sites were established across Europe and Asia to examine the impact of a multi species approach to aquaculture and create a platform to develop and deploy novel sensors and smart systems required for long-term autonomous monitoring in the field. Biometric and abiotic data from the pilot sites contributed to advanced IMTA models to examine potential yields, crop quality, circularity, socioeconomic impacts and the interaction of farm components with the environment on the scale of an ecosystem and that can be used for planning decisions by both farmers and regulators.

Results
This IMTA implementation saw the application of two low trophic species to a monoculture finfish facility. The increase in production from the site is discussed using data on biomass accumulation and crop yields to provide expected nutrient uptake rates and value of additional products to the site. The monitoring and management provided datasets to multiple arms of the IMPAQT project.

Work will examine the remediation potential throughout the growing season as well as continued molecular analysis of the crop to establish preferential harvest times dependent on the end product. Importance of increased monitoring to help establish baseline data for decision support systems to achieve better yield, less environmental impacts, less waste.

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
European Market Observatory for Fisheries and Aquaculture Products (2018). The EU Fish Market, 2018 Edition.
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

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APPLYING IMTA TO AN IRISH MONOCULTURE SITE FOR SALMON PRODUCTION

Joanne Casserly[1]*, Frank Kane[1] & Michael Sammon[2]

Marine Institute, Rinville, Oranmore, Co. Galway, Ireland[1]
Clear Seas Aqua, Co. Cork, Ireland[2]
Email: joanne.casserly@marine.ie

Introduction
Integrated Multi Trophic Aquaculture (IMTA) is acknowledged as a promising solution for sustainable development of aquaculture. By farming multiple lower trophic species adjacent to traditional aquaculture enterprises, such as salmon monoculture, the wastes and by-products of one species become the feed, fertiliser and energy source for the others. This practice is shown to reduce waste materials from fed species and lower the nutrient load in the water (FAO,2018).

This paper aims to give an overview of applying IMTA to an Irish monoculture site for Salmon production with the introduction of the lower trophic species *Alaria esculenta, Pecten maximus* and *Homarus gammarus*. Utilising low trophic products such as invertebrates and seaweeds (Barbier, 2019) would maximise the use of licensed aquaculture areas and reduce the environmental impact on the monoculture activity. If successful, this method could be adapted by other monoculture sites to provide more eco-efficient practices, creating more goods and services while using fewer resources and generating less waste. This paper can help shift the current application of IMTA to a more commercial level.

Method
In this trial the decision was made to cultivate winged kelp, *Alaria esculenta*, Scallop, *Pecten maximus* and European Lobster, *Homarus gammarus* within the current grid infrastructure traditionally established to hold salmon pens. The trial site is a pilot scale research site, with a moored grid infrastructure to support 6 pen structures. Grid spacing is 50m between cushion buoys. Seaweed longlines were attached at the cushion buoys and orientated in varying directions to the prevailing current and at different levels of exposure on the site. Lobster units and scallop lanterns were suspended from the outer ring of the salmon pens on site.

Crop yield, biomass and condition were monitored throughout the growing cycle. This data was compared to abiotic data relating to current direction and to location on the site. Molecular analysis of the harvested seaweed was carried out to look for variation in composition based on location on the site. The costs to add the additional structures, and the operating procedures cost to maintain the additional species were also considered.

Results
Utilising the existing grid structure successfully yielded a crop of seaweed. By utilising the existing grid structure, no additional moorings we required resulting in cost savings for the operator, less visual impact, and maximising use of the existing space. Waste from the salmon at the farm was remediated by the low trophic species added, through nutrient cycling in the kelp and the scallop, whilst the lobster also showed utilisation of salmon waste.

Data on biomass accumulation and crop yields provide expected nutrient uptake rates and value of additional products to the site. Although yields were not at a commercial level, a significant tonnage was achieved, providing additional value from the added products. There were further ecosystem service benefits from co-culturing novel low trophic lobster species for potential release to the wild for restocking.

This low visual impact approach and more optimal use of the limited space for aquaculture within the coastal zone proved successful.

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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

European Market Observatory for Fisheries and Aquaculture Products (2018). The EU Fish Market, 2018 Edition.

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

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PROTOCOL FOR THE LARVAE PRODUCTION OF *Patella aspera* RÖDING, 1798 (PATELLOGASTROPODA: MOLLUSCA)

Diego Castejon[1]*, José M. Cañizares[1], Natacha Nogueira[1,2] and Carlos A. P. Andrade[1,2]

1. Centro de Maricultura da Calheta. Av. D. Manuel I, n.º7. 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

Patella aspera Röding, 1798 is a true limpet species (Patellogastropoda) native from the Macaronesian Region and it is an important gastronomic and economical resource currently overexploited. Limpets represent an opportunity to diversify the local production in aquaculture promoting the consolidation of the industry through a sustainable development. The aquaculture of limpets is a recent research field with few available bibliographical and methodological resources. This study develops a reliable method for the production of larvae of *P. aspera* using NaOH-alkalinized seawater to enhance the larval production.

Material and Methods

The adults were captured in the Madeira coast during winter (2019/2020) and kept in 200 L tank with filtered seawater (FSW) at 20 ± 1 °C and 37 ± 1 psu. Oocytes were extracted by stirring the dissected gonads in 100 ml filtered seawater, and washed using a set of 200 μm and 55 μm meshes. The sperm was carefully pipetted from dissected male gonads and diluted in filtered seawater. The gametes of four females and four males were pooled. Alkalinized seawater was prepared using NaOH. The influence of the pH and bath duration was studied combining five pH treatments plus control: 8.0, 8.5, 9.0, 9.5, 10.0, and 10.2 ± 0.1; with seven bath durations (Activation Time, AT): 0:30, 1:00, 1:30, 2:00, 3:00, 4:00, and 5:00 h; three replicates *per* combined pH x AT treatment were used. The ratio of oocytes with spherical shape and chorion partial or totally removed (RAO) was determined using a microscope. The best conditions to produce RAO were used to enhance the larval production, being combined two pH treatments plus control (8.0, 9.0, 9.5), with the best AT (3:00 and 4:00 h) and a shorter AT as precaution (1:30 h). Three fecundation times were tested (FT: 1:30, 3:00, and 24:00 h). Four replicates *per* combined pH x AT x FT treatment were used. Sperm was added at 10^6 sperm cells ml^{-1}. Incubation lasted 24 h since the start of the fertilization at 21 ± 1 °C and 37 ± 1 psu. The fidelity of the results was tested repeating the experiment twice (January and February). Samples were characterized to identify normal larvae, abnormal larvae, and non-fertilized oocytes. The ratio of viable larvae was considered the "larval production".

Results

RAO was significantly influenced by the interaction pH x AT, being observed the higher RAO at pH 9.0 and 9.5 from AT = 3:00 to 5:00h. Robust ANOVA (three-way design) showed that larval production was significantly affected by the interaction pH x AT x FT in the first assay, but it was not significant in the second assay. By contrast, the interaction pH x AT on the larval production was significant in both assays. Highest larval production occurred at pH 9.0 and AT ≥ 3h; while larval production decreased significantly at pH 9.5.

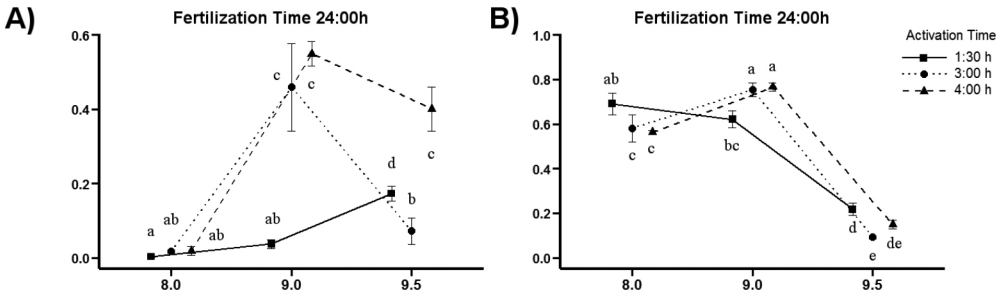

Fig. 1. *Patella aspera*. Interaction plots of the larval production at different pH x AT combinations at FT = 24h. A) First assay (January 2020). B) Second assay (February 2020).

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Discussion

NaOH-alkalinized seawater baths enhanced the larval production in *P. aspera*. The larval production varied from 50 to 75% employing NaOH-alkalinized seawater baths at pH 9.0 during 3:00h. Incubation used 10^6 sperm cells ml^{-1} lasting 24h. Higher pH apparently damages the oocytes. The differential results obtained in control treatments could be explained by a differential starting maturation state of the oocytes between both assays.

Find more details in:

Castejón, D, Cañizares, JM, Nogueira, N, Andrade, CAP. 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. DOI: 10.1111/are.15039

CULTIVATION OF *Arthrospira platensis* FOR TEXTILE WASTEWATER TREATMENT

F. Castillo Cascino*, L. Foglio, T. Bongiorno, L. Proietti, K. Parati

[I]stituto Sperimentale Italiano L. Spallanzani, loc. "la Quercia" 26027, Rivolta d'Adda (Italy)
E-mail: federico.castillo@istitutospallanzani.it

Introduction

Textile industry is one of the most important productive sectors in Italy and Europe, but unfortunately, it causes a negative impacts on the environment, above all with the advent of digital printing. This technology, on one hand, compared to traditional printing proved to be more efficient with regard to water consumption (-60%), energy (-80%) and greenhouse gases emissions (-40%), but on the other hand it is responsible for producing wastewater containing amines and dyes which are rich of pollutants like nitrogen, whose load has increased by 200% compared with that of the traditional printing technology.
In this context, the intent and the purpose of this study is to integrate the aspects of the industrial biotechnologies with those of the bioeconomy in a perspective of sustainability. One of the ways to pursue this aim is to employ microalgae able to uptake nitrogen and grow on textile wastewater.

Materials and methods

Arthrospira platensis was cultivated in round 4-liter flasks, in order to evaluate its growth and nitrogen removal in presence of textile wastewater, coming from a textile settlement near Como, in Lombardy.

A. platensis was inoculated in a culture containing 25% textile wastewater (T25) by volume, with the remaining volume being constituted of Zarrouk medium. In parallel a control (C) trial in which *A. platensis* was grown was carried out for comparison purposes. The growth conditions were room temperature at 20°C, pH 9.5-10 and irradiance at 30 μE m^{-2} s^{-1}.

The cultures were carried out in duplicate first in batch conditions, subsequently, once a peak of concentrations was reached, a phase in semi-continuous regime was performed.

Results

Both in C and in T25 *A. platensis* could grow effectively and with similar results between each other during the batch phase, with a maximum peak of 1.21 g L^{-1} and 1.07 g L^{-1} respectively. At the end of the batch phase (48 days) the nitrogen removal was 20.4% in C and 17.3% in T25. It should be noted that in T25 a small amount of total nitrogen was given by NH_4^+-N, which after few days was completely removed. After 48 days of batch growth the cultures were turned into a semi-continuous regime, setting a HRT of 20 days Moreover, a little amount (1%) of concentrated Zarrouk medium was introduced to the cultures in order to counterbalance the lack of the other elements (K, Fe, Mg etc.), which in the meantime were consumed. Nonetheless, after seven days from the beginning of the semi-continuous phase, the concentrations values decreased in both trials. Subsequently the HRT was shifted to 25 days, so that *A. platensis* could better use the nutrients and without an excessive waste of salts. In this way the concentration values increased again, until having a peak for T25 of 1.10 g L^{-1} on the 32nd day of the semi-continuous phase. Subsequently a decrease in the concentrations values was recorded until the 40th day. This was probably due to the fact that a new nutrient integration was not given to the cultures during the experimentation, though it was necessary.

During the semi-continuous phase the nitrogen removal was 32.3% in C and 31% in T25.

Table 1: Chemical composition of the wastewater coming from the plant.

NO_3^-N (mg N L^{-1})	NH_4^+-N (mg N L^{-1})	P tot (mg P L^{-1})	COD (mg O_2 L^{-1})
0-5	126.5	2.25	706

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Discussion and conclusion

The experience showed that the growth of *A. platensis* on textile wastewater is possible when diluted with the traditional synthetic medium 1:4 and when adequate growth conditions, in terms of pH and salinity, are guarantee for *A. platensis*.

It would be important to maintain a HRT over 25 days in order to reach the better results, otherwise the equilibrium between harvesting and renewal is not guaranteed.

At any case further essays with other conditions and variables are necessary to be performed, as well as it would be interesting to cultivate other microalgal species at large scale in order to obtain biomass and natural pigments

Acknowledgements

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SETTLEMENT OF *Octopus vulgaris* IN CAPTIVITY: MORPHOLOGICAL, ANATOMICAL AND BEHAVIORAL CHANGES

A. Castro-Bugallo[1], M. Martínez-Pérez[1], A. Roura*[1,2]

[1] Estación de Ciencias Marinas de Toralla, CIM, Universidade de Vigo.
[2] Instituto de Investigaciones Marinas de Vigo (IIM-CSIC), Eduardo Cabello 6, Vigo
*Corresponding author email: chiquipulpi@gmail.com

Introduction

Planktonic octopuses undergo a transitional period, known as settlement, from a pelagic lifestyle to the predominantly benthic life of the juvenile stage (Villanueva, 1995). The skin of planktonic larvae is almost transparent with only 65-80 mesodermal black pigment-filled chromatophores, or founder chromatophores (Packard, 1985). During settlement, chromatophore number increases exponentially and new chromatic cells (iridophores and leucophores) develop in the dorsal area, which help the animals in camouflage on the seafloor (Messenger, 2001). Mantle length is nearly double the arm length at the planktonic stage, but this ratio clearly diminishes during settlement owed to positive allometric arm growth (Villanueva & Norman, 2008). There are no skin sculptural components, apart from the Kölliker organs, in planktonic paralarvae (Nixon and Mangold, 1996), which are common features in benthic juveniles, essential for camouflage and communication (Messenger, 2001). The only work that described the settlement stage stopped at 60d (Villanueva 1995). In this work we studied two cohorts of *O. vulgaris* reared in captivity through the juvenile stage and summarize the main behavioural and morphological changes undergone during this transitional stage.

Methods

Paralarvae were obtained from wild females and reared in 50L dark green fibre tanks provided with filtered seawater (1μm) and a central outlet with 500μm filter. An open water system with 150% renovation per day was used with mean water temperature 19.5ºC (18.1-20.5), salinity 35.4 (34,8-36.2) and a 14:10 h light cycle provided with LED lights. The bottom of the tanks was siphoned every day. Live diet consisted of sub-adult Artemia (1-3 mm TL) at a concentration of 0.1-0.05 ind/ml, cultivated at 25ºC with a phytoplankton mix. The data presented in this study come from two different experiments (in 2018 and 2020) where octopus juveniles were obtained. Meristic data was obtained from fresh (n=48) and ethanol preserved (n=42) paralarvae/juveniles: mantle length (ML) / total length (TL), as well as number of suckers. Moreover, body changes were photographed and recorded throughout the settlement stage. A percentage of shrinkage in mantle length (9.74%) caused by fixation was considered for those individuals stored in 70% ethanol (Villanueva, 1995). An interocular area in the head, defined by the 6 dorsal founder chromatophores above and between the eyes, was used to quantify chromatophore genesis during settlement.

Results and Discussion

The shift from a planktonic to a fully benthic life implied a set of adaptations that took around 30-45 days at a mean water temperature of 19ºC, where the main mortality cause was cannibalism. Three different stages were distinguished during this transitional period based on behavioural and morphologic changes: pre-settlement (~d45-60), settlement (~d60-75) and post-settlement (~d75-90, schematically represented in Fig. 1).

During the *pre-settlement* stage or "tactile phase" the transparent octopus split between swimming and benthic crawling, touching the bottom and walls of the tank, and even hunting against these surfaces. ML/TL ratio varies from 60-55% (~20-25 suckers, Fig. 1). The paralarvae have increased number of chromatophores along the length of the arms but still do not develop chromatophores in the dorsal area.

The *settlement* stage can be easily identified by the reclusive behaviour of the pre-juveniles with the refuges provided. The length of the arms equals that of the mantle (~55-48% ML/TL), and they have ~25-35 suckers (Fig. 1). An exponential increase of chromatophores in the dorsal area starts in this stage (described by the exponential curve: $y=0.002e^{0.3705x}$; $r^2=0.84$; where "x" is sucker number and "y" chromatophore number in the interocular area). Leucophores were firstly detected at the beginning of this stage (~25 suckers, Fig.1), and pre-juveniles start to develop a pale colour.

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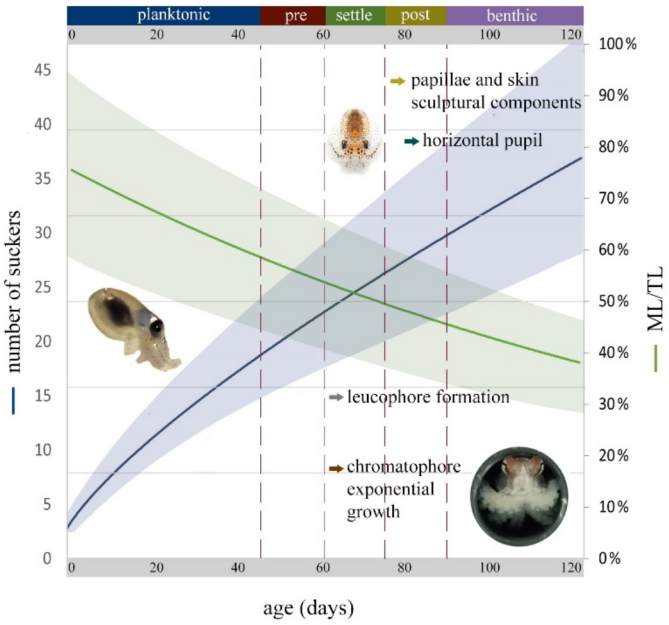

Fig.1. Evolution of suckers (blue line) and ML/TL ratio (green line) from the planktonic *O. vulgaris* paralarvae to benthic juveniles reared in captivity. The shadows surrounding the lines represent the standard deviation for each variable. The main morphological changes observed through the settlement stage are represented as colour arrows, with their location corresponding with the first time these were detected.

The *post-settlement* stage is marked by the development of skin camouflage and sculptural components (eye cirrus and dorsal papillae, Fig 1), and the horizontalization of the pupil. Despite the pupil is circular during the planktonic phase and pre-settlement, from ~85 days (>35 suckers, ~48-40% ML/TL) the pupil start to develop a horizontal pupillary response, an adaptation to a benthic mode of life. In agreement with the observations of Villanueva (1995), these settled juveniles, when disturbed, direct water fluxes to the origin of disturbance or crawl along the bottom of the tank with a cryptic posture. From this stage

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References

Nixon, M., Mangold, K. 1996. The early life of Octopus *vulgaris* (Cephalopoda: Octopodidae) in the plankton and at settlement: a change in lifestyle. Journal of Zoology. 239(2):301-327.

Packard, A. 1985. Sizes and distribution of chromatophores during post-embryonic development in cephalopods. Vie et Milieu/Life & Environment. 285-298.

Messenger, J.B. 2001. Cephalopod chromatophores: neurobiology and natural history. Biological Reviews 76(4):473-528.

Villanueva, R., Normal, M.D. 2008. Biology of the planktonic stages of benthic octopuses. Oceanography and marine biology. 46:105-202.

Villanueva, R. 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. Canadian Journal of Fisheries and Aquatic Sciences. 52(12):2639-2650.

RNA SEQUENCING ANALYSIS DEMONSTRATES THAT THE DIETARY INCLUSION OF THE PROBIOTIC PDP11 REGULATES THE HEALING PROCESS OF *Sparus aurata* SKIN

Cerezo I M.[1],Domínguez-Maqueda M.[1], Balebona, M.C[1], Esteban M.A.[2], Moriñigo M.A[1]

1) Microbiology Department, Faculty of Science, University of Málaga, 29010 Málaga, Spain
Email: b22ceori@uma.es
2) Cell biology and histology Department, Campus Espinardo s/n, University of Murcia, 30100 Murcia, Spain

Introduction

Stress in one of the most important factors affecting the health of farmed fish, and skin ulcers are recognized indicators of stressed animals (Lee et al., 2019). Mucosal surfaces are in direct contact with the external aquatic environment and epidermal integrity is vital to avoid the colonization by opportunistic pathogens (Yu et. al, 2020). It has been found that the inclusion of probiotics in the diet improves stress tolerance in fish (Thi et al., 2017), and specifically the dietary inclusion of the probiotic Pdp11 has enhanced the stress tolerance in sea bream *Sparus aurata* (Valera et al., 2010). Therefore, the aim of this work was to study the effects of Pdp11 dietary inclusion on the skin transcriptomic response of *Sparus aurata* undergoing stress induced by skin damage.

Material and methods

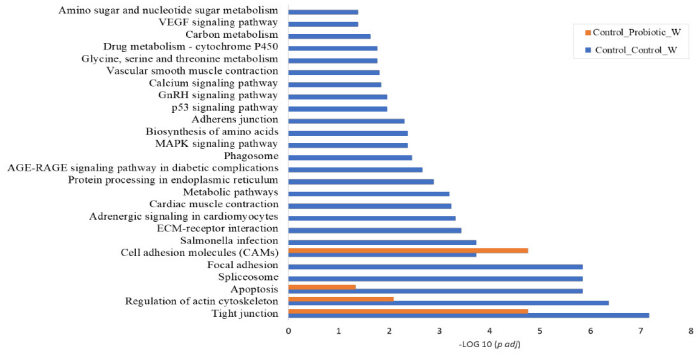
This study was carried out at the marine fishing facilities of the University of Murcia (Spain), where specimens of *S. aurata* were distributed in four fiberglass tanks of 450 L. During 30 days, fish of two tanks were fed a commercial diet (Control group), while the others received the same diet but supplemented with the probiotic Pdp11. After 30 days, a skin ulceration was produced to 6 fish/group following the methodology described by Chen et al. (2020) (Control_W and Probiotic_W groups). Samples of skin were collected 7 days after the beginning of the ulceration process.

The total RNA was isolated using Trisure protocol and purity and concentration measured using Qubit 3.0. RNA-seq libraries were generated NEBNext® Ultra™ RNA Library Prep Kit for Illumina (Illumina, San Diego, CA, USA) and generated FASTQ files were analysed. Raw reads were checked for sequencing quality and filtered to remove reads with adapter contamination or low quality. HISAT2 program was used for mapping next-generation sequencing reads and HTSeq software to count the read number mapped of each gene (FPKM). DEGseq (|log2Fold changel > 1; *p adj* < 0.005) and DESeq2 (*p adj* < 0.05) was used to show statistical differential expression analysis and the enrichment analysis was performed using KEGG database (KOBAS software to test the statistical enrichment of differential expression genes).

Results and Discussion

A total of 611,658,834 reads was sequenced, of which 88.64 ± 4.67 % were mapped. Gene expression levels were defined using fragments/Kb of transcription per million mapped reads (FPKM), confirming the expression of a total of 18,330,422.7 ± 2,742,012.15 genes in the skin tissue (expression > 0.3). After comparing differentially expressed genes between groups (fold change ≥ 2; *p adj* <0.05), 1645 genes were up or down regulated between healthy and damaged tissue of the fish that received the control diet. When comparing between the ulcerated skin of fish fed with probiotic and control group, only 288 were differentially expressed.

Figure 1. Bar chart of significant KEGG pathways (*p adj* < 0.05). Healthy fish fed control diet relative to damaged fish fed control diet (blue). Healthy fish fed control diet relative to damaged fish fed probiotic diet (orange).



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All genes were selected for further enrichment analysis of the KEGG pathways. In the case of fish fed with control diet, a total of 22 KEGG routes showed significant differences between the samples from fish healthy and injured. On the contrary, only 3 routes showed significant differences when comparing ulcerated fish fed with probiotic diet and healthy specimens receiving control diet (Figure 1).

Our results show that after 7 days of damage, ulcers in the animals fed the control diet seem to be in the inflammation and granulation phase, as shown by expression of genes related to tight junctions, cell division and inflammation, results in accordance with those reported by Sveen et al. (2020), which explain the re-epithelization process. In the case of the injured fish fed the Pd11 diet, there are only significant differences at the level of tight junctions, regulation of actin cytoskeleton and cell adhesion molecules in the KEGG pathways. The absence of significant changes in the expression of genes related to the immune system could be related to the late phase of healing. Chen et al. (2020) reported that the administration of Pdp11 diet to *S. aurata* specimens facilitate wound healing, and the results obtained in this study demonstrate that this faster healing could be related to the cellular cytoskeleton.

Conclusion

The inclusion of the probiotic Pdp11 in the diet of *Sparus aurata* produced changes in the activation of genes in the skin when it suffers an injury, it being able to improve the state of healing.

References

Chen, Z., Ceballos-Francisco, D., Guardiola, F. A., & Esteban, M. Á. (2020). Dietary administration of the probiotic *Shewanella putrefaciens* to experimentally wounded gilthead seabream (*Sparus aurata L.*) facilitates the skin wound healing. Scientific Reports, 10(1), 1-13.

Lee, J. W., Choi, H., Hwang, U. K., Kang, J. C., Kang, Y. J., Kim, K. I., & Kim, J. H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. Environmental toxicology and pharmacology, 68, 101-108.

Sveen, L., Karlsen, C., & Ytteborg, E. (2020). Mechanical induced wounds in fish–a review on models and healing mechanisms. Reviews in Aquaculture, 12(4), 2446-2465.

Thy, H.T., Tri, N. N., Quy, O. M., Fotedar, R., Kannika, K., Unajak, S., & Areechon, N. (2017). Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens*, and *Bacillus pumilus* on growth, innate immunity, and stress responses of striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology, 60, 391-399.

Varela, J. L., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arijo, S., León-Rubio, J. M., García-Millán, I., & Mancera, J. M. (2010). Dietary administration of probiotic Pdp11 promotes growth and improves stress tolerance to high stocking density in gilthead seabream *Sparus aurata*. Aquaculture, 309(1-4), 265-271.

Yu, Y., Wang, Q., Huang, Z., Ding, L., & Xu, Z. (2020). Immunoglobulins, Mucosal Immunity and Vaccination in Teleost Fish. Frontiers in immunology, 11, 2597.

WHAT IS THE ROLE OF FARM SCALE MODELS IN IMPLEMENTING THE ECOSYSTEM APPROACH TO AQUACULTURE (EAA)? OVERVIEW OF THE MODELS AND OF THEIR APPLICATIONS

Killian Chary[12*], Daniele Brigolin[3], Myriam Callier[4]

1 Independent researcher, Montpellier, France
E-mail : Killian.Chary@gmail.com
2 Aquaculture and Fisheries Group, Wageningen University and Research, Wageningen, the Netherlands
3 Department of Architecture and Arts, Università IUAV di Venezia, Venezia, Italy
4 MARBEC, Univ Montpellier, IRD, CNRS, Ifremer, Palavas-les-Flots, France

The ecosystem approach to aquaculture (EAA, Soto *et al*., 2008)a higher density of aquaculture installations and farmed individuals and greater use of feed resources produced outside of the immediate culture area. Such evolution of the sector could carry negative impacts on the environment and on portions of the society when unregulated and badly managed. In response to the explicit request of the Third Session of the Committee of Fisheries (COFI is perceived as a conceptual guide to move aquaculture development towards greater sustainability and to help to solve practical problems related to this development (Brugère *et al*., 2018). EAA listed several research priorities and management measures to face the major challenges in aquaculture at farm (e.g. reducing nutrient emissions, increasing productivity, evaluating the feasibility of integrated multi-trophic aquaculture (IMTA)), waterbody (facilitating aquaculture planning), and global scales (e.g. producing environmentally friendly aquafeeds). At each scale, models will play an important role in addressing many of these challenges, and their development is of great relevance to translate EAA principles and recommendations into practice (Soto *et al*., 2008; Ferreira *et al*., 2012; Byron and Costa-Pierce, 2013)a higher density of aquaculture installations and farmed individuals and greater use of feed resources produced outside of the immediate culture area. Such evolution of the sector could carry negative impacts on the environment and on portions of the society when unregulated and badly managed. In response to the explicit request of the Third Session of the Committee of Fisheries (COFI.

Farm-scale models (FSM) are tools of particular interest to enhance our understanding of the ecological, physical, and economical processes that occur and interact at farm scale. FSM are integrated mathematical models developed to simulate farm operations in a given culture system during a defined period in order to quantify and assess inputs flows (e.g., water, feed, fry, etc.), their use and transformation efficiency to produce fish biomass (harvests and stocks) as well as waste and by-products flows (e.g., dead fish, effluents). FSM and equivalent terms (e.g. carrying capacity tools, bioeconomic models, farm-scale production models) are regularly used in the literature (Ferreira et al., 2012; Byron and Costa-Pierce, 2013; Newell et al., 2018) but they often designate various type of models applied in aquaculture. Clear definition and/or main features that define and characterize FSM are, however, rarely specified, meaning that this type of modeling has not been applied long enough to the point where an established methodology and standard terminology are used.

This study aimed to review a set of published FSM, i) to present the common general characteristics of these models and the main modeling alternatives used to build them, ii) to provide an overview of potential applications that can contribute to challenges highlighted in the EAA. Based on the case study of aquaculture development in Mayotte Islands (French territory in the Indian Ocean) and FINS model development (Chary et al., 2019, 2020, 2021), a focus is made on the use of FSM to facilitate aquaculture spatial planning, estimate bioremediation (nutrient reuse) in IMTA and assess its global environmental impacts.

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References

Brugère, C., Aguilar-Manjarrez, J., Beveridge, M. C. M., and Soto, D. 2018. The ecosystem approach to aquaculture 10 years on - a critical review and consideration of its future role in blue growth. Reviews in Aquaculture: 1–22. http://doi.wiley.com/10.1111/raq.12242.

Byron, C. J., and Costa-Pierce, B. A. 2013. Carrying capacity tools for use in the implementation of an ecosystems approach to aquaculture. *In* Site selection and carrying capacity for inland and coastal aquaculture. FAO/Institute of Aquaculture, University of Stirling, Expert Workshop, 6–8 December 2010. Stirling, UK., pp. 87–101. Ed. by L. G. Ross, T. C. Telfer, L. Falconer, D. Soto, and J. Aguilar-Manjarrez. FAO Fisheries and Aquaculture Proceedings No. 21, Rome.

Chary, K., Fiandrino, A., Covès, D., Aubin, J., Falguière, J. C., and Callier, M. D. 2019. Modeling sea cage outputs for data-scarce areas: application to red drum (*Sciaenops ocellatus*) aquaculture in Mayotte, Indian Ocean. Aquaculture International, 27: 625–646. Springer International Publishing.

Chary, K., Aubin, J., Sadoul, B., Fiandrino, A., Covès, D., and Callier, M. D. 2020. Integrated multi-trophic aquaculture of red drum (*Sciaenops ocellatus*) and sea cucumber (*Holothuria scabra*): Assessing bioremediation and life-cycle impacts. Aquaculture, 516. Elsevier B.V.

Chary, K., Callier, M. D., Covès, D., Aubin, J., Simon, J., and Fiandrino, A. 2021. Scenarios of fish waste deposition at the sub-lagoon scale: a modelling approach for aquaculture zoning and site selection. ICES Journal of Marine Science. Oxford University Press (OUP). https://academic.oup.com/icesjms/advance-article/doi/10.1093/icesjms/fsaa238/6066594 (Accessed 27 January 2021).

Ferreira, J. G., Aguilar-Manjarrez, J., Bacher, C., Black, K., Dong, S. L., Grant, J., Hofmann, E. E., *et al.* 2012. Progressing aquaculture through virtual technology and decision-support tools for novel management. *In* Global Conference on Aquaculture 2010, Phuket, Thailand. 22-25 September 2010, pp. 643–704. Ed. by R. P. Subasinghe, J. R. Arthur, D. M. Bartley, S. . De Silva, M. Halwart, N. Hishamunda, C. V. Mohan, et al. FAO, Rome and NACA, Bangkok.

Newell, C. R., Brady, D. C., and Richardson, J. 2018. Farm-Scale production models. *In* Goods and Services of Marine Bivalves, pp. 485–506. Springer International Publishing.

Soto, D., Aguilar-Manjarrez, J., Brugère, C., Angel, D., Bailey, C., Black, K., Edwards, P., *et al.* 2008. Applying an ecosystem-based approach to aquaculture: principles, scales and some management measures. *In* Building an ecosystem approach to aquaculture. FAO/Universitat de les Illes Balears Expert Workshop. 7–11 May 2007, Palma de Mallorca, Spain, pp. 15–35. Ed. by D. Soto, J. Aguilar-Manjarrez, and N. Hishamunda. FAO Fisheries and Aquaculture Proceedings, Rome.

AN INNOVATIVE PLATFORM FOR NEW CIRCULAR AQUACULTURE MODELS: THE CASE OF MULTI-TROPHIC SYSTEMS (IMPAQT PROJECT)

Checa, D*; Escamilla, M. Sanchez, I.;

LEITAT Technological Centre C/ de la Innovació, 2 · 08225 Terrassa (Barcelona)
isanchez@leitat.org

Introduction

One of the major challenges to EU aquaculture growth is the optimization of the production systems while ensuring that environment impacts are minimized. In this context, IMPAQT is a European project that aims at validating in-situ a multi-purpose, multi-sensing and multi-functional management platform for a sustainable IMTA production. This project also seeks to validate the Integrated Multi-Trophic Aquaculture (IMTA) concept, adopted by 6 different pilot farms in Scotland, Ireland, The Netherlands, Turkey and China. IMPAQT also promotes the transition towards a circular economy business model, where the sustainability of the processes is a driver for the circularity.

A tailored made platform for IMTA production systems have been defined in the project, looking at end-users and stakeholders needs. To do so, several requirements have been defined, identifying the attributes that aim at boosting the sustainability within the multi-trophic production systems. These attributes are also expected to maximize the social, economic and environmental benefits of the new aquaculture systems.

This paper entails the assessment of the circularity associated to the new aquaculture model based on multi-trophic systems, where each attribute is analysed concluding how they are aligned with the circularity principles.

Material and methods

The circularity assessment of the new aquaculture model is firstly based on a qualitative approach for the evaluation of synergies areas between the circularity principles and IMPAQT management platform. Secondly, the attributes of the platform are prioritized through a quantitative approach to identify the most relevant requirements that promote circularity. Finally, recommendations are provided for the industry in order to increase the sustainability of the systems paving the path for a circular economy.

Table 1. Circularity of IMPAQT platform and multi-trophic production model

	Principle 1	Principle 2	Principle 3
BR-01: Structured storage and archiving of IMTA system data	✓	The multi-trophic system promotes the circularity at biological level.	✓
BR-02: Optimal Collection Time (fed)	✓		-
BR-03: Optimal seeding time	✓		-
BR-04: Optimal feeding time	✓		✓
BR-05 Maximise production	-		✓
BR-06: Optimal species grading during each growth stage	✓		
BR-07: Disease prevention and mitigation	✓		✓
BR-08: Optimal Collection Time (non-fed)	✓		✓
BR-09: Breeding / Broodstock (fed/non-fed)	✓		-
BR-10: Infrastructure, stock integrity and security and damage control	✓		✓
BR-11: Environmental and regulatory compliance	✓		✓
BR-12: Minimal environmental footprint	-		✓

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Three main principles that set the basis for the circular economy (Ellen MacArthur Foundation, 2015), can be summarized as follows:

1. Preserve and enhance natural capital controlling finite stocks and balancing renewable resource flows.
2. Optimize resource yield by circulating products, components and materials in use at the highest utility always in both technical and biological cycles.
3. Foster system effectiveness by revealing and designing out negative externalities

IMPAQT platform design is based on several business requirements, the more prominent ones to be as follows:

- BR-01: Structured storage and archiving of IMTA system data
- BR-02: Optimal collection time (fed)
- BR-03: Optimal seeding time
- BR-04: Optimal feeding time
- BR-05 Maximise production
- BR-06: Optimal species grading during each growth stage
- BR-07: Disease prevention and mitigation
- BR-08: Optimal collection time (non-fed)
- BR-09: Breeding / Broodstock (fed/non-fed)
- BR-10: Infrastructure, stock integrity and security and damage control
- BR-11: Environmental and regulatory compliance
- BR-12: Minimal environmental footprint

Results

The qualitative and preliminary evaluation of the meeting points between the circularity principles and the platform attributes are summarized in the table below:

TABLE GOES HERE

Once the principal synergies have been preliminary analysed, principle 2 is identified as the circularity dimension that is totally addressed by the multi-trophic production. Through the multi-trophic systems, some of the uneaten feed and wastes, nutrients, and by-product are recaptured and converted into harvestable and healthy seafood (Chopin, 2013). Therefore, all IMTA components have a key role in recycling processes within the systems, where the bio mitigation operates under a circular economy approach.

Conclusions

Based on the attributes of IMPAQT platform and the Multi-trophic systems, relevant synergies with Circular Economy have been identified. The contribution of the IMTA systems to the circularity of nutrients have been highlighted in the analysis, since it is totally aligned with principle 2 under a biological perspective. The assessment has also revealed that most of the management practices promote the resources consumption and this aspect should be prioritized for increasing the circularity on the aquaculture systems.

Bibliography

Ellen MacArthur Foundation. “Towards a circular economy business rationale for an accelerate transition.” 2015.
Chopin, T. (2013). Aquaculture, Integrated Multi-Trophic (IMTA).
Ellen MacArthur Foundation. (2015). Towards a circular economy business rationale for an accelerate transition.

THE AQUACULUTRE SECTOR UNDER A CIRCULARITY APPROACH

Checa, D*; Escamilla, M.; Sanchez, I.

LEITAT Technological Centre C/ de la Innovació, 2 · 08225 Terrassa (Barcelona)
dcheca@leitat.org

Introduction

Over recent decades, the EU aquaculture sector has been implementing actions towards the minimization of waste as a strategy for increasing the circular attributes of the management system. Mollusc shells valorisation, new biofouling management practices or the valorisation of by-products from seafood processing processes have all proved to be increasingly common in the industry.

In this circular context, the iFishIENCi project has been designed, and is now being carried out, in order to implement circular principles and zero waste practices by qualifying new and sustainable organic value chains for feeds and valorisation of by-products. In line with this, it seems particularly important to develop methodologies for quantifying and assessing the circularity that will ultimately be achieved by these new innovative technologies and processes along the value chain. Although several methodologies have subsequently emerged in recent years (Saidani, et al., 2017), an adaptation for assessing biological cycles is currently required.

Additionally, circular solutions might be also possible for open aquaculture systems where the collection of waste streams is not fully feasible. This is the case of the emerging IMTA (Integrated Multi-Trophic Aquaculture) in Europe, since some of the uneaten feed and wastes, nutrients, and by-product are recaptured and converted into harvestable and healthy seafood (Chopin, 2013). But, how is the IMTA aligned with the circular economy? How could IMTA products be positioned in the markets where circular principles are prioritized? These are some of the questions that the IMPAQT project is addressing through the sustainability and circular economy work package.

Both above mentioned approaches, circularity measurement and circular business development are presented and interrelated in this paper.

Material and methods

The new methodologies for circularity assessment are firstly reviewed and later adapted to measure the circularity along the value chain, focusing not only on feed production but also on the farming operation. With regard to feed formulation, ingredients sourced from waste valorisation routes are considered as recycled feedstock. The farming operation is also addressed, since the efficiency of feeding is a key element in determining the functionality of the new formulations.

Secondly, the new circular economy concept is deeply analysed in multi-trophic systems, for coastal aquaculture farms, where sludge collection and valorisation are not possible. Within this analysis, a common framework has been devised in order to design new circular models, based on IMTA systems together with the IMPAQT management platform, as a key tool for ensuring the efficiency of production from a holistic perspective.

Conclusions

Based on the work developed within both projects; iFishIENCi and IMPAQT, new tools will be provided to the aquaculture industry to meet the need to conduct circularity assessments of biological processes. Together with this, an innovative way of integrating the circular thinking into the processes and business models will also enable the delivering of added value aquaculture products.

Bibliography

Chopin, T. (2013). Aquaculture, Integrated Multi-Trophic (IMTA).

Ellen MacArthur Foundation. (2015). Towards a circular economy business rationale for an accelerate transition.

Saidani, M., Yannou, B., Leroy, Y., & Cluzel, F. (2017). How to Assess Product Performance in the Circular Economy? Proposed Requirements for the Design of a Circularity Measurement Framework. *Recycling* , 2,6 .

NORWEGIAN RED SEA CUCUMBER (*Parastichopus tremulus)* – STEPS TOWARDS LIFE IN CAPTIVITY – A VIABLE OPTION?

Gyda Christophersen* and Jan Sunde

Møreforsking AS, Ålesund, Norway
Post Box 5075, N-6021 Ålesund, Norway
gyda.christophersen@moreforsking.no

Introduction
The red sea cucumber, *Parastichopus tremulus*, is an emerging aquaculture candidate species which has recently drawn attention from fishers, farmers, manufacturers, product developers, and trade actors. *P. tremulus* may be suited for cultivation all along the Norwegian coast, as its distribution in the Eastern Atlantic ranges from the Barents Sea in the north to the Canary Islands in the south. The species was recently listed as a new marine species having potential for aquaculture in Norway (Akvaplan-NIVA 2019), but the production of juveniles is a major constraint in the development of such a new industry. *P. tremulus* is considered attractive to the Asian market (Kjerstad et al. 2015), and a possible future aquaculture production has the possibility of being sustainable and environmentally friendly, either in monoculture or as part of integrated systems (Landes et al. 2019). However, due to limited biological knowledge of the species, regulatory constraints and uncertainty of future market development, there is at this moment a high risk involved with developing this industry. A step-by-step approach to understand the requirements of the *P. tremulus* in captivity is underway as part of a bilateral research cooperation between South Africa and Norway (SANOCEAN).

Methods
The project "Emerging species for sea cucumber aquaculture" investigates the aquaculture potential of several tropical and temperate species from the two countries. As part of this project, investigations into reproduction, nutrient utilization and growth in captivity of *P. tremulus* will be investigated. Feeding studies are carried out using both typical sea cucumber aquaculture feeds as well as waste (sludge) from land-based salmon aquaculture. Møreforsking is targeting *P. tremulus* from the fjord system around Ålesund in Møre and Romsdal county in the north-western part of Norway (62°N-6°E) as study animals. Broodstock is collected annually from the wild during the natural spawning season (Christophersen et al. 2020). After arrival in the laboratory the sea cucumbers are acclimated to the conditions of incoming seawater from 40 m depth in flow-through tanks. Spawning is induced by exposure to elevated temperatures, and fertilization and larval development followed in small scale rearing systems (10 or 30 L tanks).

Results
Steps towards closing the life cycle, that is to control spawning, fertilization, and growth through the different life stages until sexual maturation, are taken, and preliminary studies have been carried out on wild *P. tremulus*. Spawning in captivity has taken place four years in a row, whereas larval development until the auricularia stage (pelagic larvae) has been achieved for two consecutive seasons. Preliminary results on handling and maintaining broodstock in captivity, and larval development will be presented. The work intends to serve as a basis for future studies on reproduction and early life stages as well as for evaluation of the viability of *P. tremulus* aquaculture.

Discussion/Conclusion
Several bottlenecks must be overcome to realize an aquaculture industry independent of collection of wild stock. One of them is a predictable supply of sea cucumber juveniles that can be sold for rearing to commercial size in land-based, sea ranching or integrated aquaculture systems. Land-based facilities are particularly well suited for collection of nutrient-rich effluents, and *P. tremulus* may be a promising candidate species for integrated culture to utilize the particulate waste fraction from these facilities (Landes et al. 2019). Biological and technological constraints related to a life in captivity still hinder the next step forward, and research is needed in the areas of reproduction, nutritional requirement, and water quality. The joint research of the SANOCEAN project team will expand existing knowledge about the performance of novel species under rearing conditions and contribute to overcoming the common constraints in developing sea cucumber aquaculture.

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References

Akvaplan-niva AS, 2019. Kunnskapsgrunnlag for nye arter i oppdrett (in Norwegian). APN-report 60679. (Accessible from: https://www.regjeringen.no/no/dokumenter/kunnskapsgrunnlag-for-nye-arter-i-oppdrett/id2685473/).

Christophersen, G., Bjørkevoll, I., Bakke, S., Kjerstad, M., 2020. Reproductive cycle of the red sea cucumber, *Parastichopus tremulus* (Gunnerus, 1767), from western Norway. Marine Biology Research 16(6-7):423-430.

Kjerstad, M., Ringvold, H., Søvik, G., Knott E.K., Thangstad, T.H., 2015. Preliminary study on the utilisation of Norwegian red sea cucumber, *Parastichopus tremulus* (Gunnerus, 1767) (Holothuroidea, Echinodermata), from Norwegian waters: resource, biology and market. In: Gundersen A.C. and Velle L.G. (eds.). Blue Bio-resources, Orkana Forlag, Norway, pp 109-132.

Landes, A.M., Sunde, J., Christophersen, G., 2019. Atlantic sea cucumber species in the spotlight – prospects for Norwegian aquaculture. In: International Perspectives on Regional Research and Practice. Akslen-Hoel L.K. and Egilsson B. (eds.). Orkana Forlag, Norway, pp 19-49.

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Møreforsking AS, Ålesund, Norway
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References

Akvaplan-niva AS, 2019. Kunnskapsgrunnlag for nye arter i oppdrett (in Norwegian). APN-report 60679. (Accessible from: https://www.regjeringen.no/no/dokumenter/kunnskapsgrunnlag-for-nye-arter-i-oppdrett/id2685473/).

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ENVIRONMENTAL FOOTPRINT OF SEABASS PRODUCTION IN THE MEDITERRANEAN SEA

Maite Cidad, Saioa Ramos

AZTI. Parque Tecnológico de Bizkaia. Astondo Bidea, Edificio 609. E-48160 Derio, 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 further shift towards a more pescatarian diet has the potential to reduce global agricultural greenhouse gas emissions and help prevent diet-related diseases. Aquaculture can contribute to the overall 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).

In 2013 European Commission proposes the Product Environmental Footprint (PEF) method as a common way of measuring environmental performance (COM2013/179/EU). The package establishes the Life Cycle Assessment (LCA) methodology methods to measure environmental performance of European Product and organizations.

Within this framework in 2018 the LIFE+ AQUAPEF project was launched with the aim to facilitate the environmental footprint calculation of Mediterranean aquaculture products. The study presented in this manuscript, describes the potential environmental impact related to the production of seabass farmed in Mediterranean Sea-cages. Results allows to identify main causes and origins of the environmental impact (hot spots identification) and therefore, to identify potential recommendations for environmental improvement for the aquaculture companies.

Methodology
To identify and quantify environmental impacts linked to the industrial aspects of fish culture, the PEF method appears as an internationally recognized methodology. The LCA is a method to assess the environmental impacts of a product encompassing the whole value chain (cradle to grave). Hence, the environmental impacts of a product are evaluated from resource extraction to material production, product manufacturing, use of the product up to the disposal of the product and the production wastes. According to the ISO 140:40:2006 the method consists of 4 steps i) Goal and scope definition; ii) Life Cycle Inventory, iii) Life cycle impact assessment and the iv) interpretation of the results.

Seabass environmental footprint results:
Step 1. Goal and scope definition
The functional unit of this study is 1 kg of gutted and packed fresh aquaculture seabass, head on, delivered to retailer.

The system boundary for the fresh aquaculture seabass follows the product from “cradle to gate”. The life cycle starts with the harvesting and cultivation of feed ingredients (fish and crops) and production of the feed at a feed mill. The feed is delivered and used at a land-based hatchery and at an aquaculture growing farm with marine Open Net-pen production system. After the harvesting of the fish in farm, the seabass is gutted and packed in EPS (expanded polystyrene) boxes. The aquaculture seabass is then transported to a retailer or for further processing. The main consumables and infrastructure for are included. However, the vaccines are out of the scope due to lack of databases.

Step 2. Life Cycle Inventory
Inventory data for seabass production at hatchery and aquaculture farm in Mediterranean region was provided by companies’ partners of the project and their suppliers. Data was provided for the operational year 2018.

When operational data is insufficient, additional data or information is obtained by using data from literature, results from other inventories or studies as well as data from databases. The primary source of background inventory data, e.g. production of diesel oil and feed ingredients used in this study, is from the “Ecoinvent 3.5 - At Point Of Substitution” database and the software used is SimaPro 9.

Step 3. Life cycle Impact Assessment
Climate change and marine eutrophication potential impacts are the most representative environmental impacts of the activity for the marine aquaculture production.

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On the one hand, climate change represents all inputs or outputs that result in greenhouse gas emissions. It is an impact affecting the environment on a global scale. The climate change environmental impact of growing European seabass in the Mediterranean region is around **6.28 kg CO2 eq. per kilogram of fish** produced at farm. Feed requirement is by far the most significant behavioral aspect affecting in more than 60 % to the total Global Warming potential. Inside the feed stage, the animal feed ingredients contribute most to the impacts (up to 60 %), followed by the plant-based ingredients (33 %) while the feed manufacturing process is less relevant (3 %).

On the other side, eutrophication impacts ecosystems due to substances containing nitrogen (N) or phosphorus (P). For the marine environment, the availability of N is a limiting factor for growth in the ecosystem, and if this nutrient is added, the growth of algae or specific plants will be increased. Eutrophication is an impact which affects the environment at local and regional scale. The marine eutrophication environmental impact of growing European seabass in the Mediterranean region is around **0.16 kg N eq. per kilogram of fish** produced at farm. The Growth phase is the major responsible (86 %) for the Marine Eutrophication impact category. Similar values, between 80 % and 93 % have been reported for different species and different systems of cultivation (García-Garcia et al., 2019). Indeed, the biggest local impact of fish farming is due to organic emissions to the ocean as a result of the non-ingested feed and the actual metabolism of fish, as feed feces or egestion. N (135 kg N) and P (25 kg P) emissions are estimated by aquaculture companies accordingly to the feed specifications and FCR.

Step 4. Interpretation of the results

Environmental impact assessment determined that the **feed composition and consumption** is the main factor affecting most of the impact categories studied. Thus, sustainable feed alternatives or optimization of feed demand could significantly reduce the environmental impact of the final products.

Conclusion

Collect all the data required for the environmental impact assessment is high effort and time consuming, particularly for the processes out of the control of the company interested in the LCA application. Innovative ways to collect this data are critically required, for the sustainability of the PEF method.

Besides, new environmental impact category to assess the specific impact of the marine eutrophication on the Mediterranean region should be developed. Currently the fate factors are given globally, however, each sea/ocean has its own characteristics, and should be modelled.

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EFFECT OF SPONTANEOUS INTAKE OF LEMNA ON PRODUCTIVE PERFORMANCE OF NILE TILAPIA JUVENILES

Luiz Augusto Cipriani*, Natalia Ha, Nandara Soares de Oliveira, Thiago El Hadi Perez Fabregat

Universidade do Estado de Santa Catarina. UDESC – Av. Luiz de Camões, 2090, Lages, SC, Brazil
Zip code 88520-000
cipriani_luiz@hotmail.com

Introduction

Lemna (*Lemna minor*) is a fresh water macrophyte that has been increasingly stablished in aquaculture for its phytoremediation potential, biomass production and nutritional value, which may be superior to well-established vegetable protein sources that have potential as alternatives to fish meal (Asimi et al. 2018; Chakrabarti et al. 2018). However, it has antinutritional characteristics, which can cause performance problems in fish when included at high substitution levels of dietary protein (El-Shafai et al. 2004). Adequate levels of lemna inclusion have shown no negative impacts on performance (Utami et al. 2018), although spontaneous consumption in intensive systems has yet to be evaluated. Fish can spontaneously modulate the consumption of dietary items to avoid nutritional deficiencies (Fortes-Silva et al. 2016). Therefore, this study evaluated the effect of spontaneous consumption of lemna on the productive performance of Nile tilapia (*Oreochromis niloticus*) reared in a recirculation system.

Materials and method

140 male Nile tilapia juveniles (average weight 21.95) were distributed in a recirculation system in 14 polyethylene tanks with 70 liters. After an acclimatization period of seven days, in which the fish received an extruded commercial diet (32% crude protein), the growth performance test was performed, lasting for 28 days. The experimental design was completely randomized with two treatments and seven replications. Fish were subjected to a control treatment with only the use of commercial feed and the treatment with constant availability of fresh lemna plus commercial feed. Fish were fed the commercial feed three times daily, until apparent satiety. In the treatment with lemna, the plants were manually collected, washed, and dried, before being weighed and supplied to the tilapia. lemna was supplied in enough quantity that made the plant always available for the animals. Fish were subjected to biometrics to determine the components of growth performance: weight gain (WG), total consumption, feed conversion (FC) and specific growth rate (SGR) (Tacon 1990), in addition to lemna daily intake index.

Results and discussion

The supply of lemna had no effect ($P>0.05$) on weight gain, feed conversion and specific growth rate of the fish. In general, lemna is offered as a partial replacement of the commercial diet, of which the use of substitution levels higher than 20% has reduced the growth performance of tilapia (El-Shafai et al. 2004). Spontaneous modulation of nutrient consumption may have mitigated negative effects on feed intake and fish performance (Fortes-Silva et al. 2016) that would be caused by lemna's antinutritional factors (Goopy; Murray 2003; Vinogradskaya; Kasumyan 2019).

The Nile tilapia juveniles spontaneously consumed the fresh lemna in the present experiment, with maximum values ($P<0.05$) of spontaneous intake being 0.5% of live weight in the first week, decreasing and stabilizing ($P<0.05$) in 0.23% of live weight throughout the experiment. Maximum levels of lemna consumption were 0.5% of live weight and were stable at 0.23% of live weight. When comparing the different periods in each treatment, the behavior of the results over time was described using a quadratic equation. In the control treatment, consumption of commercial diet was higher ($P<0.05$) in the first week when compared to the other periods. And no difference ($P>0.05$) in feed intake was shown between periods for the treatment with lemna. On the other hand, the consumption of feed proportional to the weight also decreased ($P<0.05$) throughout the experiment. Larger fish eat proportionately less food than smaller fish, due to the reduction in metabolism as a function of weight (Xie et al. 1997).

In the first week of the performance test, fish that received lemna presented lower ($P<0.05$) consumption of commercial feed, when compared to the control treatment. This reduction was compensated during the third week, and feed intake became higher ($P<0.05$) in the treatment with lemna, with no impact on the total feed consumption or productive performance. This suggests that lemna is palatable food for tilapia and it may be raised in rearing tanks while providing its services to water quality, animal welfare, and serving as food for tilapia (Vinogradskaya; Kasumyan 2019). However, the consumption compensation that occurred over time is perhaps due to lemna not being nutritionally complete (Goopy; Murray 2003).

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Conclusion

Nile tilapia juveniles grown in a recirculation system can spontaneously ingest up to 0.5% of live weight in fresh lemna with no effects on productive performance. Fish that were exposed to lemna consumed less feed in the first week of the experiment, but this reduction was compensated over time with no impact on the total consumption.

References

Asimi OA, Khan IA, Bhat TA, Husain N (2018) Duckweed (*Lemna minor*) as a plant protein source in the diet of common carp (*Cyprinus carpio*) fingerlings. J Pharmacogn Phytochem 7: 42-45.

Chakrabarti R, Clark WD, Sharma JG, Goswami RK (2018) Mass Production of *Lemna minor* and Its Amino Acid and Fatty Acid Profiles. Front Chem 6: 479. https://doi.org/10.3389/fchem.2018.00479

El-Shafai SA, El-Gohary FA, Verreth JAJ, Schrama JW, Gijzen HJ (2004) Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus* L.). Aquac Res 35: 574-586. https://doi.org/10.1111/j.1365-2109.2004.01055.x

Fortes-Silva R, Kitagawa A, Sánchez-Vázquez FJ (2016) Dietary self-selection in fish: a new approach to studying fish nutrition and feeding behavior. Rev fish boil fisher 26: 39-51. https://doi.org/10.1007 / s11160-015-9410-1

Goopy J, Murray PA (2003) Review on the Role of Duckweed in Nutrient Reclamation and as a Source of Animal Feed. Asian-Aust J Anim Sci 16: 297-305. https://doi.org/10.5713/ajas.2003.297

Tacon AGJ (1990) The nutrition and feeding of farmed fish and shrimp – A training manual. The essential nutrients. Brasília, Food and Agriculture Organization of the United Nations. 117p.

Utami RRH, Nirmala K, Rusmana I, Djokosetiyanto D, Hastuti YP (2018) The application of phytoremediation *Lemna perpusilla* to increase the production performance of Nile tilapia *Oreochromis niloticus* in a recirculation system. Journal Akuakultur Indonesia 17: 34–42. https://doi.org/10.19027/jai.17.1.34-427

Vinogradskaya MI, Kasumyan AO (2019) Palatability of Water Organisms for Nile Tilapia *Oreochromis niloticus* (Cichlidae). J Ichthyol 59: 389-398. https://doi.org/10.1134/S0032945219030196

Xie S, Cui Y, Yang Y, Liu J (1997) Effect of body size on growth and energy budget of Nile tilapia, *Oreochromis niloticus*. Aquaculture 157: 25-34. https://doi.org/10.1016/S0044-8486(97)00142-7

ASSESSMENT OF THE VIRUCIDAL EFFECT OF A COMMERCIAL BIOCIDE AGAINST TWO DIFFERENT STRAINS OF NERVOUS NECROSIS VIRUS, BETANODAVIRUS

S. Ciulli[1*], F. Errani[1], S. Zamparo[2], E. Volpe[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Viale Vespucci, 2, 47042 Cesenatico, Forlì-Cesena, Italy; [2]Veterinary practitioner
E-mail: sara.ciulli@unibo.it

Introduction

Viral nervous necrosis (VNN) is the most threatening infectious disease in Mediterranean aquaculture. VNN is caused by the nervous necrosis virus (NNV) a small naked ssRNA+ virus, highly resistant in the aquatic environment. Four genotypes of NNV have been so far described: RGNNV, SJNNV, BFNNV, TPNNV among which the RGNNV is the most spread in the Mediterranean Sea. European sea bass (*Dicentrarchus labrax*) is one of the species most affected by VNN, however the emergence of a reassortant strain RGNNV/SJNNV caused high mortality outbreaks also in gilthead sea bream (*Sparus aurata*) larvae (Volpe et al., 2020). So far, no therapy is available for VNN control and vaccination is applied limited to ongrowing facilities, therefore the control in hatchery is strongly based on direct prophylaxis aimed to prevent the entering and the spreading of the virus in the farms. In this respect the setup of effective hygiene standard procedures can greatly contribute to the reduction of the VNN impact.

The aim of this study was to assess the *in vitro* virucidal activity of a commercial compound (Virkon® S) towards the two NNV variants most spread in the Mediterranean Sea.

Materials and methods

Two NNV strains isolated during mortality outbreaks (Ciulli et al., 2006; Volpe et al., 2020) previously characterized as RGNNV genotype (It/351/Sb) and reassortant RGNNV/SJNNV (Sa-416-Dec17) were propagated in cell culture SSN-1 and tested for *in vitro* virucidal activity.

Suspension virucidal activity of a peroxy-acid commercial compound (Virkon® S) was assessed first using the European UNI EN 14675 standard "Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area" adapted to test aquatic pathogens (Verner-Jeffrey et al., 2009). Fixed temperature (20°C) and exposure time (5 min) were used, whereas variable biocide concentrations (0.5 *vs* 1% w/v) and soiling conditions (low soiling LS *vs* high soiling HS) were tested. Sea water was used as diluent.

In order to establish whether the tested biocide for use on instruments (nets, cages, etc) has a virucidal activity in the fields, a new protocol (net test) closely simulating practical conditions of application was developed. Briefly pre-drying viruses on a carrier (nylon net) were titred after incubation with or without biocide treatment. Contact time, temperature, test organisms, biocide concentrations and soiling conditions were tested as in the previous assay.

Results

A similar virucidal effect was demonstrated for Virkon® S against the tested NNV strains representing the RGNNV genotype and the RGNNV/SJNNV reassortant strain.

Suspension test: a titre reduction (TR) ≥ 4 log(10), considered effective by the EN 14675 standard, was demonstrated for the tested biocide at the concentration of 1% w/v under both low and high soiling conditions consisting in a reduction of 99.99% of the viral $TCID_{50}$ of It/351/Sb or Sa-416-Dec17 strains. Similarly, a titre reduction (TR) ≥ 4 log(10) was observed for the tested biocide at the concentration of 0.5% w/v towards both strains when tested under low soiling condition. When tested under high soiling condition at the 0.5% w/v concentration, the biocide showed a higher titre reduction towards the strain It/351/Sb, however at this condition no effective TR was reached (≤ 4 log 10).

Net test: a titre reduction (TR) ≥ 4 log(10) was obtained for the tested biocide at the concentration of both 0.5 and 1% w/v only under low soiling condition for It/351/Sb viral strain. A slightly lower TR was observed under high soiling condition (TR 3.3 and 3.5 log (10) at 0.5 and 1% w/v respectively) for It/351/Sb strain. Regarding Sa-416-Dec17 strain TR was similarly influenced by biocide concentrations and soiling conditions, even if all TRs were ≤ 4 log(10). However, Sa-416-Dec17 strain test was partially affected by the net drying process resulting in lower starting viral titres.

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Discussion and Conclusion

The tested biocide was found to be suitable for NNV inactivation being effective under at least some of the conditions tested. However, the presence of the organic matter, the concentration of the product and the application conditions (suspension *vs* net) can significantly affect the result of the disinfection procedures. For these reasons, it is of paramount importance to set up a specific disinfection protocol considering the "cleaning" level that can be reached before disinfection. Both tested strains representing the RGNNV genotype and the RGNNV/SJNNV reassortant strain were affected similarly by tested conditions, however Sa-416-Dec17 strain showed a higher susceptibility to drying process and a lower susceptibility to biocide treatment compared to It/351/Sb. The results obtained in this study led to stress the application of effective disinfection protocols preceded by surface cleaning and drying step in order to reduce the VNN impact at farms.

References

Ciulli S., Gallardi D., Scagliarini A., Battilani M., Hedrick R.P., Prosperi S. (2006). Temperature-dependency of Betanodavirus infection in SSN-1 cell line. Diseases of Aquatic Organisms, 68: 261-265.

EN 14885, Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics

Volpe E., Gustinelli A., Caffara M., Errani F., Quaglio F., Fioravanti M. L., 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. Doi: 10.1016/j.aquaculture.2020.735155

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ADVANCES IN TECHNOLOGY ARE BRINGING FIRST-FEEDING OF SENEGALESE SOLE LARVAE WITH MICRODIETS CLOSER

Luís Conceição[a*], Rita Colen[b], André Santos[a], Wilson Pinto[a], Sofia Engrola[b]

[a]SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[b]Centre of Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

*E-mail: luisconceicao@sparos.pt

Introduction

Commercial feeding protocols for Senegalese sole (*Solea sengalensis*) larvae and postlarvae have progressed considerably in recent years towards earlier and more efficient weaning into inert microdiets (Pinto et al. 2018). An introduction of inert microdiets from larvae mouth opening in co-feeding with live feed has been shown to be viable (Cañavate and Fernández-Díaz 1999), and bring long-term quality advantages (Engrola et al. 2009). Moreover, nowadays it is possible to perform an early weaning starting immediately after sole settling (15 days after hatching; DAH) and achieving 1 g at 65 DAH (Pinto et al. 2018). However, growth depression and higher mortalities have been observed depending on the level of Artemia replacement (Engrola et al. 2010). Even if most sole hatcheries currently only start applying microdiets 2-4 weeks after start feeding, for water quality and other practical purposes, Artemia replacement is desirable. Still, producing microdiet for first-feeding sole with small particles (100-200 μm) brings technological challenges. Digestibility needs to be ensured, while avoiding excessive leaching of water-soluble nutrients (e.g., protein, mineral, vitamins) to the rearing water. Also early larvae likely need easier-to-digest ingredients compared to older stages. This study aimed at testing microfeed prototypes with different ingredient and binder combinations, which may lead to a good performance of Senegalese sole in a scenario of 99% Artemia replacement.

Materials and methods

Three experimental microdiets were introduced in the feeding regime of Senegalese sole at first-feeding (3 days after hatching; DAH), being offered close to satiation and maintained until the end of the experiment (27 DAH). Microdiets were supplied to the larvae by hand in 5 daily meals during day-time. During the night, experimental diets were provided using automatic feeders, with each meal lasting a period of 2 h following a 1 h break. A small amount of live-feed was also offered to the larvae in this experiment: rotifers from 2 to 5 DAH (1 individual mL^{-1}) and Artemia from 5 to 19 DAH (0.1 individuals mL^{-1}). Larvae were reared at an initial density of 44 larvae L^{-1} in triplicate 100 L conical-cylindrical fibreglass tanks set in a partially-closed recirculation aquaculture system. Diets were analysed for proximal composition (58% crude protein, 17% crude lipid), and protein leaching following 2 and 30 min after immersion in seawater.

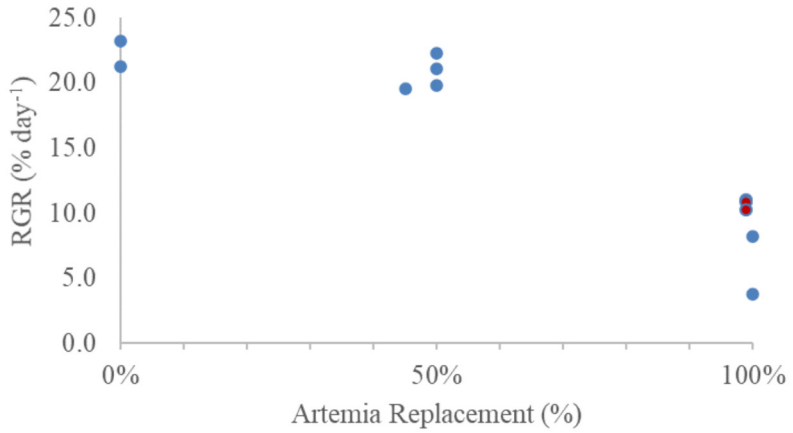

Fig. 1. Relative growth rate (RGR, % day^{-1}) as a function of Artemia replacement (% of the normal dose of Artemia provided in a standard protocol, with zero standing for only Artemia being feed, and 100 for only inert microfeed) in Senegalese sole larvae up to 30 DAH. Red dots are the results of the present study, and blue dots are published results. References: (2), (3), (4), (5), (6), (7), (8).

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Results and Discussion

Good sole growth and survival performances were observed during the trial for all microfeeds. No significant differences were obtained between treatments for larval dry weight (0.31 to 0.37 μg), total length and relative growth rate (10-11 % day $^{-1}$) at 27 DAH. Protein leaching by the 3 microdiets was below 9 and 12% at 2 and 30 min, respectively. All experimental diets tested in the current study were able to support growth and survival of Senegalese sole during the first weeks of development, at a very high Artemia replacement level (99%). Even if all diets seemed to perform equally well, one of the diet formulations allowed to reduce protein leaching.

The observed growth rates compare well with published data, especially considering the 99% Artemia replacement level (Fig 1). Senegalese sole fed on rotifers and Artemia alone during the first weeks of feeding typically have relative growth rates (RGRs) of 21 – 23 % day^{-1}, (3,4) while previous studies attempting to replace 100% of Artemia reached RGRs of 4-8 % day^{-1} (7,8). Therefore, the present study represents an improvement of over 30% compared to previous studies. It should be noted that previous studies (2 – 6) with Artemia replacements of 45-50% reach growth rates in the range of sole fed live feed. It can be expected that fine tuning of formulations and larval husbandry techniques for sole larvae will make full Artemia replacement a really in the near future.

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) 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.
(3) Engrola et al. 2009. Co-feeding in Senegalese sole larvae with inert diet from mouth opening promotes growth at weaning. Aquaculture 288, 264–272.
(4) Engrola et al 2010. Senegalese sole larvae growth and protein utilization is depressed when co-fed high levels of inert diet and Artemia since first feeding. Aquaculture Nutrition 16, 457-465.
(5) Canada et al. 2016. The supplementation of a microdiet with crystalline indispensable amino-acids affects muscle growth and the expression pattern of related genes in Senegalese sole (*Solea senegalensis*) larvae. Aquaculture 458, 158–169.
(6) Canada et al. 2017. Dietary protein complexity modulates growth, protein utilisation and the expression of protein digestion genes in Senegalese sole larvae. Aquaculture 479, 273-284.
(7) Fernández-Díaz et al 2006. Variations on development and stress defences in *Solea senegalensis* larvae fed on live and microencapsulated diets. Aquaculture 251, 573–584.
(8) Yúfera, M., Fernández-Diaz, C., Pascual, E., 2005. Food microparticles for larval fish prepared by internal gelation. Aquaculture 248, 253-262.

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LIFE CYCLE ASSESSMENT OF IRISH FRESHWATER AQUACULTURE

R. Cooney*, A. Tahar, A. Kennedy, E. Clifford

School of Engineering, National University of Ireland, Galway, Ireland, H91 HX31
Ryan Institute, National University of Ireland, Galway, Ireland, H91 TK33
E-mail: rcooney@nuigalway.ie

Introduction

In 2015 the Irish government published their plan, the "National Strategic Plan for Sustainable Aquaculture Development (DAFM, 2015). This plan aims to promote the growth and sustainable development of the Irish aquaculture sector as well as enhancing its competitiveness internationally. With these plans to expand, the sector there will be a need to increase process efficiencies. Life cycle assessment (LCA) offers the means to assess the current sustainability of on-farm aquaculture processes and can inform impact reduction opportunities and improvements in the production cycle or value chain from an environmental perspective. Four sites covering three species were benchmarked to characterise the environmental burden associated with Irish freshwater aquaculture. Two Atlantic salmon (*Salmo salar*), one rainbow trout (*Oncorhynchus mykiss*) and one Eurasian perch (*Perca fluviatilis*) sites were assessed. Combined these sites regularly produce 50% of sectoral output.

Materials and methods

The goal of the study was to determine the environmental impact of 1 kg of perch and 1 tonne of salmonids at the farm-gate, using ISO guidelines (ISO, 2006a, b). The sites consisted of flow through system (FTS) tank-based hatcheries, an FTS earthen pond system, a recirculating aquaculture system (RAS) hatchery and a recirculating aquaculture multitrophic pond system (RAMPS). The RAS and RAMPS were emerging systems at an experimental site (the focus being on fish husbandry), a number of data deficits needed to be overcome in order to develop a life cycle inventory (LCI). This required thermodynamic modelling of kerosene use, growth modelling, modelling of oxygen demand and consumption for stock and for water treatment. In all sites nutrient emissions were estimated using a nutrient digestibility model and energy use was derived from site surveys and records. The life cycle impact assessment (LCIA) was carried out using the CML method for: global warming potential (GWP), acidification (AP), eutrophication (EP), freshwater (FAETP) and marine aquatic ecoxtoxicity potential (MAETP) (Guinée, 2002). Additional methods included water use, cumulative energy demand and net primary production use (Papatryphon et al., 2004). Sensitivity, scenario and uncertainty analysis was also carried out on the results.

Results

Feed use and its production were the primary contributors to environmental burden. Energy use was the second most significant driver. There was also a high degree of variability between the cohorts monitored at the perch site notably in feed, energy and water use. The sensitivity analysis revealed that reducing the food conversion ratio to 1.0 would be the most environmentally conscious intervention for the salmon and perch sites. The intervention, which would reduce the environmental impact at the trout site, was to change the feed to one with a lower use of animal by-products (ABP) ingredients. The use of ABPs resulted in lower NPPU per tonne of fish produced but resulted in higher EP, AP and GWP emissions.

Discussion and conclusions

To the knowledge of the authors, these studies represent the first LCAs of Irish finfish aquaculture. It also represents the first LCA study of perch. The results indicate that Irish finfish production is inline with other studies. Food conversion ratios (FCRs) for smolts are almost identical to those available in the literature. Energy use and sludge production was also lower than other studies. However, liquid oxygen use was 14% higher than in the literature. Water use in Irish trout production was higher than is typical, but this is likely a result of local climatic conditions (i.e. high precipitation, low evaporation) and the location of the study site (high volume river). The influence of ABPs in increasing the impact of finfish production has been highlighted in previous studies (Parker, 2017; Pelletier et al., 2009). The study of the perch site required the development of a methodology, which can be used in data deficient aquaculture systems. This is often a limiting factor when using an LCA or eco-design approach in developing a system or product. The results of these LCAs provide a benchmark which future changes in processes, practices and supply chains of Irish aquaculture can compared.

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References

DAFM, 2015, National Strategic Plan for Sustainable Aquaculture Development, Department of Agriculture Food and the Marine.

Guinée, J. B., 2002, Handbook on life cycle assessment : operational guide to the ISO standards: Dordrecht ; Boston, Dordrecht ; Boston : Kluwer Academic Publishers.

ISO, 2006a, ISO 14040:2006 Environmental Management - Life Cycle Assessment - Principles and Framework, International Organisation for Standardisation.

ISO, 2006b, ISO 14044:2006 Environmental Management - Life Cycle Assessment - Requirements and Guidelines, International Organisation for Standardisation, Geneva, Switzerland.

Papatryphon, E., J. Petit, S. J. Kaushik, and H. M. G. van der Werf, 2004, Environmental impact assessment of salmonid feeds using life cycle assessment (LCA): AMBIO: A Journal of the Human Environment, v. 33, p. 316-323.

Parker, R., 2017, Implications of high animal by-product feed inputs in life cycle assessments of farmed Atlantic salmon: The International Journal of Life Cycle Assessment, p. 1-13.

Pelletier, N., P. Tyedmers, U. Sonesson, A. Scholz, F. Ziegler, A. Flysjo, S. Kruse, B. Cancino, and H. Silverman, 2009, Not All Salmon Are Created Equal: Life Cycle Assessment (LCA) of Global Salmon Farming Systems: Environmental Science & Technology, v. 43, p. 8730-8736.

IMPACT AND RECOVERY IN WATER QUALITY PARAMETERS DOWNSTREAM OF AQUACULTURE SITES

R. Cooney*, A. Tahar, A. Kennedy, E. Clifford

School of Engineering, National University of Ireland, Galway, Ireland, H91 HX31
Ryan Institute, National University of Ireland, Galway, Ireland, H91 TK33
E-mail: rcooney@nuigalway.ie

Introduction

The number of studies investigating the impact that freshwater aquaculture discharges have on downstream biological and chemical water quality have declined in recent years. For example, the last published article on the ecological or environmental impact of freshwater aquaculture in Ireland was 2004 (Costello et al., 2004). Prior to this, the only other work was carried out in the 1990s (Costello et al., 1994). Given the temporal gap in these studies and the waterbodies assessed (lotic versus lentic), it is timely to investigate the impact that aquaculture can have on water quality using holistic approaches such as the WFD (European Commission, 2000). The objectives of this study were to determine the degree of impact of freshwater aquaculture on water quality and the recovery of several parameters within a distance of 1,000 m. The monitoring campaign was carried out over a yearlong period and consisted of sampling during different seasons of the year to analyse water quality along the river gradient. The main parameters assessed as part of this were flow, biotic factors (macroinvertebrates and macrophytes) and water chemistry.

Materials and methods

The study site was an Atlantic salmon hatchery (*Salmo salar*), which was monitored from July 2018 – April 2019. The sites were visited in July (summer), September (autumn), December (winter) and April (spring). There were four sites monitored at Farm A, a control site (A0) located upstream of the farm, a site 10 m below the farm outflow (A1), a site 100 m downstream of the farm (A2) and a site 1,000 m below the farm (A3). On each occasion macroinvertebrate samples, data on plant abundance, hydromorphological data and physico-chemical data were collected. Water samples collected were analysed for parameters such as COD, TSS, NH_4^+, NO_2, NO_3 and PO_4^{3-}. Macrophyte abundance was also assessed using the mean trophic rank (MTR). Macroinvertebrates were identified to family and, where possible, species level. The catchment consists of an upland lake-fed river network, with low-intensity agriculture and some coniferous plantation. Several indices were used which included; abundance, richness, the percentage of Ephemeroptera, Plecoptera and Trichoptera individuals (%EPT individuals), percentage Oligochaete and Chironomidae individuals (%OC), ecological quality rating (EQR), Pielou's evenness (J), Simpson's (D') and Shannon's (H') diversities. Statistical analysis was carried out using one-way analysis of variance (ANOVA), with post-hoc Tukey tests and Bonferroni corrections. Other tests applied also included Spearman's rank correlation and Bray-Curtis dissimilarity tests.

Results

Results of the physical monitoring indicated that there was no statistically significant changes in hydromorphology or flow between A0 and the downstream sites. Chemical water quality parameters did increase in concentrations below the farm. The greatest concentrations in N and P forms were found at A3, 1,000m downstream of the farm. A3 also had the highest coefficients of variance between sampling periods. DO was very steady throughout the monitoring with a coefficient of variance of 13%. COD and TSS concentrations increased between A0 and A2 but were reduced at A3. Chemical results were not statistically significant. Changes in biological indices were statistically significant for changes in taxa at A2, %EPT at A1, %OC at A2, EQR at A2 and MTR for both all downstream sites.

Discussion and conclusions

The results for the chemical parameters were likely not significant due to the low sampling number (n=4). Despite this, there were a number of trends which could be discerned from the data. While aquaculture is a contributor to the changes in water quality, it is not the only source as evidenced by the increased nutrient concentrations at A3. These additional inputs may originate from agricultural or domestic activities. The sample size for biological results was larger (n=12), which allowed for a higher resolution dataset. Also within this dataset there were a number of positive trends which indicated that there was a high degree of recovery in biotic indices. Abundance of EPT species at A3 had increased and in some samples had exceeded those of A0. Shannon's and Simpson's diversity had both recovered at A3, with Pielou's evenness also seeing an upward trend.

(Continued on next page)

Aquaculture activities did not impair the hydromorphology of the waterbody and saw positive trends in biological parameters within 1,000m downstream of the farm. Changes in chemical water quality is driven by other activities (e.g. agriculture, water treatment) as well as aquaculture.

References

Costello, M. J., L. Collier, J. Dowse, and D. Quigley, 2004, Long-term environmental monitoring shows no impact from salmon cage farming in Lough Allen, an Irish freshwater lake: Biology and Environment: Proceedings of the Royal Irish Academy, v. 104B, p. 19-42.

Costello, M. J., E. Thompson, M. Gillooly, D. Quigley, and I. Warre-Hansen, 1994, Pollution control of freshwater fish farm effluents in Ireland, STRIDE Report No. 5,, Department of the Environment, p. 73.

European Commission, 2000, Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy, *in* E. Commission, ed., 2000/60/EC, https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32000L0060, p. 72.

NAEMO – NORTH ATLANTIC & EUROPEAN MUSSEL ORGANISATION

Ana Corrochano-Fraile*, Julie Webb, Vincenzo Alessandro Laudicella, Antonio Figueras, Paola Venier, Åsa Strand

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
E-mail: ana.corrochano-fraile@stir.ac.uk

Background

Mussels (*Mytilus spp*.) are key stone species providing valuable ecosystems services. Over the past years, mussel beds in Europe and North America have been reported to be in regression. Due to the lack of long-term monitoring, especially of unexploited mussel beds, there is no baseline for estimation of the severity of this decrease. Moreover, relatively little is known about potential threats to the welfare of mussels or if these threats are general or vary geographically.

Network objectives and roadmap

We established the North Atlantic and European Mussel Organization (NAEMO) to expand international collaboration between government, academia, industry and NGOs with the aim of providing knowledge-based support for improved management of mussels by increasing our understanding of the global and local processes affecting development of wild mussel populations and their interactions with farmed mussels. Outcomes from the initial workshop identified key questions for the network, specifically (1) evidencing and monitoring mussel bed decline, (2) identification of the causes for such decline, (3) interactions between wild mussel beds and farmed mussel populations and (4) improvements in communication between stakeholders. The roadmap (Figure 1) illustrates the future steps necessary to establish the network and to achieve the objectives, aims and goals of NAEMO. For more information or to join the network, please contact Åsa Strand asa.strand@ivl.se

Figure 1. Roadmap for achieving the NAEMO objectives and aims.

BLUE MUSSEL (*Mytilus edulis*) GENOME TO STUDY GENE UNDERGOING POSITIVE SELECTION

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* (Dias et al., 2011). 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 availability of this high-quality reference genome will serve as a valuable tool for potential studies in fundamental genetics as well as genome-scale selective breeding projects for *M. edulis*.

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.

The detection, comparison and the subsequent dN/dS evolution of key genes linked with shell formation, immunity and stress response was carried using 65 characterised genes for shell formation from *Pinctada spp., Crassostrea spp*. and *M. galloprovincialis* (Song et al., 2019); 72 genes for immunity response from *M. galloprovincialis* and *M. edulis* (Pallavicini et al., 2008; Vera et al., 2011; Gerdol & Venier, 2015); and 18 genes for stress-related responses from *M. galloprovincialis, M. edulis* and *M. coruscus* (Dondero et al., 2006; Zhang et al., 2014). Those key genes were identified in the newly annotated genome as well as M. coruscus and M. galloprovincialis.

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. This accurate reference genome of *M. edulis* will also allow the exploration of the evolutionary basis of the speciation and local adaptation of the *M. edulis* species.

Bibliography

Dias, P. J., Piertney, S. B., Snow, M., & Davies, I. M. (2011). Survey and management of mussel Mytilus species in Scotland. *Hydrobiologia*, *670*(1), 127–140. https://doi.org/10.1007/s10750-011-0664-x

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

Gerdol, M., & Venier, P. (2015). An updated molecular basis for mussel immunity. *Fish and Shellfish Immunology*, *46*(1), 17–38. https://doi.org/10.1016/j.fsi.2015.02.013

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

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Pallavicini, A., del Mar Costa, M., Gestal, C., Dreos, R., Figueras, A., Venier, P., & Novoa, B. (2008). High sequence variability of myticin transcripts in hemocytes of immune-stimulated mussels suggests ancient host-pathogen interactions. *Developmental and Comparative Immunology*, *32*(3), 213–226. https://doi.org/10.1016/j.dci.2007.05.008

Song, X., Liu, Z., Wang, L., & Song, L. (2019). Recent advances of shell matrix proteins and cellular orchestration in marine molluscan shell biomineralization. *Frontiers in Marine Science*, *6*(FEB). https://doi.org/10.3389/fmars.2019.00041

Vera, M., Martínez, P., Poisa-Beiro, L., Figueras, A., & Novoa, B. (2011). Genomic Organization, molecular diversification, and evolution of antimicrobial peptide myticin-c genes in the mussel (mytilus galloprovincialis). *PLoS ONE*, *6*(8). https://doi.org/10.1371/journal.pone.0024041

Zhang, D., Wang, H. W., & Yao, C. L. (2014). Molecular and acute temperature stress response characterizations of caspase-8 gene in two mussels, Mytilus coruscus and Mytilus galloprovincialis. *Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology*, *177–178*, 10–20. https://doi.org/10.1016/j.cbpb.2014.08.002

EVIDENCE FOR FUNCTIONAL ROLE OF A BLEND OF MICROALGAE AND MACROALGAE ON SENEGALESE SOLE POST-LARVAE ROBUSTNESS

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], 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

*E-mail: anagoncalves@sparos.pt

Introduction

Micro- and macro-algae are well recognized in human nutraceuticals antioxidant, immunostimulant and health enhancer properties. Their inclusion in aquafeeds as functional ingredients could bring significant improvements on fish robustness and resistance to disease/environmental challenges, specially at the earlier and sensitive life stages. Microalgae have been traditionally used as growth and development promoters in fish larviculture, either by the green water technique to improve water quality and prey visibility, or by serving as nutrient source for live feed such as rotifers. The inclusion of microalgae biomass in fish diet has been tested for different species during later juvenile stage with promising results, however in the larval stages research is still scarce. While some microalgae species are rich in long-chain polyunsaturated fatty acids (LC-PUFAs), such as EPA and DHA, both micro- and macro-algae are natural sources of pigments, minerals, vitamins and other bioactive compounds. However, the production of microalgae is costly, and to reduce cost and maintain nutritional and functional value of microalgae can be blended with macroalgae biomass (Batista et al 2020). This study aimed to evaluate the effects of microdiets containing an algae blend (3 and 6% *Nannochloropsis* sp. with *Gracilaria gracilis* in equal proportions) in Senegalese sole (*Solea senegalensis*) post-larvae growth, immune and antioxidant response.

Materials and methods

A growth trial was conducted with Senegalese sole (*Solea senegalensis*) post-larvae (origin: IPMA-EPPO, Olhão, Portugal) testing 3 microdiets in terms of growth, immune and antioxidant response from 34 to 63 days after hatching (DAH). In this trial, larvae were reared in triplicate tanks under standard zootechnical conditions and fed *ad libitum* on 3 microdiets produced by cold extrusion by SPAROS (Olhão, Portugal). All three microdiets used the same base formula containing premium practical ingredients (e.g., squid meal, crustacean meal, fish meal, wheat gluten, fish oil, soy lecithin). The differences between the control (CTRL), BLEND3 and BLEND6 diets are given in table 1, along with their proximal composition. Growth performance, feed conversion ratio (FCR), survival, innate immune response and antioxidant response were monitored.

Results

Senegalese sole post-larvae performance was significantly enhanced in fish fed 3% algae blend with higher total length and dry weight, reflecting in a superior RGR (see Fig. 1). Fish fed the 6% algae blend showed and initial (49 DAH) tendency for higher growth, but this was lost at the end of the trial. Moreover, a stronger immune response comparing to control was observed mainly through higher lysozyme and peroxidase activities, especially in the BLEND6 treatment. Interestingly, sole post-larvae presented a superior antioxidant response throughout the trial, but in particular at the shorter term (49 DAH), when fed the diets with algae blends.

Discussion

Results of the present study support the functional role of algae on improving fish early life stages robustness and performance. This is very relevant for tailoring optimized weaning and nursery microfeeds for Senegalese sole (Pinto et al. 2018) and other species. This follows positive results in growth performance for Senegalese sole juveniles with *Nannochloropsis* sp. biomass diet inclusion up to 15% (Vizcaíno et al 2018). The present study also suggests future research and new applications of microalgae, macroalgae and their blends as functional ingredients for farming early life stages of fish, bringing better performances and supporting sustainable fish farming.

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Table 1. Proximal composition of microdiets used in Senegalese sole post-larvae trial.

Microdiet	CTRL	BLEND 3	BLEND 6
Base formula (% feed)	94.0	94.0	94.0
Cellulose (% feed)	3.0	0.0	0.0
Crustacean meal* (% feed)	3.0	3.0	0.0
Nannochloropsis sp. (% feed)	0.0	1.5	3.0
Gracilaria gracilis (% feed)	0.0	1.5	3.0
Protein (% feed)	59.5	61.1	59.7
Fat (% feed)	17.8	18.2	17.9
Ash (% feed)	10.6	11.1	11.5
Phosphorous (% feed)	1.8	1.8	1.8
Energy (MJ/Kg)	21.8	21.9	21.6

* Additional amount, as the base formula also contains crustacean meal.

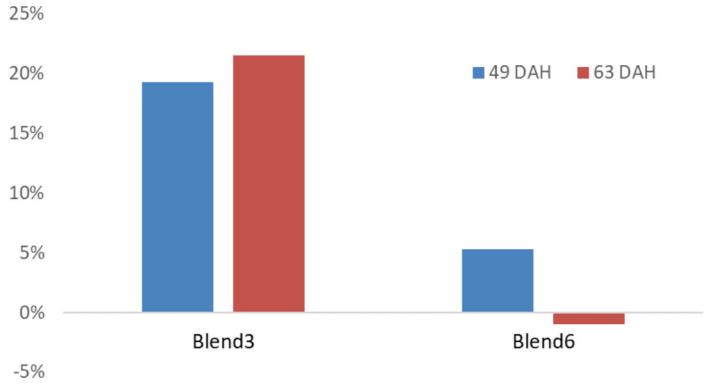

Figure 1. Variation in weight compared to the control diet at 49 and 63 DAH, in Senegalese sole post-larvae fed microdiets with 3% (BLEND3) and 6% (BLEND6) *Nannochloropsis* sp. blended with *Gracilaria gracilis* in equal proportions.

References

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.

Gonçalves, A.T., Pinto, W., Afonso, D,, Conceição, L. (2020). Microalgae as an alternative ingredient for high performance sustainable microfeeds. Hatchery Feed & Management 8(3), 34-36.

Pinto, W.; Engrola, S.; Conceição, L.E.C. (2018). Towards an early weaning in Senegalese sole: A historical review. Aquaculture 496, 1-9.

Vizcaíno, A.J.; Rodiles, A.; López, G.; Sáez, M.I.; Herrera, M.; Hachero, I.; Martínez, T.F.; Cerón-García, M.C.; Alarcón, F.J. (2018) Growth performance, body composition, and digestive functionality of Senegalese sole (Solea senegalensis Kaup, 1858) juveniles fed diets including microalgae freeze-dried biomass. Fish Physiology and Biochemistry 44, 661-677.

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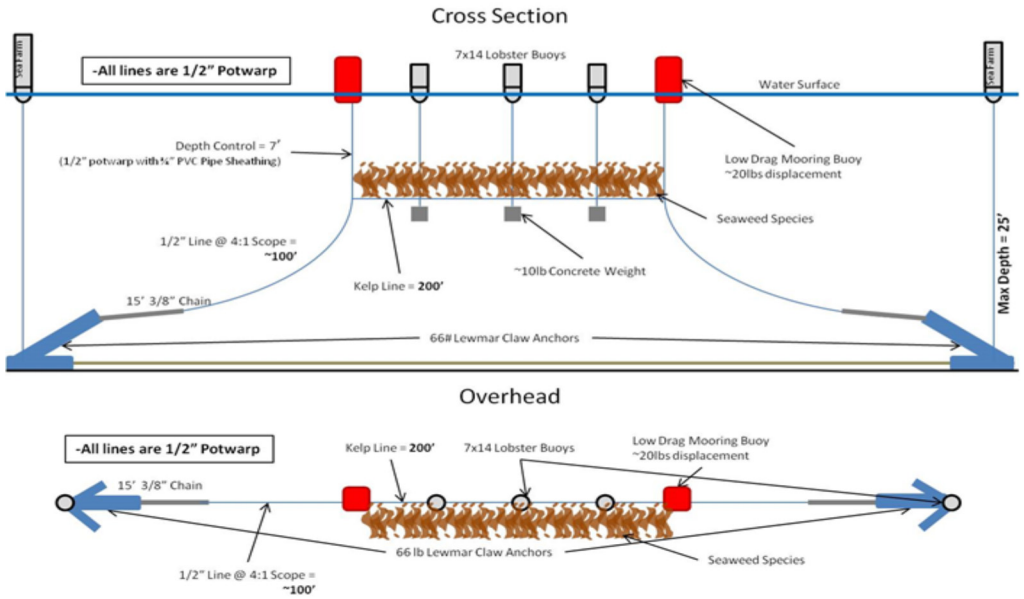
DOWNSCALING THE SEAWEED REVOLUTION: CAN VIABLE SEAWEED BIOECONOMIES BE CREATED IN ARCTIC/NORTH ATLANTIC RURAL AREAS?

B. Costa-Pierce*, A. St. Gelais, D. Fredriksson, T. Dewhurst, A. Drach

School of Marine & Environmental Programs, University of New England, ME, USA and the Swedish Mariculture Research Center (SWEMARC), University of Gothenburg, SWEDEN
Ocean Engineering Program, United States Naval Academy, Annapolis, MD, USA
Kelson Marine LLC, Portland, ME, USA
Calentis Consulting LLC, Austin, TX, USA
E-mail: bcostapierce@une.edu

Farming of seaweeds in the High North is developing but has good potential to contribute to rural and indigenous fishing/community livelihoods for additions to the food and biomanufacturing circular economies. Many government and NGO groups are promoting large scale, expansive ocean development plans for seaweed aquaculture with little equivalent planning for investments in local/regional product developments and marketing plans that would maximize regional/local impacts on job creation in fisheries dependent livelihoods. Most of the current academic and industry developments in the High North focus on "scaling up". Our bioengineering research group is focused on "downscaling" seaweed aquaculture research and development, and over the past 4 years has developed and tested in coastal Maine a simple, low cost system having a proven engineering performance in high energy nearshore areas.

Seaweed farming is an especially ideal candidate for technology transfer to seasonal pot fishermen in the High North who fish in the summer but move into land-based alternative income-producing jobs in the winter. They look to diversify their income but have no water-based alternatives. Their water based capital investment stays dormant, non-performing through almost half of the year. We examined current seaweed aquaculture designs and found them inappropriate for the fishing community, being expensive, cumbersome, immobile, and moorings more or less permanent. These aspects made scale adoption by fishermen difficult. To overcome this, an inexpensive, light weight, tensioned line system, comprised of highly mobile gear seaweed farming was developed, and its ocean engineering performance tested over four years in harsh winter conditions in Maine, USA. The system is shown below and was different completely from the widely recommended one promoted by fisheries extension leaders.



The system produced over a 4-5 month winter-spring period regular harvests of 9 to 15 kg/m of line. This low cost seaweed farming system for winter operations fits well into a "livelihood" strategy of fishing families and communities who must work multiple jobs in the offseason when their main fishery is unavailable.

REGIONAL DIFFERENCES IN ZOOPLANKTON-ASSOCIATED BACTERIAL COMMUNITIES AND AQUACULTURE PATHOGENS ACROSS TWO SHELF SEAS

K.E. Costello*, D. Haberlin, S.A. Lynch, R. McAllen, R.M. O'Riordan and S.C. Culloty

School of Biological, Earth and Environmental Sciences & Aquaculture and Fisheries Development Centre, Environmental Research Institute, University College Cork, Ireland *and* MaREI Centre, Environmental Research Institute, University College Cork, Ireland
Email: katiecostello@ucc.ie

Introduction

Pathogens can persist in the marine environment in a number of ways, for example during a transmissible free-living life stage or adsorbed onto the surface of plankton to create a more stable and transportable medium. These pathogens may include viruses, protistans and bacteria, all of which can have negative impacts on the aquaculture sector.

Ostreid herpesvirus microvariant OsHV-1µVar has caused significant mortalities in the *Crassostrea gigas* sector globally. Herpesvirus may be present in seawater in a free viral form, attached to particles or flocculated. However, herpesviruses may also be attached to, or infect, planktonic hosts.

The phylum haplosporidia is another pathogenic group of major concern to shellfish industries worldwide. Haplosporidian sequences have been detected in water and sediment samples, suggesting the possibility of either free-living stages or associations with planktonic fauna.

Molecular techniques such as Illumina-sequencing are increasingly utilised to identify distinct geographic breaks in bacterial community structure while polymerase chain reaction is used as a diagnostic tool to detect pathogen DNA/RNA.

The association of problematic bacteria and other pathogen groups is well documented in nearshore bivalve culture environments, however, very little is known about these microbial communities in offshore environments. This study combined polymerase chain reaction (PCR) and Illumina-sequencing of the bacterial V3-V4 region to screen for the presence of aquaculture associated pathogenic microbes in zooplankton samples collected offshore in the Irish and Celtic Seas.

Materials and Methods

The research cruise was conducted aboard the RV *Celtic Voyager* between 17th-26th May 2018 and diel sampling was conducted for the duration of the cruise, with sixty-five samples collected (Figure 1). Each sample was split in two, with half preserved in ethanol for molecular work and half preserved in formalin for zooplankton identification. Most pathogens associated with aquaculture are most prevalent in late spring, summer and early autumn.

Upon return to the laboratory, DNA from the zooplankton was extracted using a QIAGEN DNeasy® Blood & Tissue kit. All sixty-five samples were then screened for haplosporidia and OsHV-1 µVar using PCR. Thirty-two samples (Irish Sea, Celtic Front, Eastern Celtic and Celtic Sea groups) were then sequenced to find the entire range of associated bacteria.

This screening was conducted using the services of Novogene, Cambridge for Illumina 16s amplicon based metagenomic sequencing of the V3-V4 (466bp) region.

The zooplankton community composition for formalin-preserved samples was quantified using a Zeiss Stemi 305 darkfield stereomicroscope and all subsequent analyses were conducted in RStudio version 4.3.

Results

Analysis of Molecular Variance (AMOVA) was conducted to determine whether the difference of bacterial community structure among groups was significant and the two most significant were between the Irish Sea-Celtic Sea.

There was no detection of OsHV-1 µVar in any of the samples, however a single haplosporidian positive was detected (1.54% prevalence) and confirmed it to be 18_Haplo_BMVA_WEY (Accession number KF208557). *Vibrio splendidus* was detected in all Illumina-sequenced samples, with high abundance in the Celtic Sea but just trace amounts in the Irish Sea.

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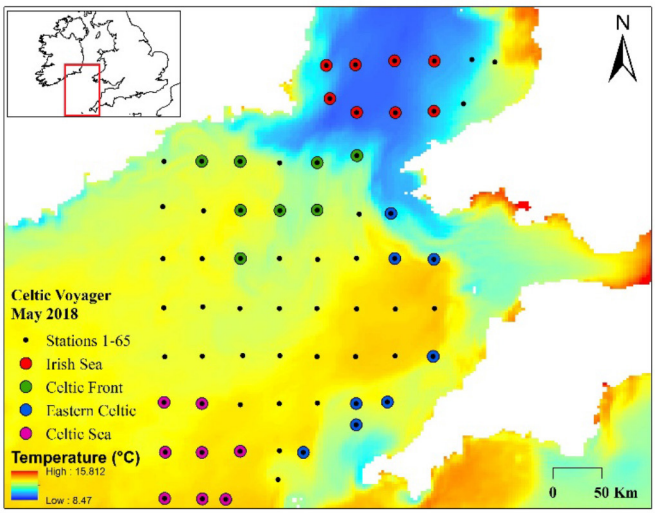

Figure 1: Map of sample sites (n=65) and groups (n=32) sent for Illumina-sequencing

Discussion

The four groups representing the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic Sea demonstrated distinct bacterial profiles, with the prevalence of cyanobacteria decreasing as the presence of proteobacteria (the phylum in which *Vibrio* spp. are found) increased. The study also detected the bacterium *Vibrio splendidus*, pathogenic to bivalves, and a low prevalence of a haplosporidian species, which has not been associated with a bivalve host.

Determining where aquaculture-associated pathogens are present and the regions in which they are found can be used to plan mitigation efforts, for example when choosing offshore locations to culture bivalves.

The haplosporidian species detected in this study was identified as a novel species first described from nearshore seawater samples collected off Weymouth, UK between 2011-2012. This finding would indicate that this species has an association with plankton and/or seawater.

Findings from this preliminary study indicate that the prevalence and diversity of known aquaculture pathogens in the offshore environment is low, however, seasonal screening at proposed culture locations would be beneficial to determine if temporal variation exists and if the seasonality of pathogen communities differs offshore compared to nearshore.

GLOBALSEAWEED STAR - SAFEGUARDING THE FUTURE OF THE SEAWEED INDUSTRY IN DEVELOPING COUNTRIES

E. J. Cottier-Cook*, C. C. Gachon, J. Brodie, L. Shaxson, F.E. Msuya, A. Buriyo, A. Hurtado, V. E. N. Ferriols, P. E. Lim, N. Nagabhatla

Science Department, Scottish Association for Marine Science (SAMS), Oban, PA37 1QA, United Kingdom
E-mail: ejc@sams.ac.uk

The cultivation of seaweed species has undergone a dramatic global expansion since the 1970s, with current production at 30 MT/year, worth €8.1 billion annually and expanding to 50 countries worldwide (FAO, 2020). ~95% of seaweed production occurs in developing countries, including China, the Philippines, Indonesia and Tanzania. Despite the apparent success of this industry to date, outbreaks of introduced pests and diseases are becoming increasingly common and are having significant negative consequences both directly on yields and more widely on the surrounding environment, both of which affect livelihoods.

Significant global losses in production of the highly valued red seaweeds, *Kappaphycus* spp and *Eucheuma* spp (>15%), which are grown for their carrageenan, equating to almost US$0.3 billion yr^{-1} in lost revenue, have been attributed to diseases and pests and have had major socio-economic impacts on communities reliant on this industry (Cottier-Cook et al. 2016; Ward et al. 2019). Key challenges include; the lack of effective legislation and farm management practices regarding biosecurity and risk management, if present at all, at both the national (Kambey et al. 2020; Mateo et al. 2020; Rusekwa et al., 2020) and international levels (Campbell et al., 2020), a poor understanding of the causal agents and environmental triggers associated with seaweed disease and pest outbreaks (Ward et al. 2019) and inequalities between genders and seaweed farming communities, in terms of access to training and government support (Suyo et al. 2020). The UK-funded 'Global Challenges Research Fund – GlobalSeaweedSTAR (GSSTAR)' (www.globalseaweed.org) programme comprises research institutes in the UK, the Philippines, Tanzania and Malaysia to build capacity to address these key challenges and to contribute the sustainable and equitable growth of this vital industry in seaweed-producing countries. Through the GSSSTAR-Research and Travel Fund we have been able to expand our network across an additional 12 countries, including Peru, Indonesia, Madagascar, Argentina, Thailand, Bangladesh, Sri Lanka, Costa Rica, Mexico, Brazil, South Africa and Colombia.

References

Campbell, I., Kambey, C.S.B., Mateo, J.P., Rusekwa, S.B., Hurtado, A.Q., Msuya, F.E., Stentiford, G.D., Cottier-Cook, E.J. (2019) Biosecurity policy and legislation for the global seaweed aquaculture industry J Appl Phycol https://doi.org/10.1007/s10811-019-02010-5

Cottier-Cook, E.J., Nagabhatla, N., Badis, Y., Campbell, M., Chopin, T, Dai, W, Fang, J., He, P, Hewitt, C, Kim, G. H., Huo, Y, Jiang, Z, Kema, G, Li, X, Liu, F, Liu, H, Liu, Y, Lu, Q, Luo, Q, Mao, Y, Msuya, F. E, Rebours, C, Shen, H., Stentiford, G. D., Yarish, C, Wu, H, Yang, X, Zhang, J, Zhou, Y, Gachon, C. M. M. (2016). Safeguarding the future of the global seaweed aquaculture industry. United Nations University (INWEH) and Scottish Association for Marine Science Policy Brief. ISBN 978-92-808-6080-1. 12pp

FAO (2020) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome, 206 pp. https://doi.org/10.4060/ca9229en

Kambey, C.S.B., Campbell, I., Sondak, C.F.A., Nor, A.R.M., Lim, P.E., Cottier-Cook, E.J. (2020). An analysis of the current status and future of biosecurity frameworks for the Indonesian seaweed industry. J Appl Phycol doi:10.1007/s10811-019-02020-3

Mateo, J.P., Campbell, I., Cottier-Cook, E.J., Luhan, M.R.J., Ferriols, V.M.E.N., and Hurtado, A.Q. (2020). Analysis of biosecurity-related policies governing the seaweed industry in the Philippines. Journal of Applied Phycology. 32:2009-2022. https://doi.org/10.1007/s10811-020-02083-7

Rusekwa, S.B., Campbell, I, Msuya, F.E., Buriyo, A.S., Cottier-Cook, E.J. (2020). Biosecurity policy and legislation of the seaweed aquaculture industry in Tanzania. J Appl Phycol https://doi.org/10.1007/s10811-020-02194-1

Suyo, JGB., Le Masson, V., Shaxson, L., Luhan, MRJ, and Hurtado, A.Q. (2020). A social network analysis of the Philippine seaweed farming industry: Unravelling the web. Marine Policy, 118 https://doi.org/10.1016/j.marpol.2020.104007.

Ward, G.M., Faisan, J.P, Cottier-Cook, E.J., Gachon, C., Hurtado, A.Q., Lim, P.E., Matoju, I., Msuyu, F., Brodie, J. (2019). A Review of Reported Seaweed Diseases and Pests in Aquaculture in Asia. Journal of the World Aquaculture Society. DOI: 10.1111/jwas.12649

LAND BASED INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) OF LOW TROPHIC SPECIES: AQUAVITAE PROJECT'S CASE STUDY

G. Courtois de Viçose[1], C. Hannon[2], C. Jones[3], M. Slater[4], S. Huchette[5], D. Weich[6], D. Taylor[7], Y. Wu[3], P. Britz[3], P. James[8]

1) Instituto ECOAQUA/GIA Universidad De Las Palmas De Gran Canaria, Juan de Quesada 30, 35001 Las Palmas de Gran Canaria Spain.
2) Marine & Freshwater Research Centre, Galway-Mayo Institute of Technology, Ireland
3) Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa.
4) Alfred- Wegener Institute, Knowledge and Technology Transfer, Aquakulturforschung, Bussestr, 2727570 Bremerhaven
5) SCEA France Haliotis, Kerazan Lilia 29 880 Plouguerneau, France
6) Marifeed Pty Ltd, 7 Gideon van Wyk Crescent, Hermanus, South Africa
P.O.Box 880, Hermanus, 7200.
7) Wild Coast abalone, KOMGA, Eastern Cape, 4950 South Africa
8) Nofima, Muninbakken 9-13, Breivika, P.O. Box 6122 Langnes
NO-9291 Tromsø, NORWAY

Introduction

Farming of aquatic species in Integrated Multi-Trophic Aquaculture (IMTA) systems has been identified of interest to foster innovative and sustainable solutions for seafood production. However, a limited number of species have been tested in production in such systems. Consequently, in a quest to develop production of new and emerging low trophic species, the AquaVitae H2020 project integrates a case study focusing on the development of Land Based IMTA systems integrating low trophic species. Diverse activities contemplated in the case study are taking place at different geographical locations across all the Atlantic Ocean to facilitate the trans national transfer of knowledge between the regions and partners while contributing to the implementation of the Galway and Belém statements which fosters the Atlantic wide EU-North America-South America-African and South African collaboration.

Material and methods

The case study aims at developing systems and processes to increase and improve Land Based IMTA production with special emphasis on low trophic species. The workplan, for the activities pulled together under the case study, addresses industry bottlenecks reported for the development of land-based IMTA integrating low trophic species at biological level (provision of seeds, production data over various periods of the cycles, identification of contributions from species integration), operational level (technology, production infrastructure) and market level.
Specific actions include the following:

- Improvement of hatchery and nursery production processes through the adoption of integrated and organic methods for these production cycles.
- Development of novel (IMTA) systems for low trophic species in land-based systems to optimise growth rates, environmental mitigation and profitability
- Assess land based produced IMTA products in terms of quality, sustainability and nutritional value.

Results

Preliminary results obtained in the first stage of experiments indicate that:

- Sporulation levels of *Ulvella lens* and plate preparation to be used as settlement substrates have a significant effect on *Haliotis tuberculata* settlement rate.
- The addition of conspecific-mucous to diatom cultures increases the larval settlement of South African abalone (*Haliotis midae*) and increases post-settlement survival.
- Higher temperature induces higher mortality and faster growth for *Haliotis tuberculata*
- The integrated production of different low trophic species in the nursery stage of *Haliotis tuberculata* production do not affect abalone growth and survival and enable the production of an additional product, the sea anemone *Anemona sulcata*.
- The production of different sea cucumber species together with abalone, during grow out, in integrated Land Based IMTA systems offers possibilities of production of an additional low trophic species when abalone are fed IMTA produced macroalgae

(Continued on next page)

- Prototype co-culture systems designed and tested to integrate sea cucumber to abalone grow out systems allow wild sea cucumbers to survive and grow while consuming abalone wastes.
- Abalone fed a mix diet of IMTA produced macroalgae and compound feed present the best growth potential not being significantly different between recirculated and open flow-through grow out systems.
- Abalone grow out feeding trials testing the feed inclusion of IMTA and non IMTA produced macroalgae allow the collection of data to establish future life cycle analysis of different grow out strategies.

Discussion

The results obtained in this case study contribute to numerous objectives of the AquaVitae nursery production, grow-out and post-harvest work packages. These results will also be further used as a source of information for other work packages of the AquaVitae project, including evaluating food safety, nutrition and market potential; environmental monitoring, ecosystem services and sustainability as well as aquaculture policy and governance.

Acknowledgements

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DISSOLVED OXYGEN CONSUMPTION AND INTRA-TANK DISTRIBUTION OF TROUT BIOMASS

A. C. Lima[1*], E. Royer[1] and R. 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

Precision fish farming (PFF) aims at improving accuracy, precision, and repeatability in farming operations by delivering reliable decision-making support tools to farmers (Føre et al. 2018). It is centred on 1) large quantitative datasets provided from sensors which feed 2) data driven models and algorithms and finally supply 3) decision-supporting tools and smart management systems based on the Internet of Things (Banhazi et al., 2012). These datasets concern both environmental variables and fish bio-responses.

Here we focus on the dissolved oxygen (DO) flux along an 8m-wide 200m-long raceway tank for rainbow trout cultivation located in Trentino Alto-Adige, Italy. Along the production cycle, liquid oxygen is supplied at constant rates into the raceway. A previous assessment (Royer el al. 2021) has demonstrated that a DO transport model can be used to implement a cost-effective automatic control of oxygen supply in this fish farm, based on short-term predictions of oxygen demand. As a step further, here we address the intra-tank spatial variability of DO, associated with a liquid oxygen supply, fish metabolism, and atmospheric exchange. By analysing the streamwise variation of DO consumption, the biomass distribution along the raceway can be estimated, allowing the assessment of preferential movements of fishes along the tank. Furthermore, assessing the DO values at different downstream positions at the raceway is suitable for improving fish growth and respiration models.

Methodology

A PFF-based model of dissolved oxygen is employed. The model introduces streamwise gradients to the previous temporal 0-dimensional framework of Royer et al. (2021) The DO flux is described by the advection equation with additional terms expressing 1) the oxygen consumption by fish respiration and 2) the capture of oxygen via exchange with the atmosphere. The DO concentration at the upstream boundary condition, characterized by a daily oscillation, includes the liquid oxygen supply which is added in the farm. The daily fluctuation of the fish respiration is described by a sinusoidal function.

This study made use of DO and temperature data collected by two probes located in the upstream and downstream ends of the raceway, and a sensor (DO only) located at the halfway section of the raceway, i.e., 100m from either the upstream or downstream boundaries of the raceway. The frequency of data collection ranged from 15 min to 1 h. Biomass weight data was acquired using Biomass Daily (BD), an 80 × 80 cm submerged frame equipped with a sensor based on infrared technology which detects a signal whenever a fish specimen moves across the frame. To the authors' knowledge, the application of BD to a trout raceway is novel.

Results and discussion

The study was targeted at two time series: July 3 to 7, 2019, and July 8 to 14, 2020, referred afterwards as case 1 and 2, respectively. In both cases the fishes were under a fasting state. In case 1 the average fish weight was 1029 g, and the total fish biomass weight was 21206 kg, whereas in case 2 those were 307g and 12201 kg, respectively. Based on the analysis of the measured data and the results of the model, the following outcomes are highlighted:

1. The DO consumption is not evenly distributed along the raceway tank, indicating a spatial variability in the fish biomass distribution. The measured data and the model results indicate that the upstream half of the raceway accounts for 57-59% of the overall DO consumption in case 1 and approximately 45% in case 2.
2. The measured data indicates a considerable time variation in the percentage of consumed DO by each half (upstream or downstream) of the raceway, following a sinusoidal daily cycle. This indicates the possibility that the preferential swimming direction (upstream or downstream) of the fish population shifts twice daily.
3. The analytical model captures the time-averaged streamwise variation in the DO consumption even if a constant biomass distribution is input, with a good agreement with the measured data.
4. Using the analytical model and the DO consumption data, the time and spatial gradients of the biomass distribution can be estimated.

AN EMERGING ENVIRONMENTAL ACCOUNTING APPROACH TO ASSESS NOVEL OR UNDEREXPLOITED INGREDIENTS: EMERGY ASSESSMENT OF FISHMEAL FROM INSECTS, POULTRY BY-PRODUCTS, & MICROALGAE

S. Maiolo[1], S. Cristiano[1]*, F. Gonella[1], and R. 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)
E-mail: silvio.cristiano@unive.it

Introduction

Fishmeal represents the optimal protein source to feed farmed fish and crustacean species. Increasing market demand and prices has been promoting the search for alternative protein sources in aquaculture. Other food supply chains currently present waste products that might be safely re-circulated in the economy. Four partial substitutes for fishmeal are here considered: two involving microalgae, one insect meal, and one the reuse of by-products from poultry farming. The results of the assessment applied to such alternative ingredients are presented and discussed in the light of their possible role in improving the integrated sustainability performances of the portion of the aquaculture sector related to protein fishmeal. Industrial innovation is mostly driven by economic reasons and consequent assessment from a receiver/consumer perspective. To counterbalance such knowledge in times of ecological crises and concerns, it is here proposed a comprehensive environmental assessment through an emerging approach, rooted in systems thinking and thermodynamics, able to offer a donor-side perspective, i.e. that of the geobiosphere. Such results are compared to the Life-Cycle Assessment of the same ingredients.

Materials and methods

The four partial substitutes for protein fishmeal are considered in operating plants located by the European coast of the Mediterranean sea, namely, in Northern Italy and Southern France. The following ingredients are here assessed: dried biomass from microalgal species *Tetraselmis suecica* and *Tisochrysis lutea*, instect meal from larvae of *Hermetia illucens*, and by-product meal from poultry farming. Such products and their related processes are elaborated through the Emergy Accounting (EMA) approach (Odum, 1996; Brown & Ulgiati, 2016). Results from the standardised Life-Cycle Assessment (LCA) (Arvanitoyannis, 2008; ISO, 2018) are also offered and discussed. Through EMA, the dependence on natural resources is further explored compared to the sole LCA. The two approaches can be seen as complementary, and are indeed increasingly used together when a comprehensive view on a process' sustainability is required or desired.

Results

The results show the insect meal has the highest environmental efficiency when expressed in emergy requirements per unit of product. The second highest efficiency is found in poultry by-product meal. This can also be found in LCA results. Microalgae seem to suffer from both low productivity and a significant use of seawater to be brought to the on-shore plants. However, some critical aspects emerge from five emergy indicators: the four processes all appear to rely on intensive industrial processes, with a poor use of local renewable sources and instead a high (99%) dependency upon resources from outer human economies (with no economy of scale being observed). For microalgae, the significant need for seawater, found through the EMA, is complemented by carbon dioxide and energy requirements, found via LCA. In the insect meal system, human labour (only measurable through EMA) plays a non-negligible role, while energy requirements are highlighted by both the approaches. Building upon such findings, possible approaches to make up for the current environmental issues are discussed.

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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.

Wackernagel, M., & Rees, W. (2004). What is an ecological footprint?. *The sustainable urban development reader*, 211-219.

FISH MORTALITY TREATMENT AND VALORISATION AS A BY-PRODUCT: ENVIRONMENTAL ASSESSMENT OF ECO-INNOVATION OPTIONS

S. Cristiano[1]* and R. 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)
E-mail: silvio.cristiano@unive.it

Introduction

Based on environmental concerns, resource optimisation, and related European policies, the aquaculture sector is not exempt from seeking circular and less impacting solutions. The main technology to farmed fish mortality is currently represented by the ensilage. According to such approach, biomass is treated with formic acid, thus requiring flammable liquids to be transported and safely disposed of while exposing humans and the rest of the environment to threats. Some eco-innovations have been developed within the Horizon 2020 project GAIN – Green Aquaculture Intensification in Europe (2018–2021). Two of them, carried out by industrial partner Waister AS, were independently evaluated by the research partner Università Ca' Foscari through a standardised well-oiled method for environmental assessment, and compared with business-as-usual. The eco-innovations' gains, potentials, limits, and margins for improvement are here presented and discussed.

Materials and methods

This study presents the environmental assessment of three scenarios. Scenario A describes the leading business-as-usual approach for fish mortality treatment and disposal, i.e. ensilage. This requires formic acid to be used at the aquaculture premises, and flammable liquids to be transported away from the plant and to be disposed of (Baarset et al., 2020).

The two eco-innovations that are here studied are aimed at avoiding to use formic acid in while processing fish mortalities. Ensilage is replaced by a superheated steam drying process through mechanical fluidisation. This is possible e.g. thanks to an eco-innovation machinery (Waister 15), able to dry and compact food waste. A dried sanitised product can be obtained for disposal or – better – valorisation as a by-product: this way, harmful waste is expected to become a resource as a secondary product to be re-circulated into the economy. In the selected eco-innovations, fish mortality is mixed with another by-product, i.e. local brewer's spent grain, used as a structure material. In scenario B, water is used as a cooling medium; in scenario C, the cooling medium is replaced by a mix of glycol (30%) and water (70%). Some subscenarios are considered for B and C, based on different end-of-life options: simple disposal or reuse as a secondary product, i.e. as an ingredient in pet food. The adopted method for environmental accounting is represented by the standardised Life-Cycle Assessment (Arvanitoyannis, 2008; ISO, 2018). Indicators are available from different calculation choices, including the Ecological Footprint (Wackernagel and Rees, 2004), the Global Warming Potential, the Cumulative Exergy Demand, the Water Footprint (Hoekstra et al., 2011), and the ReCiPe sets (Goedkoop et al., 2009).

Results

The two selected eco-innovations for mortality treatment seem to perform better than business-as-usual ensilage. Environmental gains larger than –80% are obtained in most indicators even when the product is simply disposed of. Smaller, neutral, and – according to the different calculation choices – sometimes opposing results are reached instead when talking about water consumption. 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.

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.

Baarset, H., & Johansen, J. (2020). Innovative processes for mortality disposal in aquaculture. Deliverable 2.2. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 773330. 14 pp.

Goedkoop, M., Heijungs, R., Huijbregts, M., De Schryver, A., Struijs, J., & Van Zelm, R. (2009). ReCiPe 2008. *A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level*, *1*, 1-126.

Hoekstra, A. Y., Chapagain, A. K., Mekonnen, M. M., & Aldaya, M. M. (2011). *The water footprint assessment manual: Setting the global standard*. Routledge.

ISO - International Organization for Standardization (2018). ISO 14044:2018 Environmental management - Life cycle assessment - Requirements and guidelines.

Wackernagel, M., & Rees, W. (2004). What is an ecological footprint?. *The sustainable urban development reader*, 211-219.

GENETIC VARIATION FOR CLIMATE CHANGE RESILIENCE IN GROWTH OF ATLANTIC SALMON

B.S. Dagnachew*, I. Thorland#, B. Hillestad# and A. Kettunen*

*Nofima, Osloveien 1, 1430 Ås, Norway;
#Benchmark Genetics Norway AS, Sandviksboder 3A, NO-5035 Bergen, Norway
Email: binyam.dagnachew@nofima.no

Introduction

The presence genetic by environment interaction (GxE) tells that strains respond differently to changes in environmental/ climate parameters. Existence of such variation may hinder the optimal realization of genetic gain and affects the competitiveness of aquaculture industry. However, estimates of GxE are lacking for many economically important traits (Sae-Lim et al, 2016), and need to be assessed to enable optimization of breeding programs towards development of robust genetic material for future conditions. Climate changes and increased water temperature may cause higher risk for certain disease outbreaks in aquaculture (Towers, 2015, Khaw et al., 2019), and consequently selective breeding for better robustness is of interest. The aim of this work is to assess the genetic variation for climate resilience in growth traits in A. salmon.

Materials and Methods

Two parallel experiments were conducted at Austevoll, Institute of Marine Research (as SOUTH station) and Sauaneset locality of Gildeskål Research station, GIFAS, (as NORTH station). A total of 2,190 PIT tagged fish from 75 families from Benchmark Genetics Norway population yearcalss 2019 were transported to the experiment stations at the end of October 2019 and transferred to sea cages. There were two cages at each experiment station and the fish were fed the same 'conventional' fish diet. Environmental parameters such as temperature, salinity and oxygen, mortality, lice count, and wound were recorded weekly during the experiment period. Experiment at the SOUTH station was terminated at mid May 2020 and the NORTH station was terminated at early August 2020. At termination, body weight, length, wound and deformities, and sexual maturity were recorded.

In addition to body weight, thermal growth coefficient (TGC = [(end_weight^1/3) – (initial_weight^1/3)]/day*degree) was calculated and used as phenotypes. Multivariate model treating phenotypes at each experiment location as different traits was fitted to estimate the genetic parameters using restricted maximum likelihood in BLUPF90 (Misztal et al., 2018). Genomic relationship matrix was used in the estimation.

Results and Discussion

The mortality rate and average body weight at termination were 19.4% and 965g at the SOUTH station, and 18.6% and 1580g for the NORTH station, respectively. The monthly temperature variation at each station during the experiment are presented in Figure 1.

Heritability estimates for body weight at the different location ranged from moderate to high (0.39 – 0.62, Table 1). These estimate for the thermal growth rate (TGC) were also ranged form moderate to high (Table 1). Full-sib group effect (c^2) were not significant for any of the traits and not presented here. Genetic correlation estimate was very high between body weight measured at the two locations (0.99, Table 1). The genetic correlation for TGC was also very high but lower than that of end weight (0.88, Table 1)

The results showed there is limited re-ranking for growth, which indicates that either there exits limited genetic variation in climate change resilience, or the current breeding population is robust towards climate change.

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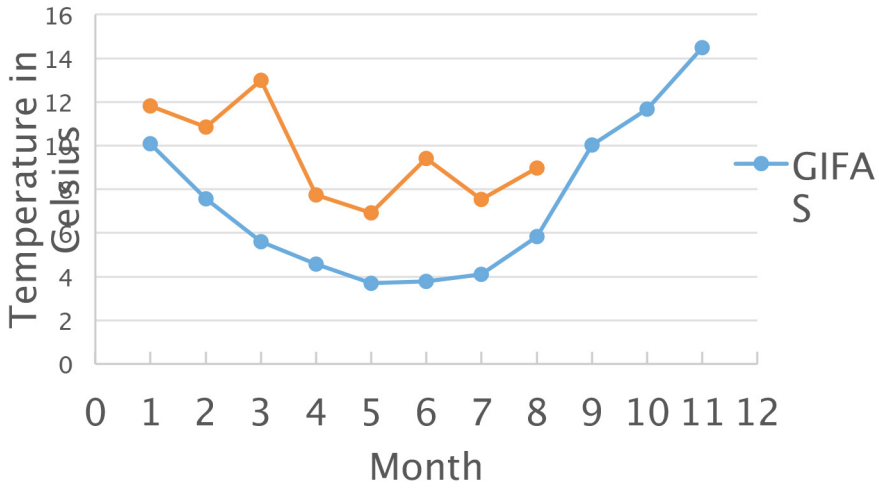

Figure 1:Average monthly temperature measurements in ºC at the experimental locations. GIFAS is the NORTH location and IMR is the SOUTH location.

Table 1: Heritabilities and genetic correlations for end weight (EW) and TGC measured at different locations.

	End weight (EW)		TGC	
Traits	SOUTH	NORTH	SOUTH	NORTH
SOUTH	0.62±0.03		0.60±0.04	
NORTH	0.99±0.06	0.39±0.02	0.88±0.08	0.49±0.03

References

Khaw, H. L., S. A. Boison, L. Johansen, M. W. Breiland, P. Sae-Lim. (2019). Genetic variation for thermal sensitivity in growth and lice tolerance of Atlantic salmon. Annual EAAP2019

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

Sae-Lim P, Gjerde B, Nielsen HM, Mulder H and Kause A (2016). A review of genotype-by-environment interaction and microenvironment sensitivity in aquaculture species. Reviews in Aquaculture 8 (4), 369-393

Towers L. (2015). Temperature a Big Factor in Vibriosis, Photobacteriosis Outbreaks in Greek Sea Bass. The Fish Site

ESTABLISHING STANDARD OPERATING PROCEDURES FOR PURGING OFF-FLAVOR FROM RAS-PRODUCED ATLANTIC SALMON *(Salmo salar)*

J. Davidson*, S. Summerfelt, C. Grimm, G. Fischer, and C. Good

The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV, USA 25443
Email: jdavidson@conservationfund.org

Introduction

A common challenge encountered when producing Atlantic salmon (*Salmo salar*) in recirculating aquaculture systems (RAS) is the tendency for fish to bioaccumulate earthy and musty off-flavors associated with the microbial metabolites, geosmin (GSM) and 2-methylisoborneol (MIB), respectively. At present, the only consistently proven method to remediate off-flavor from RAS-produced salmon is relocation of fish to separate "purge" or depuration systems where high volumes of water, low or void of off-flavor are exchanged while withholding feed. The Freshwater Institute has carried out several experiments examining variables and procedures to optimize the depuration process. Early research evaluating the effects of water aeration media and pre-disinfection of depuration systems with hydrogen peroxide will be summarized, and recent published and unpublished work assessing the effects of depuration system hydraulic retention time (HRT), fish swimming speed, and dissolved oxygen concentration on off-flavor remediation will be described in detail.

Materials and methods

Study 1 - Market-size Atlantic salmon (~6 kg) originally cultured in a semi-commercial scale freshwater RAS tank (150 m^3) were moved to a separate system and exposed to a concentrated geosmin solution to boost flesh concentrations before the trial. Thereafter, the fish were randomly stocked into twelve independent partial reuse systems (PRAS) (26 fish/tank) which had been cleaned and pre-disinfected with 250 mg/L hydrogen peroxide. Three flushing rates (37.8, 19.6, and 7.8 L/min) were applied to randomly selected PRAS by adjusting makeup water flow rates. These conditions resulted in mean system HRTs of 2.4, 4.6, and 11.3-h, respectively (N=4). Feed was withheld over a 10-day depuration period, and a representative number of fish were collected and filleted on Days 0, 3, 6, and 10 post-stocking for subsequent analysis of geosmin levels in fish flesh. Water samples were also collected from each PRAS following the same sampling regimen. Off-flavor analyses generally followed methods described by Lloyd et al. (1998) and Lloyd and Grimm (1999).

Study 2 - A second trial was carried out using similar base procedures (replicate systems, Atlantic salmon cohort, geosmin boosting), but with a 2 x 2 factorial design evaluating the effects of dissolved oxygen concentration and fish swimming speed created by adjusted water velocity on off-flavor remediation. Different combinations of dissolved oxygen (90 or 100% saturation) and fish swimming speed (0.6 or 0.3 body lengths/sec) were applied (N=3), and off-flavor concentrations were measured in water and fish flesh using a similar sampling schedule and analytical methods as used during the first trial.

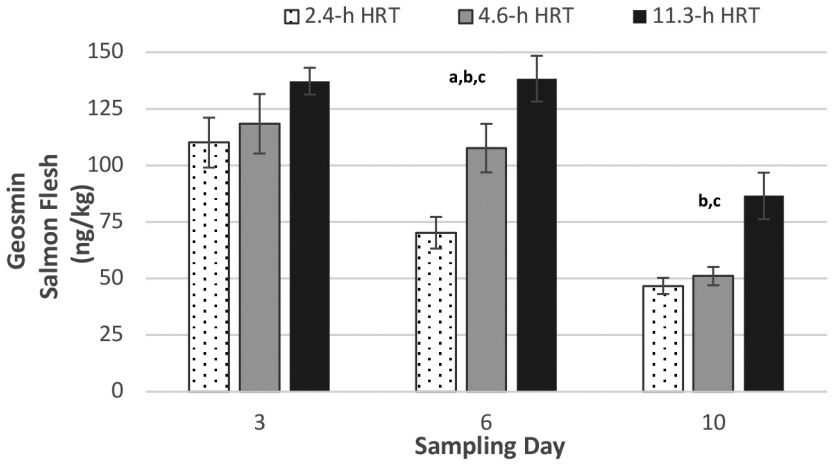

Figure 1. Mean GSM concentrations (ng/kg; mean ± standard error) in Atlantic salmon flesh after 3, 6, and 10 days of depuration in replicate partial reuse systems (N=4) operated with various hydraulic retention times. Significant differences: [a] - 2.4-h and 4.6-h HRT; [b] - 2.4-h and 11.3-h HRT; [c] - 4.6-h and 11.3-h HRT (Davidson et al., 2020)

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Results and discussion

During Study 1, waterborne GSM concentration was affected by depuration system flushing rate and associated system HRT ($P < 0.05$). Depuration systems operated with an 11.3-h HRT had higher waterborne GSM levels at days 3, 6, and 10 post-stocking compared to the 2.4 and 4.6-h HRT treatments, and a similar trend was observed in Atlantic salmon flesh. Overall, this research demonstrated that lowest residual GSM is achieved in depuration system water and Atlantic salmon fillets when increased water flushing and shorter system HRT are applied, i.e., 2.4 to 4.6-h under the described study conditions. It is important to note that Atlantic salmon exposed to each water flushing treatment were likely "on-flavor" after ten days of purging. With this in mind, selection of optimal depuration system flushing rate within this range may be partly dictated by site-specific water use and availability metrics.

Results and data analysis from Study 2 are still under evaluation but will be presented during the meeting.

References

Davidson, J., Grimm, C., Summerfelt, S., Fischer, G., Good, C., 2020. Depuration system flushing rate affects geosmin removal from market-size Atlantic salmon *Salmo salar*. Aquacultural Engineering 90, 101214.

Lloyd, S.W., Lea, J.M., Zimba, P.V., Grimm, C.C., 1998. Rapid analysis of geosmin and 2-methylisoborneol in water using solid phase micro extraction procedures. Water Research 32, 2140-2146.

Lloyd, S.W., Grimm, C.C., 1999. Analysis of 2-methylisoborneol and geosmin in catfish by microwave distillation - solid phase microextraction. Journal of Agricultural and Food Chemistry 47, 164-169.

NON INVASIVE IN-SITU RAPID TEST TO DETECT THE PRESENCE OF THE NEMATODE PARASITE, *Anguillicola crassus*, IN THE EUROPEAN EEL, *Anguilla anguilla*

De Noia M.*, Kaufmann J., Poole R. , McGinnity P., Llewellyn M.

University of Glasgow, Institute of Biodiversity Animal Health and Comparative Medicine, G12 8QQ, Glasgow, UK
E-mail: Michele.DeNoia@glasgow.ac.uk

Anguillicola crassus is an invasive nematode parasite of the European eel, *Anguilla anguilla*, and one of the primary drivers of eel population collapse. The presence of the parasite has been shown to impact many features of eel physiology and life history. Early detection of the parasite is vital to limit the spread of *A. crassus*. However, until recently accurate diagnosis of infection could only be achieved via terminal dissection. To support *A. anguilla* fisheries management in the context of *A. crassus* we developed a rapid non-lethal and non-invasive environmental DNA method to detect the presence of the parasite in the swim bladder. Screening of 128 wild eels was undertaken in 2017 and 2019 at the Burrishoole Catchment, County Mayo, Ireland to validate the procedure. DNA extractions and PCR were conducted *in situ* using Whatman™ FTA™ cards and a miniPCR DNA Discovery System™ with species specific primers were designed from the cytochrome oxidase mtDNA gene region. Primer were tested against a series of taxa to confirm their specificity. No a-specific amplification was detected after the testing PCR. The procedure took 2h 30 minutes for up to 24 individuals. Specificity and sensitivity testing of the diagnostic procedure demonstrated Positive Predictive Values at 96% and Negative Predictive Values at 87%. Zero inflation GLMM corroborates that the parasitic load increases with the length of the animal instead the most infected eels present an average fat content (AIC=488 and LolLi= -236). Longer animals are more likely to encounter the parasite during the yellow fresh water stage, increasing the risk of infection and reducing their fat accumulation process. Our method will be a powerful tool in the hands of fisheries managers to help protect this iconic but critically endangered species.

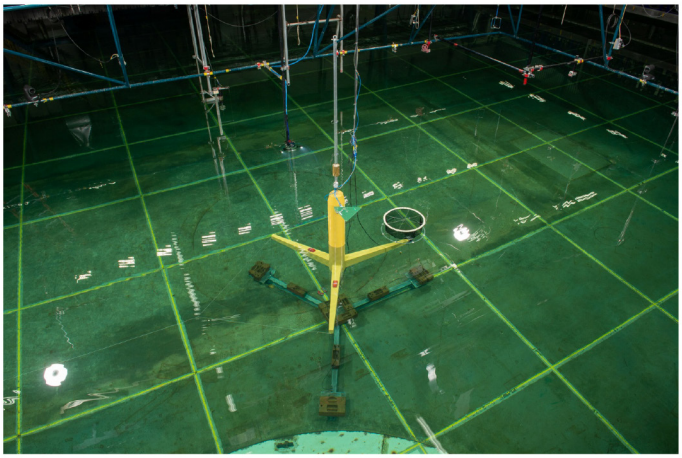
DESIGN AND MODEL TEST VERIFICATION OF NOVEL OPEN OCEAN FISH FARM FOR DUAL USE WITH OFFSHORE FLOATING WIND TURBINE

Jaap de Wilde (MARIN)
Clemens van der Nat (Bluewater), Leo de Vries (Bluewater) & Thomas Spanjaard (Jumbo Offshore)

A novel design for a large size open ocean fish farm for dual use with offshore floating wind turbines is presented. The design consists of a large diameter cylindrical fish cage for open water aquaculture which is moored to a Tension Leg Platform (TLP) design for offshore floating wind turbines. The TLP has three deeply submerged pontoons and can support three large fish cages of 16 m diameter each. Initially one TLP with one fish cage can be tested at a suitable test site in open ocean, but the future ambition is to utilize the technology for large size offshore wind farms with dozens or several hundreds of wind turbines and fish cages. Multiple use of the open sea requires innovations to allow platforms to be used for different activities. The TLP is a strong and viable floater technology for support of large offshore wind turbines. Typically, in offshore wind farms other activities like trawler based fishing is not allowed. Aquaculture may provide a commercial alternative to the fishery sector. By providing the deeply submerged TLP pontoon legs as mooring point for the fish cages, the fish farm can also be located at deep water offshore sites, without the need for heavy and expensive moorings.

The new design was established with a strong drive towards real commercial application in near future. In fact, the presented design of both the fish farm and the offshore floating wind turbine can already be deployed now as a fully operational prototype in relatively benign ocean conditions with significant wave heights up to Hs 5.5 m and wind speeds up to 32 m/s for 50-year return period. The TLP design for the offshore floating wind turbine is considered a particularly suitable solution for deeper waters between 50 to 200 m.

The paper will discuss the underlying ideas leading to the presented dual use solution, with a focus on the motions of the fish cage, the relative motions between the fish cage and the TLP, the forces in the single point mooring system and the local deformations of the fish cage. The design of the fish cage and the TLP was first rigorously tested in time domain simulation software and later verified by means of model tests in the large offshore wind, wave and current test basin of MARIN. Results of the time domain software as well as the wave basin model tests will be presented in the paper. Finally some word will be dedicated to the intended method for installation of the TLP and the fish cage.



Model test of fish cage and TLP in MARIN wind, wave and current basin

ADVANTAGES ON THE USE OF ARTEMIA IN THE LARVAL REARING PROTOCOLS OF THE 2 MAIN MEDITERRANEAN AQUACULTURE SPECIES SEABREAM (*S. aurata*) AND SEABASS (*D. labrax*)

T. De Wolf [(1*)], G. Franchi[(1)], L. Chiappi[(1)], S. Lenzi[(1)], 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: t.dewolf@inveaquaculture.com

Introduction

The use of *Artemia* has enabled commercial marine fish production to grow to the industrial levels we are at today. In the Mediterranean area, the annual production of Seabass and Seabream has increased from 400 to 1,200 million fry during the last 2 decades (CAGR of 5.95% in average). During this period, different alternatives for *Artemia* have been tested and evaluated in adjusted and optimized feeding protocols, reducing the amount of *Artemia* consumed per million fry produced. Yet until today it is clear that *Artemia* is of vital importance for the larvae to allow them to develop and grow into high-quality fry. This study is showing the results obtained using different amounts of *Artemia* together with high-quality larval diets applied in Seabream and Seabass commercial rearing protocols, showing the effects on survival, larval growth and fry quality.

Materials and methods

Seabass experiment

Nearly hatched Seabass larvae, originating from the same pool of eggs, were stocked at the same density (50-75larvae.l^{-1}) in 6,000l larval rearing tanks. At 25dph, the larvae were transferred to 10,000l tanks, diluting the original population. Three different feeding protocols were used, changing mainly the quantity of *Artemia* nauplii fed to the larvae. The live food control (LFC) was fed the normal quantities of *Artemia*. In the partial *Artemia* replacement treatment, the *Artemia* amount was reduced with 70-72% and no *Artemia* at all was fed in the total *Artemia* replacement protocol. The production scale trial was done in duplicate, stocking over 2 million of eggs. During the larval rearing period, growth was followed as well as the quality of the fry (deformities and stress resistance). At 56dph, larval survival and biomass produced per tank was evaluated and *Artemia* consumptions were calculated.

Seabream experiment

A similar experiment was set-up in duplicate using more than 4 million Seabream eggs. The stocking density was around 100larvae.l^{-1} and larvae were transferred from 6 to 10,000l tanks at 30dph. Partial *Artemia* substitution was done, reducing the normal *Artemia* consumption with almost 90%, next to a full *Artemia* replacement protocol. The same parameters were evaluated as in the Seabass trial.

Results

In the Seabass experiment, the growth of the larvae in the partial *Artemia* substitution trial was slightly lower compared to the Live food Control (LFC) while the growth in the 100% *Artemia* Substitution (AS) was significantly lower. Average survival rate at 56ph was highest for the LFC (47%), slightly lower for the partial AS (42%) and lowest for the full AS (32%). Therefore, the produced tank biomass at 56dph was highest for LFC, 25% less for the partial AS and 70% lower in the full AS. The average *Artemia* consumption was 117kg.million^{-1} fry for the live food control, 32kg. million^{-1} fry in the partial AS and 0kg. million^{-1} fry in the total AS treatment. Regarding the stress resistance, the LFC has always shown the best resistance. Few differences were noted between the partial and full AS. Early deformity levels showed a higher percentage of head and spinal deformities in the partial and full AS treatments compared to the LFC.

In the Seabream experiment, similar survival rates were obtained between the 3 treatments but also for this species the differences in growth were visible: in the partial and full AS, growth was almost stagnant between 35 and 42dph and caught up later. There were no big differences in growth between the partial and full AS. But the reduction of *Artemia* in the partial AS treatment was very drastic, reducing the amounts with more than 90%. The final tank biomass at 56dph was 25% lower for the partial AS and 40% lower for the full AS compared to the LFC. Regarding the stress resistance, Seabream larvae under partial or complete AS regimes scored similar to the LFC at 30dph. Only at 56dph, the LFC scored better. Deformity levels were low in general, but a higher incidence of operculum deformities was observed in the AS treatments compared to the LFC.

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Conclusions

Based on this study it became clear that *Artemia* is still an important component in the Mediterranean larval rearing protocols. Even if important progress has been made over the years, the production output following protocols with strongly reduced amounts of *Artemia* is still inferior when compared to a larval rearing protocol that includes the normal amounts of *Artemia*. Differences are mainly observed in growth rate, survival and stress resistance, confirming *Artemia* as an essential live prey in the production of high-quality Seabass and Seabream fry.

TRACING THE GEOGRAPHICAL ORIGIN OF THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) IN FOOD AUTHENTICATION USING STABLE ISOTOPE AND TRACE ELEMENT ANALYSIS

Ane del Rio[1*], Jan Weber[2], Joachim Molkentin[3], Elisa Jiménez[1], Miguel Ángel Pardo[1]

[1]AZTI- Tecnalia, Food Research Unit, Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609 E-48160, Spain
[2]National Reference Centre for Authentic Food, Max Rubner-Institut, Hermann-Weigmann-Str. 1, 24103, Kiel, Germany
[3]Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institute, Hermann-Weigmann-Str. 1, 24103 Kiel, Germany

Email: adelrio@azti.es

Introduction

The global production of molluscs, mainly bivalves, reached 17.7 million tonnes (USD 34.6 billion) in 2018 with consistent growth in production (FAO, 2020). Among them, mussels are an important product, which is globally traded and with strict requirements and standards, particularly when entering the European Union (EU) market. On a global scale, Europe is a major producer of mussels, supplying over a third of the total production, with *Mytilus edulis* and *M. galloprovincialis* being the two main species harvested (FAO, 2019).

Considering the economic relevance of this extensively farmed species and its importance in international trade, the verification of the geographic origin is necessary for labelling, traceability and food safety purposes (Luque and Donlan, 2019). Among the exiting methods for seafood traceability, stable isotope and elemental fingerprinting have been recognized as useful origin discriminants (Li et al., 2016). In the case of mussels, stable isotope ratio analysis (SIRA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has been used to trace their origin (Deudero et al., 2009; Zhao et al., 2019). Additionally, trace element fingerprinting (TEF) has also been successfully applied to trace the geographical origin of bivalves (Dunphy et al., 2015; Ricardo et al., 2015; Bennion et al., 2019; Morrison et al., 2019).

Moreover, the combination of both methodological approaches, organic stable isotope and inorganic trace element analysis, is an innovation in seafood authentication. In this sense, we present a combined study of stable isotope ratio and trace element analysis for the authentication of the geographical origin of *M. galloprovincialis* samples from the Mediterranean Sea, the European Atlantic coast and the Chilean Pacific coast.

Material and Methods

Mytilus galloprovincialis samples for SIRA and TEF were collected between autumn 2018 and autumn 2019. Samples were obtained from 11 different locations in 7 different countries namely Spain, Portugal, France, Italy, Ireland, Tunisia, and Chile. The sample set for SIRA comprised 183 individual mussels, whose tissue (without digestive gland) was dried and mortared before stable isotope analysis. Simultaneous $\delta^{13}C$ and $\delta^{15}N$ measurements were performed using a Flash EA 1112 elemental analyser coupled to a Delta Plus XL isotope-ratio mass spectrometer (Thermo Scientific, USA) according to Molkentin 2018. For TEF analysis, a total of 106 mussel shells were processed following a methodology similar to previous studies (Ricardo et al., 2015; Bennion et al., 2019). After organic tissue removal, microwave-assisted digestion procedure was carried out. The quantitative analysis of trace metals (B, Al, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Ba, Pb) was performed by using ICP-MS (7700x, Agilent Technologies, USA). All statistical analyses were performed using R software. PERMANOVA was used to test the significance of differences in isotopes and trace elements. Differences in sampling points were examined using non-metric multidimensional scaling (nMDS). To test whether isotope and element fingerprinting could be used to successfully assign samples to their harvesting locations, random forest (RF) classification method was used.

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Results

Significant differences were recorded among locations for both methodological approaches. The RF analysis revealed that the combination of stable isotope ratio and trace element analysis was able to assign significantly the samples to each respective farming location, with successful classification in 97% of samples. Most relevant elements for provenance discrimination were $\delta^{15}N$, Pb, $\delta^{13}C$, Ba, Mn, Zn and Al.

Conclusions

In conclusion, this study reveals that the combiantion of stable isotope ratio and trace element analysis is an effective technique for the authentication of the geographical origin of *M. galloprovincialis* mussels farmed in Mediterranean Sea, the European Atlantic coast and the Chilean Pacific coast. The technique described here provides a reliable traceability tool applicable for labelling and seafood safety purposes.

Acknowledgments

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References

Bennion M, Morrison L, Brophy D, Carlsson J, Abrahantes JC and Graham CT (2019). "Trace Element Fingerprinting of Blue Mussel (*Mytilus Edulis*) Shells and Soft Tissues Successfully Reveals Harvesting Locations." Sci Total Environ 685: 50-58.

Deudero S, Cabanellas M, Blanco A, & Tejada S (2009). Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis*. Journal of Experimental Marine Biology and Ecology, 368(2), 181–188.

Dunphy B, Silva C and Gardner JPA (2015). "Testing Techniques for Tracing the Provenance of Green-Lipped Mussel Spat Washed up on Ninety Mile Beach." New Zealand Aquatic Environment and Biodiversity Report No. 164.

FAO (2019). "GLOBEFISH - Analysis and information on World Fish Trade: New product development for bivalves" FAO. http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1189970/

FAO (2020). "The State of World Fisheries and Aquaculture 2020". FAO. https://doi.org/10.4060/ca9229en

Li L, Boyd CE and Sun Z (2016). "Authentication of Fishery and Aquaculture Products by Multi-Element and Stable Isotope Analysis." Food Chem 194: 1238-44.

Luque, GM, and Donlan, CJ (2019). "The characterization of seafood mislabeling: A global meta-analysis." Biological Conservation, 236, 556–570.

Molkentin, J (2018). "Detection of Leguminous Protein in Casein by Nitrogen Stable Isotopes." Journal of Food Research 7, 1-7.

Morrison L, Bennion M, Gill S and Graham CT (2019). "Spatio-Temporal Trace Element Fingerprinting of King Scallops (*Pecten Maximus*) Reveals Harvesting Period and Location." Science of the Total Environment 697.

Ricardo F, Genio L, Costa Leal M, Albuquerque R, Queiroga H, Rosa R and Calado R (2015). "Trace Element Fingerprinting of Cockle (*Cerastoderma Edule*) Shells Can Reveal Harvesting Location in Adjacent Areas." Sci Rep 5: 11932.

Zhao X, Liu Y, Wang G, Tao W, Lou Y, Li N, & Liu Y (2019). Tracing the geographical origins of Yesso scallop (*Patinopecten yessoensis*) by using compound-specific isotope analysis: An approach for overcoming the seasonal effect. Food Control, 102, 38–45.

RESPONSIBLE PRODUCTION FOR SHRIMP (*Penaeus vannamei*) IN ECUADOR – HOW MR.GOODFISH PROGRAMME AND EARTHWORM FOUNDATION WORK WITH TO PROPOSE SUSTAINABLE SHRIMP TO FRENCH MARKET ?

J. Delettre*, F. Girard, F. Huron Blond, F. Loth Ovine, K. Marcano, P. Vallette

Nausicaá, Centre National de la mer, Boulevard Sainte Beuve, 62203 BOULOGNE SUR MER
Email : mrgoodfish@nausicaa.fr

Who is Mr.Goodfish ?
Launched in March 2010 by three major european aquaria – Nausicaá National sea center, France, Acquario di Genova, Italy, and aquarium Finisterrae, Spain – Mr.Goodfish is a programme created to raise awareness of the importance of the sustainable seafood consumption among the general public and professionals.

The programme intends to stimulate the active engagement of consumers to preserve marine resources by publishing, for each season, a list of seafood products recommended by an expert committee : scientists, regional fisheries committee, chefs, consumer associations, retailers, wholesalers, etc.

The programme is based on the entire fishing industry. It includes fishermen, wholesalers, but also restaurant owners, fishmongers and supermakets, who play a key role in the buying process.

Today, more than 2,500 professionals have joined the Mr.Goodfish programme. A variety of tools have been developed to help them to communicate about the programme: leaflets, slates, recipes, newsletters...

The programme is also advising on responsible farmed seafood : through technical specifications on feed / breedind practices / impact on ecosystem.

Who is Eartworm Foundation ?
Earthworm Foundation is a non-profit organisation who helps companies transforming their value chain. Earthworm Foundation is driven by the desire to positively impact the relationship between people and nature.

Founded in 1999, Earthworm Foundation have an expertise on social and environmental issues. With most of their staff operating directly on the ground where the issues are, they work with their members and partners (over 100) to make value chains an engine of prosperity for communities and ecosystems. They work across 5 continents.

In fact, they see a world where forests are a boundless source of materials and a home for biodiversity; communities see their rights respected and have opportunities to develop; workers are seen as productive partners; and agriculture becomes the instrument to feed a hungry planet and keep our climate stable.

Abstract :
The Ecuador farming shrimp on French market represents 41% of the total shrimp importation. To develop a sustainable supply in France, Earthworm Foundation and Mr.Goodfish had join their expertise. The aim of this alliance is to develop a responsible industry in Ecuador by co-build a code of conduct to identify best practices and support farm improvements. Earthworm Foundation have a team working on the ground and checking the respect for communities and workers, the preservation of ecosystems and the environmental practices, and the breeding conditions of *Penaeus vannamei*.
In parallel, 3 areas on which we want to develop specific and deeper projects :
- Deforestation and restauration of mangroves
- Traceability and small producers resilience
- Shrimp feed : delink from marine resources overexploitation and deforestation.

The aim is to establish a code of conduct as guidance to improve practices with the technical expertise based on the Mr.Goodfish programme for shrimp in Madagascar (*Penaeus monodon*) on breeding practices, ecosystem impacts, feed and social impact.

DISTINGUISHING THE IMPACTS OF REARING DENSITY VERSUS TANK VOLUME ON THE SKELETAL QUALITY AND DEVELOPMENT OF *Sparus aurata* DURING THE HATCHERY PHASE

Z. Dellacqua*[1,3], C. Di Biagio[1,4], A. Martini[2], M. Izquierdo[3], 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', Via Cracovia Rome (Italy)
[3] Aquaculture and Marine Ecosystems Department, University of Las Palmas de Gran Canaria Parque Científico Tecnológico Marino de la ULPGC. Carretera de Taliarte S/N. 35200-Telde, Gran Canaria (Spain)
[4]Laboratory of Evolutionary Developmental Biology, University of Gent, Belgium
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. Data from 2017 and 2018 has revealed increased production while the price of seabream and seabass have been in decline due to the oversupply. 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 (⌀>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 juveniles. The design envisages to test the effects at a commercial scale of: A) larger and smaller tank volumes on seabream larvae, stocked at the same density; and B) higher and lower stocking densities on seabream larvae maintained in the same tank volume.

Materials & Methods

Experimental rearing was conducted in the EcoAqua facilities at the University of Las Palmas, Gran Canaria (Spain). Following a spawning event from seabream brooding tanks containing 23 females and 25 males of High Growth Fish-oil fed broodstock, eggs were collected and incubated for 24hrs in a mesh net held in 500L tanks with an elevated renovation of seawater (125L/h). Eggs were then transferred at 3 different densities (Low Density (LD): 25 eggs/L; Medium Density (MD): 100 eggs/L; High Density (HD): 250 eggs/L) into three 500L tanks (Small Volume) and three 1000L tanks (Large Volume). Natural seawater was pumped into the system at a rate of 25% replacement/h, after the first feeding. Photoperiod was based on natural light. Larvae were reared with the 'Green water' technique (phytoplankton *Nannochloropsis spp*., rotifers *Brachionus sp*. and *Artemia sp*. nauplii) until 25dph when fish were weaned to a Gemma microdiet feed (Skretting, France) and fed *ad libitum* hourly during the daylight hours. Throughout experimental rearing, tanks had an average water temperature of 22.4°C and average Dissolved Oxygen was 5.4 (mg/L) which was regularly monitored in order to maintain a constant saturation level above 70% SAT. Seabream were reared up to ~60dph in order to be sure that the complete skeletal mineralization had occurred. Juveniles were euthanized with an overdose of clove oil, 100 individuals/condition were photographed and measured (length and weight); other 300 individuals/condition were fixed (1.5% PFA & 1.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4) for subsequent anatomical analyses.

Samples were whole-mount stained with Alizarin red (Park and Kim; 1984). Mass monitoring of meristic counts and skeletal anomalies was conducted using an AxioZoom V.16 (Zeiss) stereomicroscope. Data on skeletal anomalies was recorded following an adapted alphanumeric code from Prestinicola et al. (2013).

All statistical analyses and graphs were done using Microsoft Excel and Past 4.02.

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Results

Obtained data validates that juveniles reared in the LD conditions (both 500L and 1000L) had a significantly greater total length, wet and dry weight than seabream from the other rearing conditions ($P<0.05$, Kruskal-Wallis, Dunn's post hoc with Bonferroni correction). Both of the LD conditions also had the greatest survivorship (at 60dph), as well as the lowest malformative index and frequencies of severe anomalies affecting the vertebrae. The highest frequency of anomalies affecting the vertebral column was found in the HD 500L condition while the lowest frequency was found in the LD 1000L condition. From this data it can be inferred that the extreme density conditions (LD and HD) undoubtedly impact the development of seabream, leading to considerably different effects on their size, survivorship and skeletal quality, regardless of tank volume.

On the other hand, samples from the MD conditions do indeed suggest a 'tank volume' effect. While the initial densities were the same, a greater survivorship in the large volume (MD 1000L) was recorded. This consequentially entails that the densities in MD 500L and MD 1000L were not the same. The lower density established in MD 500L, could explain the fact that the specimens' length and weight measurements were greater in MD 500L than in the MD 1000L. Similarly, the malformative index and the frequency of severe anomalies affecting the vertebral column was greater in the MD 1000L condition.

This preliminary data suggests that reducing the stocking density, rather than scaling up the tank volume can effectively enhance the quality of hatchery reared seabream fry.

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References

Boglione, C., et al. (2009). *Aquaculture*, *291*(1-2), 48-60.

Koumoundouros, G., et al. (2004). *Aquaculture*, *240*(1-4), 165-173.

Llorente, I., et al. (2020) *Marine Policy*, ISSN 0308-597X (online), 117, 2020, p. 103876, JRC117066.

Park, E. H., & Kim, D. S. (1984). *Stain Technology*, *59*(5), 269-272

Prestinicola, L., et al. (2013). *PLoS One*, *8*(2), e55736.

Scientific, Technical and Economic Committee for Fisheries (STECF) (2018). ISBN 978-92-79-79402-5, doi: 10.2760/45076, JRC114801.

IMPORTANCE OF DISPERSAL MODELLING TOOL FOR SHELLFISHERIES AQUACULTURE

J. Demmer*; P.E. Robins; A.J. Goward Brown; S. Ward; S.P. Neill and S. Jenkins

Address: School of Ocean Sciences, 4 Askew St, Menai Bridge LL59 5EG
Email: osp816@bangor.ac.uk

Introduction

Shellfish production in Wales (mussels, oysters, clams, scallops) represents £12.5M and the UK government want to double aquaculture production by 2030. The blue mussel (*mytilus edulis L.*) represents between 40 to 50 % of the total gross turnover of Welsh shellfish industries, which have been operating sustainably for 50 years. Most of this aquaculture is based on wild spat collection, which are harvested to then be seeded in suitable areas. Consequently, it is both of scientific and economic interest to study the mechanisms experienced by mussel larvae from the release of the gametes to the settlement of juveniles. The complex interactions between physical processes (tidal and wind-driven currents) and biological processes (e.g. pelagic larval duration (PLD)) need to be better understood to characterise population dynamics and to help shellfisheries companies manage their stock sustainably and assess their contribution to the ecosystem. Within the ECOSTRUCTURE project, we developed biophysical modelling techniques to help predict and understand the dispersal of marketable marine organisms in the Irish Sea. The tool offer the possibility to define the likelihood dispersal: 1) for different period of release (e.g. spring, summer and autumn); 2) for different pelagic larvae duration; and 3) from different release sites (6974) along the Irish Sea coast. Here we will outline how the results from the biophysical model experiments will be used in a rapid-response tool that can be used by stakeholders to collect species and manage their stock efficiently.

Material and methods

A hydrodynamic approach using Roms 3D model has been applied for the year 2014 coupled with a particle tracking model (PTM) developed with Python. The spatial resolution of the hydrodynamic model is 1000 m and output (velocity) were recorded hourly. Particles were released from the coast, which surrounds the Irish Sea, every 1 km, which represents 6974 particles. Fifteen-release time were applied separate by 24 hours. The larvae were released for a PLD of two month. Every particle position were recorded until the end of simulation. No mortality was considered as this would reduce the number of samples for statistical analysis, and because there is insufficient information of mortality rates during the larval phase. Furthermore, if particles were advected onto land, they were reflected back to their previous position.

Results

Results highlight the importance of: 1) the period of release; and 2) the area of release (Figures 1 and 2, respectively). The example of Isle of Man showed that a spawning event occurring at two weeks interval dispersed larvae differently. Indeed, larvae released the 1st of March (Figure 1.A) showed a higher dispersal than larvae releases two weeks later (Figure 1.B). Indeed, the dispersal is higher in term of distance from the release site, but also in terms of space occupation. The figure 2 highlights that released sites selected can either present similar dispersal (Figures 2.B and 2.C) and different dispersal (Figures 2.A and 2.C).

Discussion

The management of shellfisheries aquaculture based on wild spat is dependent on several factors such as larval recruitment and weather condition. Indeed, farmers experienced some year with high recruitment of larvae and other with no recruitment (Pers. Comm. with Trevor Jones; observation made between the year 2014 and 2018). It is essential for this industry to be able to predict where they can find spat from a year to another. Dispersal modelling tool presented here showed the importance of both period of release and area of release on larval dispersal and can be used by stakeholders. Furthermore, with the increase of need for renewable energy and the anthropic pressure on coastal environment, this tool will be useful to define the pathway of larvae from different species and study the interaction with offshore structure and possibly develop multi-use platforms at sea.

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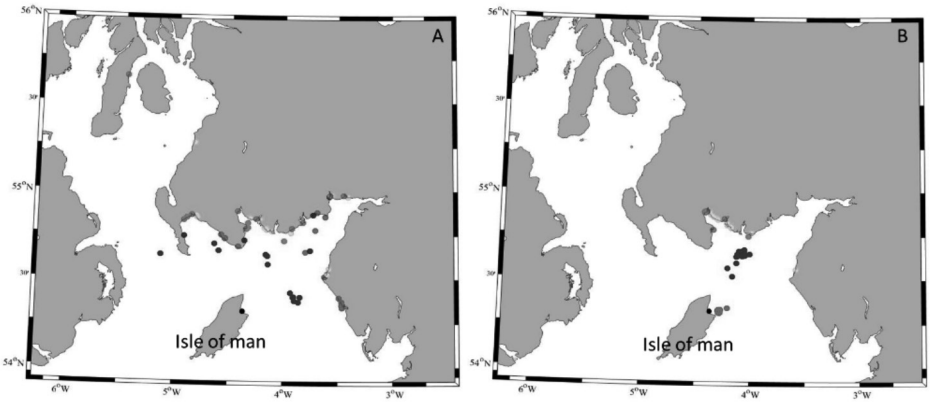

Figure 1: Position of larvae released form the Isle of Man (black dot) after 2 weeks (red dots), 4 weeks (blue dots), 6 weeks (green dots) and 8 weeks (magenta dots). Particles were released the 1/03/2014 (A) and 15/03/2014 (B)

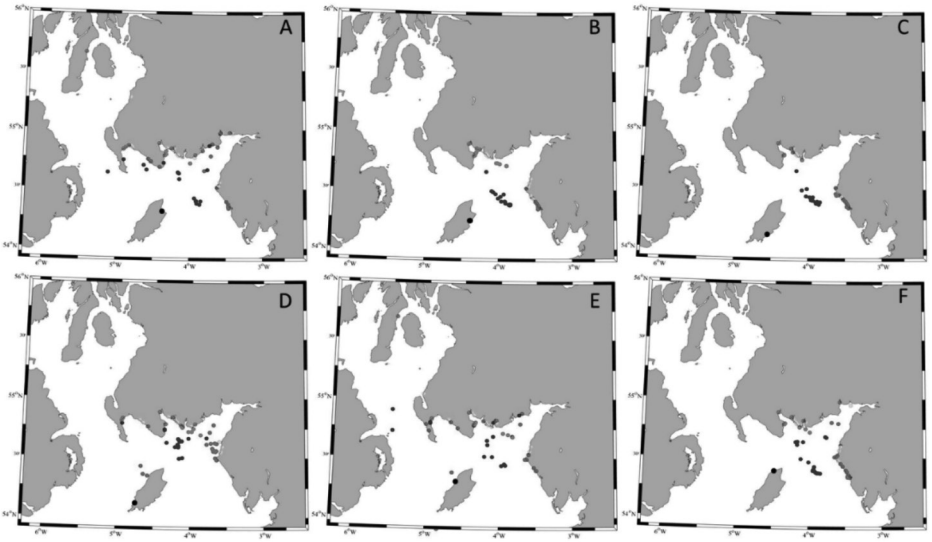

Figure 2: Position of larvae released form the Isle of Man (black dot) after 2 weeks (red dots), 4 weeks (blue dots), 6 weeks (green dots) and 8 weeks (magenta dots). Partilces were released 01/03/2014 from: Ramsey (A); Laxey (B); Port Soderick (C); Dalby (D); Kirk Michael (E) and Smeale (F).

EFFECT OF THE REARING ENVIRONMENT AND DIETARY PROBIOTIC ON THE GROWTH AND GUT MICROBIOTA OF NILE TILAPIA (*Oreochromis Niloticus*) LARVAE

Yale Deng*, Fotini Kokou, Ep Eding, Marc C.J. Verdegem

Aquaculture and Fisheries Group, Wageningen University and Research, The Netherlands
Email: yale.deng@wur.nl

Introduction

Stable and predictable commercial production of high quality juveniles is a bottleneck for many aquatic species. Unpredictability of mortality and individual growth cause low reproducibility in larvae cultivation even under exact the same rearing conditions (Verner-Jeffreys et al., 2004). There is increasing evidence that the observed variation might be caused by interaction between the host larvae and their microbiota (Vdastein et al., 2018). Rearing water and feed inputs are sources of bacteria potentially colonizing the gut (Dehler et al., 2017). In flow-through systems (FTS), the constant inflow of clean water keeps the microbiota density low, while removing feed wastes. This favors the development of opportunistic bacteria after each feeding in FTS. On the contrary, recirculating aquaculture systems (RAS) harbor a matured and stable microbial community (De Schryver and Vadstein, 2014). How different rearing conditions during early-life affect fish growth and gut microbiota composition in Nile tilapia larvae remains unclear. Nile tilapia embryos were incubated until first feeding in either FTS or RAS, and then transferred to aquaria operated as FTS or as part of a RAS and fed a control diet for 25 days. In another recirculating system, the control diet coated with *B. subtilis* was also tested (RASB). *Bacillus* sp. has been widely applied as a probiotic to improve fish performance (Kuebutornye et al., 2019). The effects of the rearing environment and dietary probiotics on the survival, fish growth and gut microbiota were evaluated.

Material and methods

The three rearing systems FTS, RAS and RASB shared the same water supply. The water temperature was maintained at 27°C. Each system contained three replicate 70-L tanks to culture hatched embryos. One batch of 2835 male Nile tilapia embryos (3 day post fertilization, dpf) was subdivided over 3 incubators. At 10 dpf (referred as day 0), swim-up larvae were counted and randomly divided into 3 tanks per treatment connected to the respective rearing system. On day 4, the number of tilapia larvae in each tank was reduced to 200 fish and batch weighted. A commercial tilapia diet (F-0.5 GR Pro Aqua Brut-Trouw Nutrition, France) was used as control diet and the experimental diet was produced by coating the control diet with *B. subtilis* spores at the density of 10^8 CFU g^{-1} feed (Monteiro et al., 2005). On day 25, three fish per tank were randomly collected, anesthetized and the whole gut was removed. The DNA from gut samples was extracted and send for 16S rRNA sequencing. Raw sequencing data was cleaned using package DADA2 in R to get the amplicon sequence variant (ASV) table. The statistical analysis of the gut microbiota was performed by Primer software (Version 6). Principle Coordinate Analysis (PCoA) was conducted based on Bray-Curtis distance. Linear Discriminant Analysis (LDA) was applied to calculate the effect size (LEfSe) of each differentially abundant taxa identified by non-parametric factorial Kruskal-Wallis sum-rank test. The similarity and difference among the different treatments were tested by ANOSIM and PERMANOVA, respectively.

Results

The final survival rate in FTS (62%) was significantly lower ($P < 0.05$) than in RAS (86%) and RASB (90%). There was no treatment effect on the average individual body weight on days 4 and 25, and the specific growth rate (SGR) and apparent feed conversion ratio (FCR) between days 4 and 25. Each treatment exhibited a distinct distribution in the gut bacterial community according to PCoA and ANOSIM (Figure 1). PERMANOVA analysis showed both treatment and tank within treatment had a significant effect on gut bacterial community composition. We further investigated separately the tank effect within each treatment, which revealed no differences between the replicate tanks in RAS and RASB while a tank effect was observed in FTS. Besides, the body weight (BW) and standard body length (SBL) of tilapia were positively correlated to gut microbiota with the two RAS treatments and negatively correlated with the FTS treatment. A total of 39 genera were significantly different in the gut of the three treatments (Figure 2). *Shinella*, *Cetobacterium* and *Bacillus* were enriched in the FTS, RAS and RASB, respectively.

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References

De Schryver, P., Vadstein, O., 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. *ISME J.* 8, 2360–2368. https://doi.org/10.1038/ismej.2014.84

Dehler, C.E., Secombes, C.J., Martin, S.A.M., 2017. Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar L.*). *Aquaculture* 467, 149–157. https://doi.org/10.1016/j.aquaculture.2016.07.017

Kuebutornye, Abarike, E.D., Lu, Y., 2019. A review on the application of Bacillus as probiotics in aquaculture. *Fish Shellfish Immunol.* 87, 820–828.

Monteiro, S.M., Clemente, J.J., Henriques, A.O., Gomes, R.J., Carrondo, M.J., Cunha, A.E., 2005. A Procedure for High-Yield Spore Production by *Bacillus subtilis*. *Biotechnol. Prog.* 21, 1026–1031. https://doi.org/https://doi.org/10.1021/bp050062z

Vdastein, O., Attramadal, K.J.K., Bakke, I., Forberg, T., Olsen, Y., Verdegem, M., Giatsis, C., Skjermo, J., Aasen, I.M., Gatesoupe, F.J., Dierckens, K., Sorgeloos, P., Bossier, P., 2018. Managing the microbial community of marine fish larvae: A holistic perspective for larviculture. *Front. Microbiol.* 9, 1–22. https://doi.org/10.3389/fmicb.2018.01820

Verner-Jeffreys, D.W., Shields, R.J., Bricknell, I.R., Birkbeck, T.H., 2004. Effects of different water treatment methods and antibiotic addition on larval survival and gut microflora development in Atlantic halibut (*Hippoglossus hippoglossus L.*) yolk-sac larvae. *Aquaculture* 232, 129–143. https://doi.org/10.1016/S0044-8486(03)00525-8

MEDAKA, *Oryzias latipes*, A MODEL FOR MARINE REARED FISH SPECIES: EFFECT OF REARING DENSITY AND VOLUME ON THE SKELETON DURING LARVAL REARING

C. Di Biagio[1,3]*, Z. Dellacqua[1,4], A. Martini[2], P.E. Witten[3] and C. Boglione[2]

[1]PhD Course in Evolutionary Biology and Ecology, Biology Dept, University of Rome 'Tor Vergata', Rome (Italy)
[2]Dept. of Biology, University of Rome 'Tor Vergata', Italy
[3] Laboratory of Evolutionary Developmental Biology, Biology Dept, University of Gent, Belgium
[4] Parque Científico Tecnológico Marino de la ULPGC, Gran Canaria – España
Email: claudiadibiagio@gmail.com

Introduction

Zebrafish *(Danio rerio)* and medaka *(Oryzias latipes)* have increasingly gained attention as model fish in the field of skeletal biology. Although evolutionarily related as Teleosts, phylogenetic differences exist between these two taxa. Zebrafish is a basal Teleost with cellular bone, whereas medaka is an advanced Teleost evolved from marine ancestors characterized by acellular bone, this is bone without incorporated bone cells (Boglione et al., 2013, Witten et al., 2017). Consequently, while zebrafish is an appropriate model for studies on basal Teleosts (i.e. Salmonidae), medaka is a more suitable model to tackle unanswered questions related to the prevalence of skeletal anomalies in farmed marine fish species. The aim of this study was to verify the utilization of medaka as a model for studying bone malformations in farmed marine species. This was done by conducting laboratory experiments with medaka, that mimicked experimental conditions in fish farms carried out on Gilthead seabream (*Sparus aurata*). This study tested if the use of larger tank volumes and lower stocking densities can reduce the incidence of skeletal malformations. Laboratory studies with medaka provide the possibility to characterise skeletal malformations in depth, on the level of microstructure, cells, genes, skeletal proteins and cell signalling factors. In addition, it possible to monitor the animal movements, patterns, plasticity and larval behaviour.

Materials and Methods

The effects of density and volume were tested by rearing medaka larvae from hatching to 40dph held at different rearing densities and tank volumes. All of the experimental trials were carried out under standard rearing conditions (T: 26°C, photoperiod: 14H light: 12H dark, fed *ad libitum* with commercial feed ZEBRAFEED® (Sparos, Portugal)). The oxygen levels were maintained between 98-100%. Ammonia, water hardness and pH were monitored and regulated weekly. Three densities were tested in 3L aquaria: low (LD, 5 larvae/L), medium (MD, 15 larvae/L) and high (HD, 45 larvae/L). While the effect of the aquarium volume was tested by rearing larvae at HD in 3L (small volume, SV) and 6L (large volume, LV) tanks. Videos (1hr) of medaka interactions were recorded in the LV and SV tanks every 4 days at 2-3pm. At the end of the experimental rearing the individuals were euthanized with an overdose of MS-222, fixed and whole mount stained with Alizarin Red S. From these individuals, the standard lengths S_L, meristic counts, bone development and skeletal malformations were recorded.

Results

Rearing density had a clear effect on the S_L of the medaka, as also observed in zebrafish (Martini et al. 2020): juveniles from the HD group were significantly smaller and their length measurements are characterized by a wider distribution (Fig. 1A, $p<0.01$ Kruskal-Wallis, Bonferroni corrected). Rearing density, however, did not produce fish with differences in meristic counts. Moreover, no effect on the mineralization of skeletal elements was observed. Mineralization was correlated with the standard length of HD-reared juveniles, indicated by the logistic regression analysis. The reduced number of vertebral bodies found in HD group animals was caused by increased vertebral body fusions in the region of the caudal complex. The analysis of 86 types of malformations, including variations in numbers, shape and presence/absence of specific skeletal elements, revealed that the caudal complex is the most affected region (Fig. 1C). This is in accordance with height degree of variability of the Teleost caudal complex (caudal fin exoskeleton and pre-caudal vertebrae) observations made on gilthead seabream (Ford 1937, Witten & Hall 2015, Prestinicola et al. 2013). The paired and unpaired fins were the least affected skeletal structures. In contrast an increased amount of caudal fin ray malformations was observed. In this study larger tank volumes did not mitigate the effects of high rearing density: individuals reared in larger volumes were significantly

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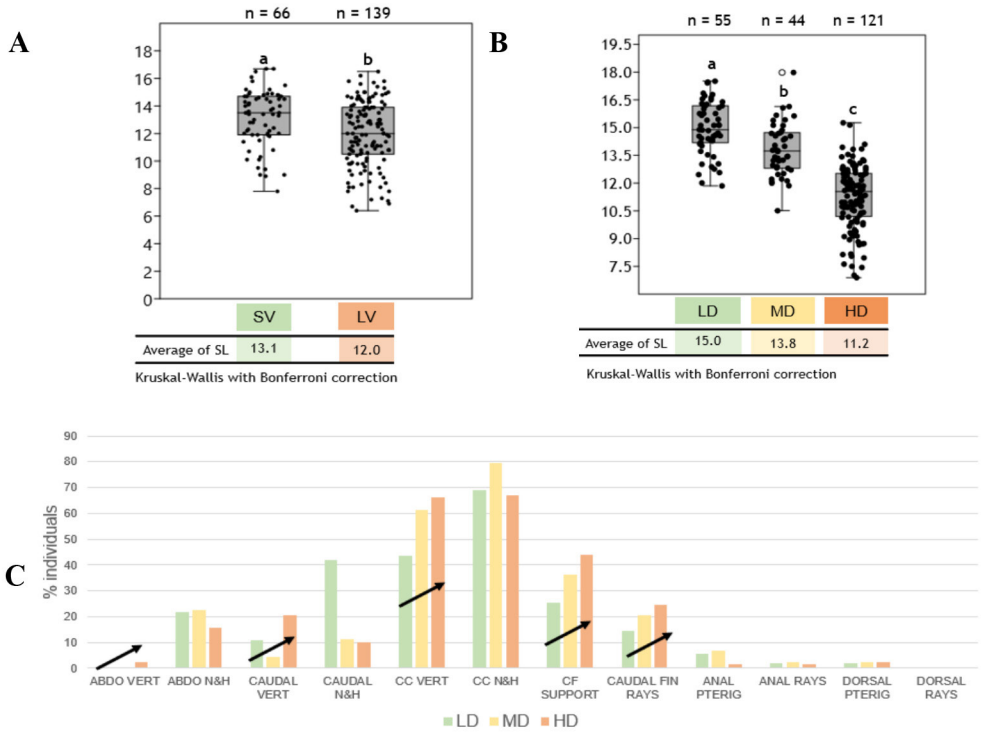

Fig.1 S_L variation according to rearing density (A) and volume (B). The variation of the skeletal elements according to the region is presented in C for the density study.

smaller than the individuals in the small volume group (Fig. 1B) and no significant differences in the distribution of skeletal anomalies within the axial skeleton were found. In contrast, medaka from the low volume (LV) group reveal an increase in vertebral body fusions as well as anomalies of the centra and arches in the caudal complex region. The analysis of videos revealed that medaka in LV did not fully exploit the space availability in the tank. Likely since medaka are a gregarious species, they simply prefer swimming on the bottom and top of the tank, avoiding the middle section of the water column. Due to the animals' aggregation in the tank, the density in effect, is augmented under low volume conditions.

Acknowledgements

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References

Boglione, C. et al. (2013) Reviews in Aquaculture 5, S99-S120. doi: 10.1111/raq.12015.
Ford, E. (1937) Journal of the Marine Biological Association of the UK 22 (1): 1-60
Martini, A. et al. (2020) Journal of Fish Biology https://doi.org/10.1111/jfb.14272
Prestinicola, L. et al. (2013) PLoS One 8, e55736. doi: 10.1371/journal.pone.0055736
Witten, P.E. & Hall, B.K. (2015) Copeia 103 (4):727-739
Witten, P.E. (2017) Methods in Cell Biology 138, 321-344

ENVIRONMENTAL PARAMETERS INFLUENCING VALVE MOVEMENTS OF MANILA CLAM FARMED IN VENICE LAGOON: PRELIMINARY RESULTS

Federica Di Giacinto [(1)*], Bertolini, C. [(2),], Carbone, L. [(3),], Mascilongo G. [(1),], Di Renzo L.[(1),], Berti M., [(1),] Giansante C, [(1),] Ferri N. [(1),] Roberto Pastres [(2)]

1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", via Campo Boario, snc, 64100 Teramo, Italy
2) Università Ca' Foscari, Campus scientifico, Via Torino, 155, 30170 Mestre, Venezia, Italy
3) Officine Inovo, engineering & design studio, 64100 Teramo, Italy
E-mail: f.digiacinto@izs.it

Introduction

In the recent years, mass mortalities of Manila clam, *Ruditapes philippinarum*, were recorded in the shellfish farms of Northern Adriatic lagoons. During the summertime events, they could be related to thermal stress as demonstrated applying a thermal tolerance landscape model (Bertolini & Pastres, in review). High mortalities in autumn may be due to the low salinity levels, caused by extreme precipitation events and related high freshwater discharges.

In order to elucidate the sublethal effects of temperature and salinity on Manila clam, the behaviour of molluscs have been investigated under controlled environmental conditions. The valve movements of bivalves were recorded in lab using the sensor "SmartShell", a system developed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM) (Di Giacinto et al, 2019).

Methodology

Fifty (50) *R. philippinarum* specimens were collected from a shellfish farm located in the Venice Lagoon, Chioggia (VE) and transferred within 24 hours at the IZSAM laboratory. All individuals were kept in a closed system (400 Liter aquaria) at 20 °C for a first acclimatization period of 1 month. Artificial seawater was prepared using Instant Ocean® sea salt dissolved in deionized water (33 g/L). Molluscs were fed with microalgae suspension of a mixture of *Tetraselmis* spp. and *Dunaliella* spp. Experimental setting up was settled in four experimental groups composed by 2 individuals inserted in the SmartShell sensors in closed aquaria at different temperature and salinity conditions:1) 20°C ± 2 °C at 22 psu; 2) 20°C ± 2 °C at 36 psu; 3) 28°C ± 2 °C at 33 psu; 4) 20°C ± 2 °C at 33 psu (control group). The duration of the test was 7 days. Two replicates were carried out.

Data were grouped in five classes of valve gapes (VG), namely: VG ≤ 20%, 21–40%, 41–60%, 61–80% and ≥ 81% (Redmond et al., 2017). Both in control and exposed mollusc groups, the average amount of time percentage spent in each VG class has been evaluated.

Results and discussion

All molluscs had clearly distinguishable periods of activity and inactivity, repeated within the 24 hours. The control group showed regular pattern, with 1 long cycle of activity/inactivity per day. At lower salinity (22 psu), the number of periods was higher (almost no 4) and shorten than the control group. At 36 psu, the patterns showed almost no 1 cycle per day, shorten than the control one. At high temperature, the molluscs spent more time opened than the control group, showing almost no 2 activity/inactivity cycles.

In the control group, molluscs showed a flapping behaviour, spending more time (28%) at VG category of 41–60%. At lower salinity, the animals spent more time (42%) at VG of 61–80%. The most represented VG categories at higher salinity (32%) and temperature (33%) were 41–60% and 0 – 20%, respectively.

These preliminary results confirmed that environmental parameters influenced the behavioural patterns of molluscs, consistently. Further replicates will be carried out and final results will be analysed using the tolerance landscape model to study their potential impact on the mollusc production.

References

Redmond Kirsten J., Berry Mark, Pampanin M. Daniela, Andersenb Odd Ketil (2017) Valve gape behavior of mussel (*Mytilus edulis*) exposed to dispersed crude oil as an environmental monitoring endpoint. Marine pollution bulletin 117:330-339.

Di Giacinto F., Carbone L. Mascilongo G., Berti M., Ferri N. (2019) A novel sensor for behavioural toxicity testing with freshwater and marine bivalves: preliminary results. Proceedings of the 55th Congress of the European Societies of Toxicology, Helsinki, Finland, 8th – 11th of September, Toxicol. Lett. 314S1, S97-S98.

Bertolini C., Pastres R. (in review) Beyond the marine heatwave concept: using tolerance landscape models for an ecologically meaningful classification of extreme climate events.

LUMPFISH WELFARE AND NUTRITION IN ON-GROWING FARMS: DEVELOPING OPTIMAL NUTRITIONAL REQUIREMENTS FOR JUVENILE LUMPFISH IN FARM CONDITIONS AND WHEN DEPLOYED IN SALMON SEA FARMS BASED ON A SURVEY OF WILD POPULATIONS

Di Toro, J.[1]*, Betancor, M.B.[1], Johannesen, A[2]., Rey-Planellas, S. [1]

[1]Institute of Aquaculture, University of Stirling, FK7 4LA Stirling, Scotland, United Kingdom
[2]Fiskaaling, Við Áir 11, 430 Hvalvík, Faroe Islands
E-mail: jessica.ditoro@stir.ac.uk

Introduction

Sea-lice infestations by ectoparasitic copepods (*Lepeophtheirus salmonis and Caligus sp*) represent the major health and welfare threat for Atlantic salmon aquaculture. The use of lumpfish, *Cyclopterus lumpus*, as a biological control offers a green alternative to medicinal treatments. However, both the health and the welfare of lumpfish are major issues that need to be improved in the farm environment. Lumpfish can arrive at the deployment stage to the salmon sea cages under compromised nutritional and welfare conditions. Those juveniles lumpfish, show high levels of fin damage with pale liver colours than the wild ones. This suggests that fish are nutritionally compromised when they are deployed. Currently there is a huge knowledge gap upon the optimal nutritional requirements for lumpfish that leads to poor welfare and health. This investigation aims to identify nutritional deficits in the feed provided and hence to improve the welfare status of deployed lumpfish, based on a survey on wild populations. Sampling wild lumpfish and the immediate environment informs on the natural and seasonal variation in diet, it gives indication of how healthy lumpfish ought to look and it provides some information on potential prey selection and feed preferences. These results will help to identify key differences between wild lumpfish and the ones living in farms. They will be used to formulate a new feed that resembles the wild diet of lumpfish aiming to improve the health and welfare of lumpfish under farm conditions.

Materials and Methods

The lumpfish used for this study were collected at different salmon farming sites, lumpfish hatcheries and also from the wild and divided according to six size classes (<50g, 50-150g, 150 – 300g, 300g-1kg, 1-3 kg and 3-5kg). Regarding wild specimens, both coastal and pelagic lumpfish were sampled. Coastal lumpfish were collected along the coast, by shaking seaweed, and collected by using a hand net. Pelagic lumpfish were sampled by pelagic fisheries and by a research survey vessel conducted in the Faroe Islands (name the vessel in here). Farmed lumpfish data and samples were collected at three Atlantic salmon farming sites, during the seasonal lumpfish health monitoring (4 times/year). Twenty to thirty lumpfish in the sea cages were harvested with a hand dip net from the pen edges. Thirty pre-deployment juvenile lumpfish were collected from two Faroese tank-based sites, and they were used as control. At each sampling occasion, a suite of morphometric measurements and operational welfare indicators (OWI) were recorded for every fish, e.g., body condition, fin damage, deformities, eyes integrity, skin status, liver colour and stomach contents were recorded. OWIs were categorised using the method reported by Eliasen et al. (2020). Ten livers were also weighed out to determine the Hepatosomatic Index (HSI) and frozen on dry ice during the sampling and stored at -80°. Further nutritional analyses were carried out such as total lipid content (Folch et al., 1957), fatty acid methyl esters (FAME) (Christie, 2003), lipid classes (Henderson and Tocher, 1992) and total carotenoid (Barua et al., 1993). A subsample of ten fresh livers, anterior and distal intestine, spleen was fixed in 10% neutral buffered formalin (NBF) for posterior histopathological analysis and in RNAlater for gene expression studies. Also, a subsample of ten whole lumpfish was kept for posterior proximate analysis according to standard procedures (AOAC, 2000).

Results

Only some of the nutritional analyses were finished and the rest are still in progress. Here we present only the data obtained for the wild individuals. Total lipids from livers of wild lumpfish were extracted and ranged from 14.9% to 35% and results showed a decreasing trend with an increase of size. The main fatty acids found in wild lumpfish livers are the monounsaturated (MUFA) that account for 50 % of the total profile, of which the most predominant is 18:1n-9 that ranged from 31% to 37%. The total n-3 PUFA are the second most predominant (21 – 28 %), followed by the saturates (SAFA) and finally the total n-6 PUFA. There was a trend towards a reduction of DHA (22:6n-3) for the different size classes. In the proximate analysis of wild whole fish, moisture ranged from 85 to 86.8%, oil content from 3.4 to 4.4 %, protein from

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6.06 to 8.31%, and ash from 1.47 to 1.6 %. Statistical differences were found only in the ash content of bigger fish (3-5 kg) compared to small fish (300g – 1kg) (one-way ANOVA, P-value = 0.05). The results of the carotenoid analysis showed that astaxanthin is the main carotenoid that strongly influence the colour of lumpfish liver (93.4-101.7 mg/kg), followed by small quantities of astacene (2.5-3.3 mg/kg) and lutein (0.8-1 mg/kg). Canthaxanthin, lutein and zeaxanthin were not found in these samples. Lipid class analysis was performed on total lipid extracted from lumpfish livers and results show that the main lipid classes found were triacylglycerols (TAG) (55.7%), free fatty acids (9.3%) and Phosphatidylcholine (PC) (8.3%). Preliminary results of the OWIs and the farmed lumpfish will be presented in the final poster as analysis is ongoing.

References

AOAC (2000). Official Methods of Analysis. Washington, DC: Association of Official Analytical Chemists.

Barua, A. B.; Kostic, D.; Olson, J. A. (1993). New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum. J. Chromatogr., 617, 257- 264.

Christie, W. W., & Han, X. (2003). Lipid Analysis: Isolation. Separation, Identification and Structural Analysis of Lipids (Barnes, Bridgwater, UK).

Eliasen, K., Patursson, E. J., McAdam, B. J., Pino, E., Morro, B., Betancor, M., ... & Rey, S. (2020). Liver colour scoring index, carotenoids, and lipid content assessment as a proxy for lumpfish (Cyclopterus lumpus L.) health and welfare condition. Scientific Reports, 10(1), 1-12.

Folch J, Lees N, Sloane-Stanley GH. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem.; 226: 497–509.

Henderson, R. J., & Tocher, D. R. (1992). Thin-layer chromatography. Lipid Analysis: A Practical Approach, 65-111.

NOVEL MICRODIETS FOR LARVAE OF PURPLE SEA URCHIN *Paracentrotus lividus*

D. Dias[1]*, A. Rebotim[1], R. Colen[1], A. Santos[2], W. Pinto[2], L.E.C. Conceição[2], S. Engrola[1]

[1]Centre of Marine Sciences. University of Algarve. Gambelas campus. 8005-139 Faro, Portugal
[2]SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
*E-mail: ddias@ualg.pt

Introduction

Sea urchin gonads are considered a delicacy comparable to caviar, that goes by the name of *roe* or *uni* (Sun & Chiang, 2015). The growing demand for *roe*, has led to the depletion of natural stocks of the purple sea urchin, *Paracentrotus lividus*, all over Europe (Sartori et al., 2016). To establish commercial aquaculture of *P. lividus* that can significantly contribute to meet the global demand for *roe*, high-quality dry feeds that ensure a healthy somatic growth are required (Lourenço et al., 2020). Furthermore, larval rearing is dependent on the use of accessory microalgae cultures (Brundu et al., 2017) which can be costly and unpredictable (Oostlander et al., 2020). The aim of this work was to develop larvae microdiets to improve output and control at early production stage of the lifecycle of *P. lividus*.

Materials and methods

A sea urchins broodstock was kept under optimised abiotic conditions and fed *Ulva* sp. and inert diet. Spawning was induced by injection of 1ml of KCl 0.5M through the peristomial membrane. Female gametes were collected in filtered natural seawater and male gametes were collected in vials and cold stored. Fertilization was done using a ratio of 20μl of semen per 10^6 eggs in a beaker. Fertilization rate was assessed by comparing the number of embryos undergoing cellular division against the unfertilized eggs. Larvae were reared in 250L circular tanks, with an initial larvae density of 8000 larvae/L, kept in static conditions until the 8 days after hatching (DAH). Medium aeration was provided to ensure optimal oxygen values as well as to prevent both larvae and feed sedimentation. Three different feeding regimes were tested: 1) CTRL, a 50:50 mixture of microalgae (*Tisochrysis lutea* and *Chaetoceros calcitrans*) and two formulated diets produced by spray-drying – 2) MARINE; and 3) ALGAE. The MARINE diet was formulated to include fishmeal, squid meal and whey as main protein sources. In the ALGAE diet, the same ingredients were used, but *Chlorella* sp. and *Nannochloropsis sp*. biomasses were also included. The microdiets were fed exclusively without the addition of microalgae to the rearing tanks. The larvae were fed daily, and the amount of fed was adjusted based on visual inspection according to larval development stage. This corresponded to 120 000 cell/ml/day from 2 to 8 DAH and 200 000 cell/ml/day onwards for the microalgae and a progressive increase from 2 to 3 g/tank/day for the experimental feeds. To assess growth and survival, samplings were performed at hatching and 8 DAH. Larval total length (n=30) at each sampling stage was determined by image analysis using a stereo microscope with camera and data analysis was performed using ImageJ software.

Results

At 8 DAH, length results (figure 1) indicate that larvae fed on MARINE microdiet presented a similar growth to the larvae fed exclusively with microalgae (CTRL treatment). However, larvae under the feeding regime composed of the ALGAE microdiet displayed a slight growth impairment when compared to the larvae from CTRL and MARINE diets (p-value=0.000).

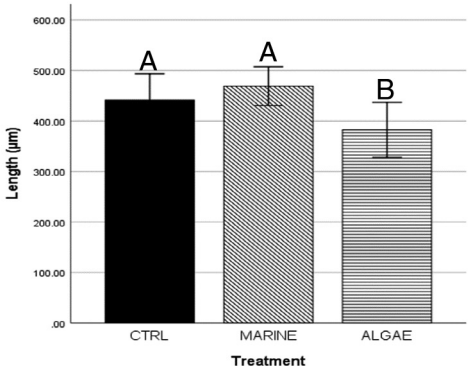

Figure 1 – Larvae length at 8 DAH. Presence of capital letters indicate the presence of statistical differences (p-value<0.05)

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Conclusion

This short trial suggests that spray dried microdiets, specifically designed and formulated, are adequate for the initial stages of the purple sea urchin and can sustain growth at similar rate of the traditionally used microalgae. Furthermore, it can be stated that, despite the extremely low pellet size, feeds have the necessary characteristics in terms of permanence in the water column and nutrient leaching. Nonetheless, formulation clearly plays a major role on the growth of purple sea urchin larvae, and there seems to be no clear benefits on the inclusion of microalgae on the microfeeds. Overall, this study contributes to the development and optimisation of the culture and feed technologies which may, in the future, play a key role in the way in which sea urchin roe is obtained, ultimately contributing to the preservation of wild populations.

References

Brundu, G., Vallainc, D., Baroli, M., Figus, A. M., Pinna, A., & Carboni, S. (2017). Effects of on-demand feeding on sea urchin larvae (Paracentrotus lividus ; Lamarck, 1816), development, survival and microalgae utilization. *Aquaculture Research*, *48*(4), 1550–1560. https://doi.org/10.1111/are.12990

Lourenço, S., José, R., Andrade, C., & Valente, L. M. P. (2020). Growth performance and gonad yield of sea urchin Paracentrotus lividus (Lamarck, 1816) fed with diets of increasing protein: energy ratios. *Animal Feed Science and Technology*, *270*, 114690. https://doi.org/10.1016/j.anifeedsci.2020.114690

Oostlander, P. C., van Houcke, J., Wijffels, R. H., & Barbosa, M. J. (2020). Microalgae production cost in aquaculture hatcheries. *Aquaculture*, *525*, 735310. https://doi.org/10.1016/j.aquaculture.2020.735310

Sartori, D., Pellegrini, D., Macchia, S., & Gaion, A. (2016). Can echinoculture be a feasible and effective activity? Analysis of fast reliable breeding conditions to promote gonadal growth and sexual maturation in Paracentrotus lividus. *Aquaculture*, *451*, 39–46. https://doi.org/10.1016/j.aquaculture.2015.08.037

Sun, J., & Chiang, F.-S. (2015). Use and Exploitation of Sea Urchins. In *Wiley Online Books*. *Echinoderm Aquaculture* (pp. 25–45). https://doi.org/10.1002/9781119005810.ch2

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HIGH THROUGHPUT PHENOTYPING REVEALS THE HERITABLE LANDSCAPE OF NEAR-INFRARED AND RAMAN SPECTROSCOPIC MEASUREMENT TO GENETICALLY IMPROVE LIPID CONTENT IN ATLANTIC SALMON

Gareth F. Difford*, S.S. Horn, K.R. Dankel, B. Ruyter, B.S. Dagnachew, B. Hillestad, A.K. Sonesson and N.K. Afseth

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-1433 Ås, Norway
[2]Benchmark Genetics Norway AS, Sandviksboder 3A, NO-5035 Bergen, Norway
Email: gareth.difford@nofima.no

Introduction

Product quality and production efficiency of Atlantic salmon are, to a large extent, influenced by the deposition and depletion of lipid reserves. Fillet lipid content is a heritable trait and is unfavourably correlated with growth, thus genetic management of fillet lipid content is needed for sustained genetic progress in these two traits. The laboratory-based reference method for recording fillet lipid content is highly accurate and precise but, at the same time, expensive, time-consuming, and destructive. Here, we test the use of rapid and cheaper vibrational spectroscopy methods, namely near-infrared (NIR) and Raman spectroscopy both as individual phenotypes and phenotypic predictors of lipid content in Atlantic salmon.

Materials and methods

As part of commercial breeding operations Benchmark Genetics AS created 194 full sibling families using 92 sires and 194 dams. All families were reared in a single sea cage after smoltification. At a mean weight of 3605 g and 12 months at sea the fish were harvested and filleted. Four lipid traits were recorded on each individual: (1) reference method lipid content ($Lipid_{True}$), (2) commercial NIR system lipid content ($Lipid_{FieldNIR}$), (3) laboratory-based NIR system ($Lipid_{NIR}$), and (4) laboratory-based Raman system ($Lipid_{Raman}$) lipid contents. Muscle samples were taken from the Norwegian Quality Cut (NQC), frozen and stored at -20°C. Total lipids were extracted from homogenized NQC muscle samples from each fish, and lipid content was determined in g of lipid per 100 g of muscle tissue calculated according to the method described by (Folch et al., 1957). Then, homogenized muscle samples were recorded for laboratory-based Raman and NIR spectroscopy analysis. Raman spectra were obtained using a Kaiser RamanRXN2™ Multi-channel Raman analyzer on the homogenized samples, culminating in 1300 Raman spectral variables ranging from 500 to 1800 cm^{-1} range. Diffuse reflectance near-infrared spectra of homogenized salmon samples were obtained using the FOSS NIRSystems XDS Rapid Content Analyzer, resulting in 2300 NIR spectral variables in a range from 1150 to 2500 nm for further analysis. In total 523 individuals were phenotyped with all methods. Variance components were estimated by applying univariate animal models using average information criterion restricted maximum likelihood models in DMU version 6 (Madsen and Jensen, 2014):

$$\mathbf{y} = \mathbf{X}\mathrm{b} + \mathbf{Z}\mathrm{a} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ is the vector of phenotypes for individual $i = 1,2,3 \ldots n$; i.e. 1300 phenotypes for Raman shift values, 2300 phenotypes for NIR absorbance values, and the four lipid content phenotypes ($Lipid_{True}$, $Lipid_{FieldNIR}$, $Lipid_{NIR}$, and $Lipid_{Raman}$). Heritability (h^2) estimates were calculated as the ratio of additive genetic variance to total phenotypic variance $\sigma_a^2/(\sigma_a^2 + \sigma_e^2)$ and their standard errors by using a Taylor series approximation.

Results

Remarkably, 827 of the 1500 individual Raman variables (i.e. Raman shifts) of the Raman spectrum were significantly heritable (heritability () ranging from 0.15 to 0.65) (Figure 1). Similarly, 407 of the 2696 NIR spectral landscape variables (i.e. wavelengths) were significantly heritable (= 0.27-0.40) (Figure 1). Both Raman and NIR spectral landscapes had significantly heritable regions, which are also informative in spectroscopic predictions of lipid content. Partial least square predicted lipid content using Raman and NIR spectra were highly concordant and highly genetically correlated with the lipid content values (= 0.91-0.98) and were significantly heritable (= 0.52-0.67) (Difford et al., 2021).

Conclusion

Both NIR and Raman spectral landscapes show substantial additive genetic variation and are highly genetically correlated with the reference method. These findings lay down the foundation for rapid spectroscopic measurement of lipid content in salmonid breeding programmes.

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References

Difford, G.F., Horn, S.S., Dankel, K.R., Ruyter, B., Dagnachew, B.S., Hillestad, B., Sonesson, A.K., Afseth, N.K., 2021. The heritable landscape of near-infrared and Raman spectroscopic measurements to improve lipid content in Atlantic salmon fillets. Genet. Sel. Evol. https://doi.org/https://doi.org/10.1186/s12711-021-00605-6

Folch, J., Lees, M., Sloane Stanley, G.., 1957. A simple methof for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

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.

FAMILY DIFFERENCES IN FEED INTAKE AND FEED COVERSION RATIO OF ATLANTIC SALMON

Gareth F. Difford[1]*, B. Hatlen[1], B. Gjerde[1], J.P. Wold, A.T. Norris[2], A.K. Sonesson[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-1433 Ås, 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 amount of edible product produced relative to the feed input of Atlantic salmon (i.e. feed efficiency) offers considerable potential to improve both profitability and environmental sustainability (Kause et al., 2006).

Selective breeding is one possible strategy for improving the feed efficiency of Atlantic salmon production. There is considerable evidence of genetic variation in feed efficiency of Atlantic salmon (Kolstad et al., 2004; Thodesen et al., 2001, 1999)"id":"ITEM-1","issued":{"date-parts":[["1999"]]},"page":"237-246","title":"Feed intake, growth and feed utilization of offspring Salmo, from wild and selected Atlantic salmon (Salmo salar as well as other salmonids (Kause et al., 2006). A necessity of breeding for improved feed efficiency is the need to record feed intake and growth on thousands of family structured individuals under the commercial conditions they are expected to perform (Falconer and Mackay, 1996). One approach is to culture families in replicates and record the feed given and the feed refusals. This approach allows for fine phenotyping of feed intake and growth and ensure that the between family genetic potential is unlocked. Our objectives are to use family-based tank feeding to quantify the family differences in feed intake, growth, lipid deposition in Atlantic salmon.

Materials and methods

A total 1,750 Atlantic salmon parr from 35 families of the MOWI Genetics AS (MOWI ASA, ***Øyerhamn***, Norway) elite nucleus material were cultured in mixed groups and genotyped at PIT tagging, at an average weight of 40 grams were transported to the Nofima Research Station for Sustainable Aquaculture (Sunndalsøra, Norway). The fish were acclimated in mixed tanks for one month until they reached a mean weight of 50 grams. The fish were sorted by family into two tanks, each with 25 individuals and allowed to further acclimate for two weeks. The trial was conducted over 7 weeks to ensure doubling of body weight, the daily feed rations and refusals collected for each tank and record. At the end of the trial period the fish were euthanized and recorded for length, weight and body composition using a commercial NIR system for recording lipid and energy content.

For each tank the cumulative phenotypes for feed intake (FI), initial weight (IW), specific growth rate (SGR), lipid content (FLC) were computed for each tank. In additional feed conversion ratio (FCR) was calculated for the entire growth period. Linear mixed models were run using DMU version 6 (Madsen and Jensen, 2014). A full five trait model of the following for was run until convergence:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e}, \qquad [1]$$

where $\mathbf{y}$ is the vector of phenotypes for at the tank level (i.e. FI, IW, SGR, LC & FCR). Fixed effects for tank location were included and the random effect of family was estimated. Heritability in the broad sense (H^2) estimates were calculated as the ratio of between family genetic variance to total phenotypic variance $\sigma^2_b/(\sigma^2_b + \sigma^2_e)$ and their standard errors by using a Taylor series approximation

Results and Discussion

The descriptive statistics and the broad sense heritabilities are presented in Table 1. In general, all traits showed considerable and significant broad sense heritability. Although the broad sense heritability is not a preferred proxy for the narrow sense heritability (h^2), as possible common environmental effects and maternal effects cannot be partitioned and removed. As a result, broad sense heritability are typically an over estimation of the narrow sense heritability. However, broad sense heritability estimated do represent an upper limit to the narrow sense heritabilities and they do demonstrate the genetic potential between families. If the broad sense heritability is not significantly different from zero it is unreasonable to expect the narrow sense heritability to be different from zero.

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Table 1. Descriptive statistics the feed intake complex traits and their heritability in broad sense

Trait (Tank level)	Mean ± SD*	Min - Max	H^2 ± SE**
Feed intake (g)	1167.4 ± 189.12	628.6 - 1525.9	0.86 ± 0.04
Initial Weight (g)	1254.6 ± 132.9	939.0 - 1541.0	0.95 ± 0.02
Specific Growth rate (g/d)	1.53 ± 0.16	1.01 - 1.79	0.82 ± 0.06
Lipid content (%)	11.3 ± 0.63	10.1 - 12.9	0.68 ± 0.09
FCR	0.78 ± 0.04	0.72 - 0.95	0.76 ± 0.07

* SD = standard deviation, H^2 = broad sense heritability and SE = standard error

Conclusion

There is considerable and significant between family variation in all traits within the feed efficiency complex demonstrating that there is genetic potential between families which can be exploited to genetically improve feed efficiency. However, the between family genetic correlations and selection index theory are needed to determine the relative amount of genetic variation available after partitioning of energy to maintenance, growth and lipid deposition.

Acknowledgement of Funding

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References

Falconer, D., Mackay, T., 1996. Introduction to quantitative genetics, Fourth edi. ed. Longman Scientific and Technical, Harlow, Essex, United Kingdom.

Kause, A., Tobin, D., Houlihan, D.F., Martin, S.A.M., Mäntysaari, E.A., Ritola, O., Ruohonen, K., 2006. Feed efficiency of rainbow trout can be improved through selection: Different genetic potential on alternative diets. J. Anim. Sci. 84, 807–817. https://doi.org/10.2527/2006.844807x

Kolstad, K., Grisdale-Helland, B., Gjerde, B., 2004. Family differences in feed efficiency in Atlantic salmon (Salmo salar). Aquaculture 241, 169–177. https://doi.org/10.1016/j.aquaculture.2004.09.001

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.

Thodesen, J., Gjerde, B., Grisdale-Helland, B., Storebakken, T., 2001. Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (Salmo salar). Aquaculture 194, 273–281. https://doi.org/10.1016/S0044-8486(00)00527-5

Thodesen, J., Grisdale-Helland, B., Helland, S.J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring Salmo, from wild and selected Atlantic salmon (Salmo salar). Aquaculture 180, 237–246.

Winther, U., Hognes, E.S., Jafarzadeh, S., Ziegler, F., 2020. Greenhouse gas emissions of Norwegian seafood products in 2017.

DEVELOPMENT OF A COMMERCIAL MICROALGAE DIET FOR BIVALVES APPLIED TO PORTUGUESE OYSTERS (*Crassostrea angulata*) JUVENILES

Patricia Diogo[1*], Gonçalo Bastos[1], Cristiana Gastão[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.com

Introduction

Commercial microalgae diets specifically formulated for bivalve's nutrition are still underdeveloped. Lower trophic aquaculture species, represent currently half of the world's aquaculture production, being this number expected to grow in the upcoming years mainly due to its market and non-market value. Microalgae produced in aquacultures to feed bivalves is highly demanding and subjected to fluctuations potentially threatening its production. Therefore, microalgae diets industrially produced, specifically developed for bivalves is a valuable asset. Oysters (*Crassostrea* spp.) depict the world's most produced mollusc species. Portuguese oyster (*Crassostrea angulata*) is a valuable species, even though its natural populations suffered a decline, requiring subsequently a grand effort in the conservation of this species[1]. Aquaculture production of *C. angulata* is essential for this sector development and natural populations restocking. This project aims to develop a commercial diet for bivalves, formulated with a blend of microalgae species commonly used for oyster nutrition in aquacultures.

Material and methods

Juvenile oysters were placed in nets on the natural environment (Rio Mira, Odemira, Portugal) in duplicate (n=250 oysters per replica) as control or conditioned in the nursery in recirculation tanks (experiment beginning in December 2020). Juvenile oysters in the nursery were fed with two distinct diets in duplicate (n=250 oysters per replica). Pilot commercial diets were formulated containing 8% of industrially produced microalgae biomass in dry weight (DW), and juveniles were fed daily with an amount equivalent to 4% of the oyster dry meat (g) in DW of microalgae (mg)[1]. Both diets contained a blend of microalgae species commonly used in oysters nutrition[1]. Diet 1 was composed by *Tetraselmis* sp., *Skeletonema* sp., *Tisochrysis lutea* and *Pavlova* sp. (8: 6:1:1). Diet 2 was composed by *Tetraselmis* sp., *Skeletonema* sp. and *Tisochrysis lutea* (T-ISO clone) (1:11:4). Oysters were sampled (n=50) monthly for at least 3 months (survival, weight, valve length, width and growth difference between valves). Oyster shells have two asymmetrical valves joined by a ligament at their hinges. The difference between both valves represent a sclerochronologic record of the hinges growth[2]. Samples were collected for further dry weight analysis (n=5) and condition factor evaluation. The survival percentage of all treatments was also evaluated. The environmental conditions of the tanks and of Rio Mira were daily monitored (temperature, oxygen, pH, salinity, ammonia and nitrates). IBM SPSS Statistics 26.0 software was used for statistical analysis, ANOVA was used to compare differences between groups. A cluster analysis will be applied to the data and subsequentially, a decision tree approach will allow to understand the growth pattern in the different size groups of oysters in the treatments.

Results

After 9 weeks of experiment, preliminary results showed that juvenile oysters of control group had significantly higher weight and biometric values than the ones in the indoor tanks. Although in the beginning of the experiment oysters fed with Diet 1 showed no significant differences compared to Diet 2 except in wet weight. However, Diet 2 showed higher growth throughout time and in the last sampling point Diet 2 showed significantly higher weight, width and difference between valves than oysters fed with Diet 1. The parameters increased significantly throughout time, even though water temperature and salinity were low (13.75 ± 0.75 °C, 19.99 ± 5.53).

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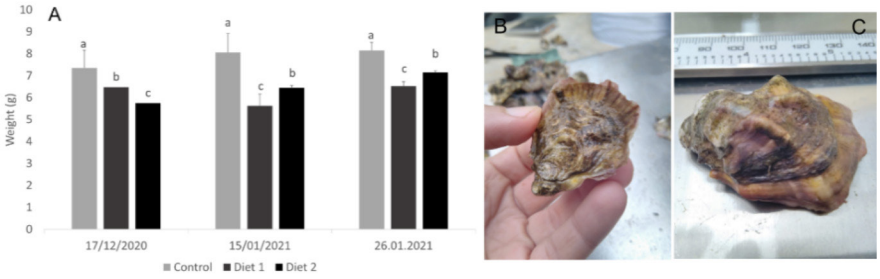

Figure 1 – A) Wet weight of *C. angulata* of control group and fed with Diet 1 and 2. Different letters indicate significant differences (one-way ANOVA, post hoc Tuckey $p < 0.05$). B) Right valve of a juvenile oyster. C) Left valve of a juvenile oyster.

Discussion

Juvenile oysters showed constant growth throughout time. Oysters in their natural environment, with constant microalgae availability, exhibited the highest growth values. This fact suggests that juvenile oysters may have higher capacity of food intake than the daily ration provided in indoor tanks, additionally the low temperatures and salinity observed during this period could be responsible for reduced growth. Therefore, this preliminary data suggests that the daily food quantity can be increased to improve oyster's growth. Oyster nutrition requires a combination of microalgae species, particularly diatoms and flagellates[1]. Diet 2 displayed the best biological performance of juvenile oysters throughout time amongst the tested diets. This diet had high concentration of the diatom *Skeletonema costatum* and the flagellate *Tisochrysis lutea*, which have previously shown good results in oysters nutrition[1,3]. The present work showed promising results since the prototype commercial diets with the blends of microalgae species selected promoted successfully juvenile oysters' growth.

References

1.Anjos, C. *et al.* Broodstock conditioning of the Portuguese oyster (*Crassostrea angulata*, Lamarck, 1819): influence of different diets. *Aquac. Res.* **48**, 3859–3878 (2017).

2.Surge, D., Lohmann, K. C. & Dettman, D. L. Controls on isotopic chemistry of the American oyster, *Crassostrea virginica*: Implications for growth patterns. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **172**, 283–296 (2001).

3.Pennarun, A. L., Prost, C., Haure, J. & Demaimay, M. Comparison of two microalgal diets. 1. Influence on the biochemical and fatty acid compositions of raw oysters (*Crassostrea gigas*). *J. Agric. Food Chem.* **51**, 2006–2010 (2003).

AQUACULTURE VIRTUAL CAREER DEVELOPMENT PLATFORM FOR THE SOUTH BALTIC REGION

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

There is a significant demand for a high-qualified personnel and knowledge in modern aquaculture. To develop an innovative aquaculture sector and move the focus into the South Baltic region, competencies and knowledge are crucial. This is where AquaVIP has a field for action. A three-year project is led by Klaipeda Science and Technology Park, accompanied by University of Rostock, University of Gdańsk and Klaipeda University. AquaVIP project objective is 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. AquaVIP summer schools are aimed to introduce participants to background theoretical skills in modern aquaculture biotechnology: main types, biological and technological processes and development trends. The courses delivered by the University of Gdańsk and Klaipeda University provide participants with practical hands-on experience on modern aquaculture technology and innovative blue biotechnology-based approaches to increase aquaculture development potential. They are based on real ongoing AquaVIP experiments in RAS in research facilities, and partner aquaculture companies. The topics are set according to the needs defined by the sector.

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.

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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.

STUDY OF THE ENZYMATIC ACTIVITIES AND ANTAGONISTIC EFFECT PRESENT IN THE EXTRACELLULAR PRODUCTS OF THE PROBIOTIC STRAIN *Shewanella putrefaciens* PDP11

M. Domínguez-Maqueda[(1)], Isabel M. Cerezo[(1)], M. I. Sáez[(2)], M.C. Balebona[(1)], M.A. Moriñigo[(1)]

(1) Departamento de Microbiología, Universidad de Málaga, Andalucía Tech, Campus de Excelencia Internacional del Mar (CEIMAR), 29071 Málaga, Spain
E-mail: martadm@uma.es
(2) Departamento de Biología y Geología, Universidad de Almería, CEIMAR, 04120 Almería, Spain

Introduction

Shewanella putrefaciens Pdp11 has been demonstrated as probiotics for farmed species such as *Sparus aurata* and *Solea senegalensis* (Tapia-Paniagua et al., 2015). Benefits provided by probiotics may be associated to a diverse array of extracellular products (ECPs) produced by the bacteria and named as postbiotics. Postbiotics are soluble factors (products or metabolic by-products) either secreted by live bacteria (i.e., probiotic or non-probiotic) or released after bacterial lysis that might offer positive effect to the host (Cuevas-González et al., 2020) mediating certain metabolic processes and interacting directly with mucosal cells, such as epithelial and immune cells on the host (Hossain et al., 2020). The aim of this work is identifying Pdp11 postbiotic activities linked to the bacterial secretome and related to improved feed digestibility, through enzymatic hydrolysis, and defense against fish pathogens. For this, characterization of *S. putrefaciens* Pdp11 ECPs obtained under different growth conditions (temperature, incubation time or growth medium) has been carried out.

Materials and methods

S. putrefaciens Pdp11 cells were cultured in tryptic soy broth (Oxoid Ltd., Basingstoke, UK) added with NaCl (1.5%) at 23ºC and 15ºC for 36h. Then, 1mL from each culture was spread on plates containing on basal medium (1.5% agar) added with tryptic soy broth and NaCl (1.5%) (TSBas), basal medium added with (160g/L) aquafeed added with and without NaCl (1.5%). Plates were incubated as shown in Table 1. Aquafeed composition is described by Ayala et al., (2020).

ECPs were obtained by the technique described by Liu et al., 1957. Bacterial cells were harvested, after 24h and 48h, with 2mL of sterile phosphate-buffered saline (PBS) and centrifuged (10000xg, 20min, 4ºC). The supernatants were filtered through 0.45- and 0.2 μm pore-size membrane filters and kept at -80ºC until use. ECPs were also concentrated by Amicon Ultra centrifugal filters (10 K) (Merck Millipore, USA) and protein concentration determined with Qubit Protein assay kits and the Qubit 2.0 (Thermo Fisher Scientific, USA). A total of 19 enzymatic activities of the ECPs were evaluated with the API ZYM system (BioMerieux, Spain). Phytase, tannase and cellulase activities were assayed according to Kumar et al., 2010. In addition, protease, collagenase, lipase, amylase activities as well as the antagonistic effect on fish pathogens were assayed according to Chabrillon et al., 2005. In all cases, 50 μL of ECPs (0,5μg protein/μL) were inoculated into 6 mm-diameter wells made in the plates and incubated at 22ºC for 24-48h.

Table 1. Different conditions for ECPs extraction.

ECPs extraction conditions		
Medium	Incubation (T and time)	Nomenclature
TSBas (1.5% NaCl) (1.5% agar)	23ºC-24h	T23C24h
	23ºC-48h	T23C48h
	15ºC-24h	T15C24h
	15ºC-48h	T15C48h
Aquafeed (1.5% NaCl) (1.5% agar)	23ºC – 24h	FB23C24h
	23ºC – 48h	FB23C48h
	15ºC-24h	FBS15C24h
	15ºC-48h	FBS15C48h
Aquafeed (1.5% agar)	23ºC – 24h	FB23C24h
	23ºC – 48h	FB23C48h
	15ºC-24h	FB15C24h
	15ºC-48h	FB15C48h

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Results and discussion

Screening of ECPs secreted by *S. putrefaciens* Pdp11 from 12 different extraction conditions revealed different API ZYM and hydrolytic enzymatic profiles. ECPs from the same source culture medium showed similar APY ZYM profiles, regardless of incubation time and temperature. On the other hand, hydrolytic activities of nutritional and anti-nutritional compounds showed more variation. In all the cases, protein, lipid and collagen hydrolysis were detected, with the exception of T15C24h, with only collagen hydrolysis activity. No condition hydrolyzed antinutritional compounds (phytate, tannins and cellulose). In general, ECPs obtained after incubation at 23ºC, 24h and at 15ºC, 48h showed a more intense activity regardless of the culture medium. In addition, FBs23C24h is the only one capable of hydrolyzing starch. In any case, antagonistic effect was observed for pathogens at the ECPs concentrations studied.

Postbiotics can interact with the host, helping the degradation of substances, improving the quality of feed that may not be used by the animal as a whole, due to the lack of digestive activity (Cuevas-González et al., 2020). In this term, the enzymatic pattern obtained from the probiotic's ECPs could contain potential activities to enhance the feed utilization and intestinal functionality previously observed on *S. senegalensis* (Tapia-Paniagua et al., 2015) specimens when whole Pdp11 cells are dietary administered as supplement. Thus, postbiotics are leading an alternative to improve the performance of culture animals when the inclusion of viable live cells is not commercially possible.

References

Ayala, M. D., Galián, C., Fernández, V., Chaves-Pozo, E., García de la Serrana, D., Sáez, M. I., ... & Arizcun, M. (2020). Influence of low dietary inclusion of the Microalga *Nannochloropsis gaditana* (Lubián 1982) on performance, fish morphology, and muscle growth in juvenile gilthead seabream (*Sparus aurata*). Animals, 10(12), 2270.

Chabrillón M, Rico RM, Arijo S, Díaz-Rosales P, Balebona MC, Moriñigo MA. (2005) Interactions of microorganisms isolated from gilthead sea bream, Sparus aurata L., on *Vibrio harveyi*, a pathogen of farmed Senegalese sole, *Solea senegalensis* (Kaup). Journal of Fish Diseases 28, 531–537.

Cuevas-González, P. F., Liceaga, A. M., & Aguilar-Toalá, J. E. (2020). Postbiotics and paraprobiotics: From concepts to applications. Food Research International, 109502.

Hossain, T. J., Chowdhury, S. I., Mozumder, H. A., Chowdhury, M. N., Ali, F., Rahman, N., & Dey, S. (2020). Hydrolytic exoenzymes produced by bacteria isolated and identified from the gastrointestinal tract of Bombay duck. Frontiers in Microbiology, 11, 2097.

Kumar, R., Kumar, A., Nagpal, R., Sharma, J., & Kumari, A. (2010). A novel and sensitive plate assay for screening of tannase-producing bacteria. Annals of Microbiology, 60(1), 177-179.

Liu, P. V. (1957). Survey of hemolysin production among species of pseudomonads. Journal of bacteriology, 74(6), 718.

Tapia-Paniagua, S.T., Vidal, S., Lobo, C., García de la Banda, I., Esteban, M.A., Balebona, M.C. & Moriñigo, M.A. 2015. Dietary administration of the probiotic SpPdp11: effects on the intestinal microbiota and immune-related gene expression of farmed *Solea senegalensis* treated with oxytetracycline. Fish Shellfish Immunology 46, 449-458.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF DEFORMITIES AT THE EMBRYONIC STAGES OF THE PROTECTED MARINE GASTROPOD *Charonia seguenzae* (ARADAS & BENOIT, 1870)

C.K. Doxa[a,b*], D.G. Sfakianakis[a], A. Sterioti[a,c], and M. Kentouri[a]

[a] Biology Department, University of Crete, P.O. Box 1470, 71110 Iraklio, Crete, Greece,
[b] Cretaquarium, H.C.M.R , P.O. Box 2214, 71003 Iraklio, Crete, Greece, Fax + 30-2810-337882,
Tel. + 30-2810-337705, e-mail chrisadoxa@cretaquarium.gr
[c]Institute of Marine Biology Biotechnology and Aquaculture, H.C.M.R., P.O. Box 2214, 71003 Iraklio, Crete, Greece

Introduction

Aquaculture is commonly considered as an excellent method for sustaining populations of endangered and intensively exploited aquatic species. In the case of the marine gastropod *Charonia seguenzae* (Aradas & Benoit, 1870), the population declined due to over-fishing for shell trade (Katsanevakis *et al*., 2008)but still exploited by Man, the present study attempts to gather and review the available scientific information on molluscs of minor commercial importance in order to assist in the adequate management and protection of their populations. Forty one species (18 gastropods, 13 bivalves, and 10 cephalopods which led to its protection in the Mediterranean Sea (Bern convention, Barcelona convention). Re-establishment of *C. seguenzae* populations in the depleted habitats requires knowledge of its biology and successful maintenance and breeding in captivity.

Controlling the reproductive cycle and producing offspring of good quality is the first and most important step of breeding in captivity. Deformities have a huge impact both on offspring survival and quality and, although poorly studied, temperature has been recorded to affect the development of deformities in some marine gastropods (Cancino *et al*., 2011). The present study aims to identify the stage of development at which deformities occur, under four temperature regimes; the optimum (23^{o}C) at which tritons spontaneously reproduce (Doxa *et al*., 2019), the minimum (17^{o}C) and maximum (26^{o}C) temperature of the tritons' natural environment (Shaltout and Omstedt, 2014) and that of 29^{o}C that will probably be the environmental maximum in a few decades due to climate change (IPCC, 2014).

Materials and Methods

The development of deformities at the embryonic stages of the Mediterranean Triton *Charonia seguenzae* (Aradas & Benoit, 1870), was studied under 4 temperature conditions (17, 23, 26 and 29^{o}C). Sixty-six (66) egg capsules laid on the same day, by the same individual and of the same developmental stage (1 cell stage) were collected (23,4^{o}C). At the stage of morula (5th day after deposition) 60 egg capsules were separated into four groups of 15 capsules. One group remained at 23^{o}C and the other three were acclimated at 17, 26 and 29^{o}C. The stage of development was checked under stereoscope every other day. At the stages of trochophore, veliger and free veliger larvae, three capsules were collected, opened and 50 larvae per capsule were photographed under stereoscope. The percentage of deformities was calculated and the dimensions of the larvae were measured from microphotographs.

Results

The lower percentage of deformities at every stage occurred at 23^{o}C (Fig 1.). The highest rate of deformities in that temperature condition was observed at the trochophore stage (15.33 ± 5.03%), but it subsequently declined significantly at eclosion (0.67 ± 1.15%). The higher tested temperature (29^{o}C) was lethal since embryos survived only until the trochophore larva stage at which 82.66 ± 2.31 % of the larvae were deformed. The lower tested temperature (17^{o}C) initially exhibited a relatively low rate of deformities (22 ± 3.46%) but until the free veliger stage, almost every specimen was deformed (96.66 ± 5.77%). At 26^{o}C, an increased incidence of deformities was initially observed at trochophore larva stage (50.66 ± 22.7%) but it gradually decreased until eclosion (17.33 ± 7.02%).

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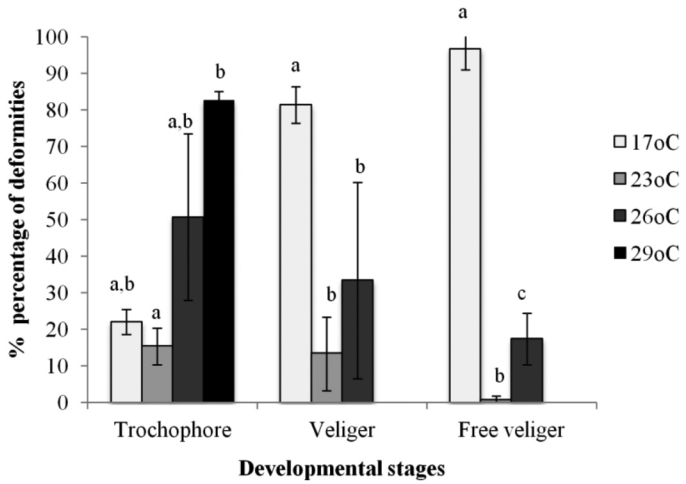

Fig.1: Percentage (%) of deformed larvae of *Charonia seguenzae* larvae developed at four different temperature regimes (17. 23, 26 and 29°C) at three developmental stages (trochophore, veliger and free veliger). Bars represent mean±SD; values indicated with different letter are significantly different ($p<0.05$).

Discussion

Mediterranean Triton's optimum developmental temperature seems to be the same as its optimum reproductive temperature, 23°C (Doxa et al., 2019). A possible temperature rise due to climate change will significantly affect the already endangered condition of *Charonia seguenzae* since the elevated temperatures of the present experiment led to high rates of deformities and, at 29°C, total mortality. Questions are raised about the reasons of the occurrence of such high deformity rates at 17°C, a temperature that prevails in the natural environment during early winter (Shaltout and Omstedt, 2014) when it is estimated that the egg capsules hatch. The high deformity rates at 17°C could be associated with the time window at which the lower temperature was applied.

Acknowledgments

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References

Cancino, J.M., Gallardo, J.A., Torres, F.A., 2003. Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus* (gastropoda: Muricidae). Mar. Biol. 142, 133–139.

Doxa, C.K., Sterioti, A., Divanach, P., Kentouri, M., 2019. Reproductive behavior of the marine gastropod *Charonia seguenzae* (Aradas & Benoit, 1870) in captivity. Mediterr. Mar. Sci. 20, 49–55.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp

Katsanevakis, S., Lefkaditou, E., Galinou-Mitsoudi, S., Koutsoubas, D., Zenetos, A., 2008. Molluscan species of minor commercial interest in Hellenic seas: Distribution, exploitation and conservation status. Mediterr. Mar. Sci. 9, 77-118.

Shaltout, M., Omstedt, A., 2014. Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia 56, 411–443.

VERTEBRAL COLUMN ADAPTATIONS IN ATLANTIC SALMON *Salmo salar,* L. PARR AS A RESPONSE TO DIETARY PHOSPHORUS

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, Ledeganckstraat 35, 9000 Ghent, Belgium
E-mail: Lucia.Drabikova@ugent.be

Introduction

Dietary phosphorus (P) deficiency is considered as a risk factor for the development of vertebral column deformities in farmed Atlantic salmon *Salmo salar*, L. Few studies have evaluated dietary P supply as a single experimental factor on bone formation, bone mineralisation and structure of the vertebral bodies in Atlantic salmon (Baeverfjord et al., 1998; Fjelldal et al., 2016; Smedley et al., 2018, Witten et al., 2016, 2019). The aim of the current study was, therefore, to investigate the morphology, structures and microstructures of cells and connective tissue in vertebral bodies of Atlantic salmon parr. Since vertebral centra compression and fusion, the most commonly observed deformity in Atlantic salmon (Fjelldal et al., 2007), arise within the intervertebral spaces (Witten et al., 2005, Ytteborg et al. 2010), particular attention was paid to the effects of low and high dietary P on the intervertebral joints and ligaments. The effect of varying dietary P levels was examined through the analysis of bone and plasma mineral content, vertebral centra stiffness, vertebral centra deformities as well as bone microstructure and cellular composition.

Materials and methods

The experiment was carried out at Lerang Research Station (Forsand, Norway). Triplicate groups of Atlantic salmon parr with a mean initial weight of 13 g were distributed over nine tanks (100L) at a density of 50 fish/ tank. The animals were fed three experimental diets on a continuous basis over the course of 85 days until the animals reached an average final weight of 44 g. Diets were based on a Skretting pre-smolt diet formulated to differ by the concentration of P only. Low P diet contained 0.68% of total P, 0.35% of soluble P, regular P diet contained 1.0% of total P, 0.56% of soluble P and high P diet contained 1.3% of total P, 0.93% of soluble P.

Results

Feed conversion ratio (0.78 ± 0.11) and feed intake (1.32 ± 0.18 % body mass day^{-1}) were similar in all diet groups. X-ray analysis showed high and regular P animals with fully mineralised vertebral bodies while distal ends of the vertebral body end plates in low P animals were not-mineralised (radiolucent). The area of radio dense vertebral bodies was thus reduced compared to regular and high P diet groups. Whole mount Alizarin red S stain-based diagnosis demonstrated that bone growth in low P animals continues with the development of extensive areas of non-mineralised (radiolucent) bone matrix. Despite a 50% reduction of mineral content of vertebrae and opercula and a 3-fold reduction of plasma inorganic phosphate in low P animals, the specific growth rate (SGR) was not reduced compared to the regular and high P animals (average SGR in all diet groups, 1.57 ± 0.12% body mass day^{-1}). The vertebral stiffness of the low P vertebral centra was reduced compared to regular and high P diet groups. Prevalence of deformities on a gross morphological (radiographic) level was low in all diet groups and types of vertebral centra deformities were not associated with dietary P levels. A histological examination of the vertebral centra revealed regularly formed vertebral body structures with well-developed intervertebral spaces and intervertebral ligaments in all diet groups. Intervertebral spaces did not show any alterations that foreshadow vertebral body fusion (Witten et al., 2005, Ytteborg et al. 2010). However, intervertebral ligaments in low P animals were lengthened and thickened, possibly to compensate for the reduced bone stiffness caused by the lack of minerals. Surprisingly, continuous feeding of the low P diet maintained growth without apparent immediate adverse health effect. Previous studies have shown similar results for post-smolts, albeit with reduced growth (Witten et al. 2016, 2019). The long term effects of dietary P deficient freshwater history are currently evaluated.

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References

Baeverfjord, G., Asgard, T., Shearer, K.D., 1998. Development and detection of phosphorus deficiency in Atlantic salmon , *Salmo salar* L., parr and post-smolts. Aquac. Nutr. 4, 1–11.

Fjelldal, P.G., Hansen, T.J., Berg, A.E., 2007. A radiological study on the development of vertebral deformities in cultured Atlantic salmon *(Salmo salar* L.). Aquaculture 273, 721–728. https://doi.org/10.1016/j.aquaculture.2007.07.009

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. https://doi.org/10.1111/anu.12238

Smedley, M.A., Migaud, H., McStay, E.L., Clarkson, M., Bozzolla, P., Campbell, P., Taylor, J.F., 2018. Impact of dietary phosphorous in diploid and triploid Atlantic salmon (*Salmo salar* L.) with reference to early skeletal development in freshwater. Aquaculture 490, 329–343. https://doi.org/10.1016/j.aquaculture.2018.02.049

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. https://doi.org/10.1242/jeb.188763

Witten, P.E., Gil-Martens, L., Hall, B.K., Huysseune, A., Obach, A., 2005. Compressed vertebrae in Atlantic salmon *Salmo salar*: Evidence for metaplastic chondrogenesis as a skeletogenic response late in ontogeny. Dis. Aquat. Organ. 64, 237–246. https://doi.org/10.3354/dao064237

Witten, P.E., Owen, M.A.G., Fontanillas, R., Soenens, M., Mcgurk, C., Obach, A., 2016. A primary phosphorus-deficient skeletal phenotype in juvenile Atlantic salmon *Salmo salar*: The uncoupling of bone formation and mineralization. J. Fish Biol. 88, 690–708. https://doi.org/10.1111/jfb.12870

Ytteborg, E., Torgersen, J.S., Pedersen, M.E., Baeverfjord, G., Hannesson, K.O., Takle, H., 2010b. Remodeling of the notochord during development of vertebral fusions in Atlantic salmon (*Salmo salar*). Cell Tissue Res. 342, 363–376. https://doi.org/10.1007/s00441-010-1069-2

ADAPTATION OF THE INDUSTRY 4.0. PRINCIPLES TO REALIZE THE NEXT GENERATION OF SMOLT PRODUCTION UNITS

E. Eilertsen*, E. Kelasidi, E. Svendsen, M. Føre, L.M. Sunde

Email: Espen.Eilertsen@sintef.no

Introduction

Automation and use of advanced technological solutions in different industries have made significant headway the past decades. This has consequently contributed to increased profits, more optimal production, reduced production time, better health and safety for employees and helped keep production in more expensive countries competitive against traditional low-cost production countries. Lately, there is trend both in the sea-based and land-based aquaculture industries to adapt automated system and advanced technological tools with the overall goal to increase the production, reduce the risk factors, be profitable and sustainable. However, while integral to the aquaculture industry, the smolt production facilities have not seen a proportional growth in the integration of automated systems and advanced technological tools, when compared to similar industries and its sea-based counterpart. Currently, there is a general trend in aquaculture industry shifting production methods from manual operations and experience-based reasoning towards a more objective approach. This is accomplished using intelligent sensors in combination with mathematical models and decision support- and autonomous systems in different stages of production. The present day smolt production plants, however, are still based on the same management principles and methods as the first generation of such facilities established in the 1980's, just in bigger scales.

Apart from some notable exceptions such as automation of vaccine distribution, RAS systems and some automated sorting and grading systems, manual labour and experienced based decision still dominates most of the day to day operations. Several of daily operations (e.g. tank cleaning, smoltification tracking, catching fish, removing dead fish, filling automated feeders, inspection of the population and welfare tracking) are labour dependent in current smolt production units. Therefore, we can see that there is lack of adapting a holistic approach to smolt production through development of novel technology for data collection and -analysis, autonomous operations and maintenance. To address these challenges, this paper presents results obtained in AUTOSMOLT2025 project [1] aiming to enable the future realization of standardized, predictable and intelligent smolt production by applying the principles of Precision Fish Farming (PFF) at different stages of the smolt production cycle [2], thus bringing smolt production closer to realization within the framework of Industry 4.0. This entails increasing the level of autonomy and objectivity in smolt production operations to reduce dependencies on manual labour and subjective assessments, and to improve accuracy, precision and repeatability.

Method

The AUTOSMOLT2025 project investigates the state of automation in the smolt production industry and derives the direction on how, and in which parts of the production phase, to increase the level of automation. The project focuses its research efforts towards three research areas: 1. Optimized smolt production, 2. Self- monitoring rearing tanks and 3. Autonomous tank operations. Some of the key elements in targeting these research challenges will be to facilitate a remote central control room, employ sensor technology to monitor fish and environmental conditions, and introduce actuators for interaction with the production process. In addition, such solutions will include autonomous maintenance policies determining how important and frequent operations such as grading, feeding, vaccination and cleaning/ disinfection of rearing tanks are to be handled by the autonomous systems. The obtained research outcomes from the project will lay the foundation for future unmanned, self-rearing and cost effective smolt production. In this paper we present results where the current state of the industry is analysed using SINTEFs "Seatonomy" method [3] and compared to other, similar industries like the oil and gas industry, the automotive and agriculture industries. The "Seatonomy" method was established by SINTEF to provide tools in order to identify and tackle challenges related to increase of level of automation in maritime industry. The main purpose of this method is to establish guidelines, principals and best practice methods when analysing and designing autonomous systems. The project also applies the principles of Precision Fish Farming (PFF) [2] when analysing the current state and designing the future systems for smolt production. PFF outlines how technologies and automation principles can be used to industrialise, digitise and improve operations in the fish farming industry. Even though PFF primarily discussed the principles related to sea-based farming operations, it can also be applied to land-based smolt production.

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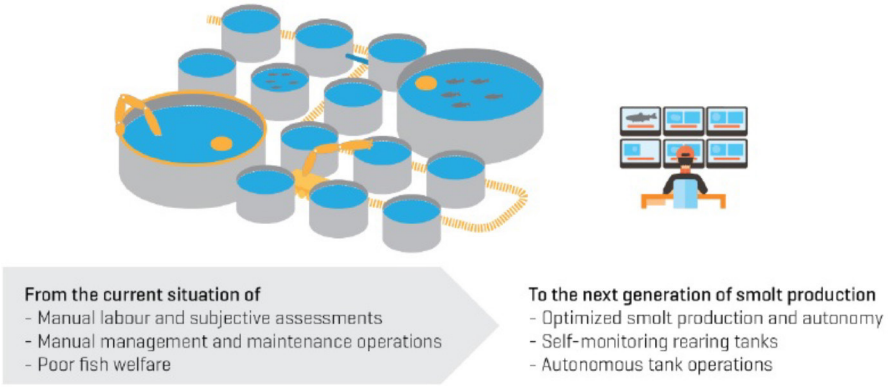

Figure 1: Foundation for the next generation of autonomous smolt production

Results

The results from this initial study and analysis of the current state and challenges is a holistic approach towards designing future smolt production facilities. In particular, the conditions and relevant rules for the transition from today's manual and labour dependent operations, to the fully autonomous smolt production facilities of the future, are identified. Based on this, a mathematical model for optimized and autonomous smolt production is derived with an overall vision to improve the internal logistics and realize just-in-time bio production. Furthermore, this paper includes results regarding the identification of system design requirements and specifications with respect to sensors and other instruments, system architecture, process control software, error management and safe modes. This holistic approach also derives the direction for robotic tool and sensor development in order to realize in the future optimized and sustainable smolt production. Figure 1 shows what the foundation for such a facility may look like. Consequently, the obtained results both contribute to increase the level of autonomy in smolt facilities by replacing manual labour with new technological solutions, and it improves the current decision making in the facility by gathering, processing and displaying relevant data in a comprehensive manner. The decision-making process could be further improved by also developing and implementing decision support systems based on machine learning to aid site managers to make more informed decisions on a daily basis. Therefore, by applying the derived principles, specifications and guidelines from Seatonomy method, it is expected that the project will reduce feed waste by tracking fish appetite, increase fish welfare by reducing the amount of handling operations necessary during a life cycle within the facility, secure a water quality, reduce spread of disease, track smoltification and reduce mortalities connected to mechanical damage and handling.

Discussion and conclusion

Nowadays there is a general trend in aquaculture industry to integrate more and more new technological tools and automated systems. However, most of the research and innovations have been focused on the sea-based production. This has led to land-based smolt production not being able to keep up with the industries increasing level of automation and push towards industry 4.0. Through targeted research and current state analysis, the need for new technologies and innovations have been identified, and a new roadmap toward the future of smolt production has been presented. This paves the way towards a paradigm shift in how smolt is produced, moving the industry away from the current trend of manual labour and experience-based decision making, and towards a production model based on autonomy and data driven decision making, based on the principals of Industry 4.0, instead.

References

[1] AUTOSMOLT2025, Partners: Scale Aquaculture AS (Project Owner), SINTEF Ocean AS (Project Leader), Wago Norway AS, Brimer AS, Festo AS, Posicom AS, Sinkaberg-Hansen AS, Andfjord Salmon AS, Salmon Evolution AS https://www.sintef.no/en/projects/autosmolt2025/ *Supported by the research counsel of Norway*

[2] M. Føre *et al.*, "Precision fish farming: A new framework to improve production in aquaculture," *Biosystems Engineering*, vol. 173. Academic Press, pp. 176–193, 01-Sep-2018.

[3] E. I. Grotli *et al.*, "SEATONOMY," in *OCEANS 2015 - MTS/IEEE Washington*, 2016

NUTRITIONAL VALUE OF FAT IN FILLETS OF COMMON CARP (*Cyprinus carpio* L.) FED n-3 PUFA ENRICHED DIETS

Sobczak M.[1], Panicz R.[1], Eljasik P.[1], Sadowski J.[2], Tórz A.[2], Barbosa V.[3], Marques A.[3], Dias J.[4]

[1] Department of Meat Science, Faculty of Food Science and Fisheries, West Pomeranian University of Technology, Szczecin, Poland
[2] Department of Aquatic Bioengineering and Aquaculture, Faculty of Food Science and Fisheries, West Pomeranian University of Technology, Szczecin, Poland
[3] Divisão de Aquacultura, Valorização e Bioprospecção. Instituto Português do Mar e da Atmosfera, I.P. Lisboa. Portugal.
[4] Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
Email: malgorzata.sobczak@zut.edu.pl

Introduction

Fish fat is a valuable source of essential fatty acids (EPA and DHA) in human diet. The highest levels are found in marine fish while freshwater species usually have considerably lower content of those acids in fillets. However, in case of freshwater fish, such as common carp (*Cyprinus carpio*), it is possible to profile quality of fat by appropriate feeding programme. Changes in composition feed fed to *C. carpio* affect proximate composition and profile of fatty acids and eventually nutritional value of a raw material. The aim of this study was to assess the influence of feed enriched with sustainable and natural feed ingredients (vegetable and salmon oil) on fat quality and nutritional value of common carp meat.

Material and methods

The study included two trials. First, Pilot Scale Trial (PST), tested four feeds fed to *C. carpio*: i) control (with 3% of soybean and 3% of rapeseed oil), ii) CB1 (with 4.1 % of rapeseed oil), iii) CB2 (with 5.1 % of rapeseed oil) and iv) CB3 (with 2% of soybean oil, 2% of rapeseed oil and 2.1% of salmon oil). Second, Farm Scale Trial (FST) in which fish were fed with two feeds: v) control (the same composition as PST control feed) and vi) experimental (with 6.1% of salmon oil). Fish were kept in cuboid cages of 3 m^3 (3 cages per feed, 100 fish per cage) The feeding trial was conducted for 100 days (PST) and 116 days (FST) during which fish were fed with the feed blends in equal portions at 9:00 and 15:00 hrs. At the end of the trials, n = 10 fish from each cage were slaughtered, following commercial practices, and filleted. Filleting was performed by one individual. All fish samples were stored at 4°C until analysis.

The chemical composition of minced fillets was determined according to AOAC procedures (Latimer, 2019). FA profiles were quantified by gas chromatography (GC) with a flame ionization detector (FID) in accordance with PN-EN ISO 12966-1:2015-01. Energetic value was calculated using the relative percentage of each nutrient (protein and fat) which was multiplied by the correction factors, 4 kcal g-1 (17 kJ g-1) and 9 kcal g-1 (37 kJ g-1) for protein and fat, respectively, as described in the Regulation (EU) No 1169/2011. Fat quality was described by the following factors: SFAs (sum of saturated FAs), MUFAs (sum of monounsaturated FAs), PUFAs (sum of polyunsaturated FAs), h/H (hypocholesterolemic/hypercholesterolemic ratio) calculated according to Fernández et al. (2007), IA (index of atherogenicity) calculated according to Ulbricht and Southgate (1991) and Fehily et al. (1994) and IT (index of thrombogenicity) calculated according to Fehily et al. (1994).

Results and Conclusions

The results from the Pilot Scale Trial showed highly significant ($p \leq 0.01$) influence of feed on the level of protein and fat in carp meat, its energetic value and fat quality. The highest content of protein, and the lowest level of fat and energetic value were observed in the control, while in CB3-fish fed feed enriched with salmon oil the opposite results were obtained. Fat in fillets of carp fed experimental feeds (CB1, CB2, CB3) had the highest levels of PUFA, especially EPA and DHA, the lowest levels of SFA and MUFA, and higher levels of quality indices (PUFA:SFA, UFA:SFA, IA, IT, h:H) comparing to nutritional recommendations.

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The information obtained during first trial were used to design finishing diets used for the ongrowing stage (Farm Scale Trial). In the experimental feed the source of fat was salmon oil retrieved from salmon by-products. Similarly, to PSC, feed significantly influenced proximate composition, energy value of meat as well as nutritional quality of fillet fat. Meat of carp fed CB diet had higher level of crude protein and lower content of crude fat and energy value comparing to control group. Moreover, fat had higher share of SFA and PUFA fatty acids, EPA and DHA content, n-3/n-6 PUFA ratio, PUFA:SFA ratio and IA but lower share of MUFA fatty acids, MUFA: SFA and UFA:SFA ratios, IT and h:H ratio. However, some of the indices of fat quality (i.e., IT, PUFA:SFA) were lower comparing to nutritional recommendations.

Irrespectively of feed fed to fish, meat of carp in FST had higher content of crude protein and 2-3-fold lower mount of crude fat comparing with PST. Muscular fat of FST carps was characterized by worse quality parameters than fat of PST fish, i.e., higher content of SFA, lower content of PUFA, sum of EPA and DHA, PUFA and SFA ratio, UFA and SFT ratio, indexes of atherogenicity and thrombogenicity as well as hypocholesterolemic/hypercholesterolemic ratio (h:H). In order to fulfil daily reference intake of EPA and DHA (250 mg) between 160 g and 240 g of meat of carp fed PST experimental diets is recommended, while in case of FST it is even up to approx. 840 g.

Concluding, the trials and subsequent chemical analysis showed that both source and quality of the fat in feeds significantly influenced proximate composition, energy level and nutritional value of carp meat. It should be also underlined, that fortification efficacy of fish meat with essential nutrients (EPA and DHA) might be also dependent on farming conditions (e.g., water temperature).

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References

Fehily, A.M., Pickering, J.E., Yarnell, J.W.G., Elwood, P.C. 1994. Dietary indexes of atherogenicity and thrombogenicity and ischaemic heart disease risk: The Caerphilly Prospective Study. Br. J. Nutr. 71, 249–257, DOI: 10.1079/bjn19940131.

Fernández, M., Ordoñez, J.A., Cambero, I., Santos, C., Pin, C., De la Hoz, L. 2007. Fatty acid composition of selected varieties of Spanish dry ham related to their nutritional implications. Food Chem. 101, 107–112, DOI: 10.1016/j.foodchem.2006.01.006.

Latimer, G.W. 2019. Official methods of analysis of AOAC international. (21st ed.). AOAC International: Gaithersburg, USA.

PN-EN ISO 12966-1:2015-01. 2015. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 1. Guidelines on modern gas chromatography of fatty acid methyl esters.

Regulation (EU) No 1169/2011. European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. *Official Journal of the European Union* 22/11/2011.

Ulbricht, T.L.V., Southgate, D.A.T. 1991. Coronary heart disease: Seven dietary factors. Lancet 338, 985–992, DOI: 10.1016/0140-6736(91)91846-M.

DEVELOPMENT OF A LOW-POWER UNDERWATER NFC-ENABLED DATA ACQUISITION FOR SEAWEED MONITORING

Caroline Peres[1], Masoud Emam*[1], Hamed Jafarzadeh[1], Marco Belcastro[1], Joanne Casserly[2], Frank Kane[2], Brendan O'Flynn[1]

[1] Tyndall National Institute, University College Cork, Cork, Ireland.
[2] Marine Institute, Rinville, Oranmore, Co. Galway, Ireland. H91 R673

E-mail: caroline.peres@tyndall.ie

Introduction

Aquaculture farming faces challenges to increase production while maintaining welfare of livestock, using resources efficiently, and being environmentally sustainable. To achieve this, remote and real-time monitoring of the farm environmental and biological conditions is highly important (Føre et al. 2018). The H2020 IMPAQT project (https://impaqtproject.eu/), funded by the European Union aims to develop and validate in-situ a multi-purpose, multi-sensing and multi-functional management platform for sustainable Integrated Multi-Trophic Aquaculture production (Troell et al. 2009)industry and policy makers as a promising opportunity for large-scale expansion of the aquaculture industry. Simultaneously, there has also been increased interest in both land-based and nearshore aquaculture systems which combine fed aquaculture species (e.g. finfish. As part of an IMTA Paradigm, seaweeds are at the intersection of many topical trends. They provide many inter-sectorial benefits: 1) they are a source of food and many other applications; 2) they provide several key ecosystem services; 3) they allow local diversification of a more balanced aquaculture industry; and 4) they participate in the dietary shift toward more decarbonized ocean-based sources of protein (Chopin and Tacon 2020). Global demand for seaweed is growing 8.1% per annum (Barbier et al. 2019) reflected in the industry with the establishment of new culture sites.

Environmental monitoring in an aquaculture setting is already well supplied by commercial off-the-shelf sensors that measure parameters such as temperature, light radiation, and water quality (dissolved oxygen, pH, salinity, nitrogen). However, these sensors usually measure only single parameters, which increases the power consumption and project cost. The collection of local data monitoring hydrodynamics and abiotic conditions combined with crop yield and crop quality throughout the growing cycle will help to maximise yields, optimise use of space, site selection and orientation, species selection, planting time and harvest dates. Wave and water movement are also important environmental factors that affect the production of seaweed and kelp, but the specifics are not completely understood (Hurd 2000). Wave sensors (such as wave-rider buoys) can be too expensive for some projects, which results in researchers and farm operators using computer modelling to estimate wave conditions that lack accuracy and specific local data (Focht and Shima 2020)two depth strata at each of six sites. Current monitoring solutions for seaweed and kelp also include satellite and aerial sensing, which cover large areas effectively. However, these methods do not offer high-resolution, specific local data for growing sites, and are usually limited by turbidity and weather conditions (Bennion et al. 2019)Ochrophyta.

Some research has been done to more finely monitor and log wave and water movement related to macroalgae, such as the work of (Stevens et al. 2002) in which an accelerometer was attached to a seaweed blade, and (Mullarney and Pilditch 2017)tilting of the stipe is largest toward the holdfast, whereas at infragravity frequencies, the stipe tilting is largest closer to the water surface. It is postulated that the stretching of blades and subsequent pull on the stipe is, in part, responsible for these patterns. This conclusion is supported by results of manipulative experiments, which show a more along-stipe uniform response after removal of blades from the kelp. The length of the kelp also exerts a strong control on the relative magnitudes of movements in the different frequency bands, with the swell band becoming more important relative to the infragravity band for shorter length kelp. These results indicate that kelp will differentially dissipate energy over both frequencies and varying depths within the water column. The variety of movement responses over differing wave forcing frequencies may also imply that there exist differing rates of breakage for kelp exposed to hydrodynamics stressors of multiple frequencies.","container-title":"Limnology and Oceanography","DOI":"https://doi.org/10.1002/lno.10587","ISSN":"1939-5590","issue":"6","language":"en","note":"_eprint: https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.1002/lno.10587","page":"2524-2537","source":"Wiley Online Library","title":"The differential response of kelp to swell and infragravity wave motion","volume":"62","author":[{"family":"Mullarney","given":"Julia C."},{"family":"Pilditch","given":"Conrad A."}],"issued":{"date-parts":[["2017"]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} in which an accelerometer logger was attached to kelp. Nonetheless, in these cases only an accelerometer was attached to the macroalgae, and additional sensors such as pressure and temperature had to be deployed separately. An integrated solution that monitors different biotic and abiotic factors would lessen the cost and time to deploy the system and provide useful information on the dynamic forces affecting the plants.

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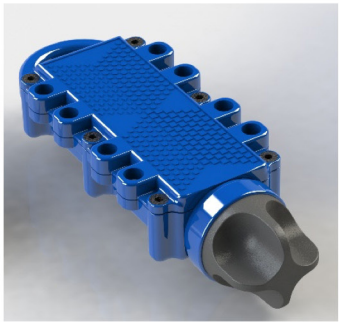
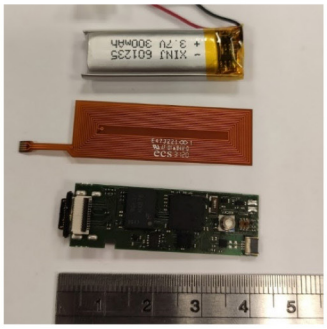
(a)  (b) 

Figure 1 - (a) 3D design of the waterproof enclosure with texture for gluing to macroalgae blades and eyeholes for threading attachment lines; (b) Internal components of the sensor device: rechargeable battery, custom-designed NFC antenna, and custom-designed printed electronic circuit board with pressure, temperature, light and motion sensors.

System

In this work, we present a novel miniature low-power NFC-enabled data acquisition system to monitor seaweed in an IMTA setting. This sensor system monitors temperature, light intensity, depth, and motion, logging the data collected internally. The sensor device can communicate with NFC-enabled readers (such as smartphones) to configure the sensors with custom sampling frequencies, communicate status, and to download data. It also has an on-board machine learning enabled microcontroller, which can be used to perform data analysis internally. The device is designed to be attachable to a variety of seaweed types, kelp blades or stipes: it has a textured surface on the bottom side for gluing the system onto the blades of the seaweed directly; it also has holes for threading safety threads to secure the device to the mooring line or to tie it to the stipe.

Results

The device was manufactured and is currently undergoing testing and characterisation. Deployment results will be presented. **Error! Reference source not found.** show the waterproof enclosure for the device and the embedded system that we designed and fabricated for this seaweed growth parameter monitoring device.

Future Work

Future work involves incorporating data analytics and machine learning algorithms to process data internally, allowing for lower transmission requirements and enabling autonomous decision making in regards to optimum growth parameters for the seaweed species under investigation.

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References

Barbier, M., Charrier, B., Araujo, R., Holdt, S.L., Jacquemin, B., Rebours. 2019. C. PEGASUS-PHYCOMORPH European Guidelines for a Sustainable Aquaculture of Seaweeds. COST Action FA1406: Roscoff, France.

Bennion, M., J. Fisher, C. Yesson, and J. Brodie. 2019. Remote Sensing of Kelp (Laminariales, Ochrophyta): Monitoring Tools and Implications for Wild Harvesting. Reviews in Fisheries Science & Aquaculture 27(2):127–141.

Chopin, T., and A. G. J. Tacon. 2020. Importance of Seaweeds and Extractive Species in Global Aquaculture Production. Reviews in Fisheries Science & Aquaculture 0(0):1–10. Taylor & Francis.

Focht, R. C., and J. S. Shima. 2020. Acceleration loggers reveal fine-scale heterogeneity in wave exposure along an open coast. Estuarine, Coastal and Shelf Science 233:106507.

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, and D. Berckmans. 2018. Precision fish farming: A new framework to improve production in aquaculture. Biosystems Engineering 173:176–193.

Hurd, C. L. 2000. Water Motion, Marine Macroalgal Physiology, and Production. Journal of Phycology 36(3):453–472.

Mullarney, J. C., and C. A. Pilditch. 2017. The differential response of kelp to swell and infragravity wave motion. Limnology and Oceanography 62(6):2524–2537.

Stevens, C. L., C. L. Hurd, and M. J. Smith. 2002. Field measurement of the dynamics of the bull kelp Durvillaea antarctica (Chamisso) Heriot. Journal of Experimental Marine Biology and Ecology 269(2):147–171.

Troell, M., A. Joyce, T. Chopin, A. Neori, A. H. Buschmann, and J.-G. Fang. 2009. Ecological engineering in aquaculture — Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297(1):1–9.

SUSTAINABLE DIETS TO PROMOTE AQUACULTURE ACROSS THE ATLANTIC VIA AQUAVITAE PROJECT

Sofia Engrola*[1], Luis T. Antelo[2], Gercende Courtois de Viçose[3], Clifford L.W. Jones[4], Luís André Sampaio[5], Lucas Simon Torati[6], Felipe do Nascimento Vieira[7]

[1] Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal;
[2] CSIC Institute of Marine Research (IIM - CSIC). Eduardo Cabello 6, 36208, Vigo, Spain;
[3] IU-ECOAQUA/Grupo de Investigación en Acuicultura (GIA), Universidad de Las Palmas de Gran Canaria (ULPGC), Las Palmas, Canary Islands, Spain;
[4] Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa;
[5] Laboratório de Piscicultura Estuarina e Marinha (LAPEM), Instituto de Oceanografia (IO), Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil
[6] EMBRAPA Pesca e Aquicultura, Palmas, TO CEP 77008-900, Brazil;
[7] Laboratório de Camarões Marinhos, Universidade Federal de Santa Catarina, Florianópolis 88061-600, Santa Catarina, Brazil;

* Presenting author: sengrola@ualg.pt

Introduction

There is a growing concern for the ability to produce enough food to feed the global human population in the future. If the global population reaches 9.6 billion by 2050, the equivalent of almost three planets will be required to sustain current lifestyles. To maintain the actual *per capita* average fish consumption without further improvements expected from fisheries, aquaculture production will have to increase by 70% and this will depend on its capacity to expand while reducing environmental impact. New biomasses able to accommodate aquaculture expansion need to be explored, so tackling this food demand in a transdisciplinary approach is vital for a better protection of the environment for future generations.

Intensive research made possible to increase aquaculture production using low environmental footprint diets that rely partially on land produced ingredients. In addition, currently farming carnivores' species with formulated diets is more acceptable since this strategy ensure a high conversion efficiency of fish-in fish-out ratio. The replacement of fishmeal in fish and shellfish diets by low trophic levels ingredients is a possible approach to create an aquaculture industry independent of fishmeal and agriculture ingredients and simultaneously animal production may be brought down several trophic levels. This will increase not only the environmental sustainability of the sector, but also its economic and social sustainability by adding value to new biomasses, decreasing the imports of fishmeal, and fomenting the use of good practices in the sector.

Objectives

The present work aims to increase system biological efficiency by including low trophic levels ingredients in diets to feed abalone, shrimp, freshwater and marine fish. The novel concepts of AquaVitae such as harvesting low trophic levels species, that additionally can be produced as side species associated to aquaculture production and included in animal diets to tackle this food demand, will be supported by the most advanced research and technology through the Atlantic Ocean. This challenge is being addressed in Brazil (EmBraPa, FURG, UFSC), South Africa (RhU), Spain (IIM-CSIC, ULPGC) and, Portugal (CCMAR).

All the research will contribute to the national and international sustainability agendas, mainly by promoting the achievement of the UN SDGs 2 (Zero hunger), 13 (response to climate change) and 14 (Life below water), although the results will also benefit progress in SDGs 1 (No poverty), 3 (good health and well-being), 8 (Decent work and economic growth) and 12 (Responsible consumption and production).

The proposed aims present a high potential to implement the findings into innovative products and services for the aquaculture industry and to increase the competitiveness of worldwide aquaculture, promoting the concept of modern aquaculture as an environmentally and economically sustainable practice to society.

Acknowledgements

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AquaVitae website: https://aquavitaeproject.eu

NANOPARTICLES IN AQUACULTURE: IS IT SOMETHING TO WORRY ABOUT?

Begoña Espiña[1,*], Mário J. Araújo[2], Nuria Barros[1], Pilar Bermejo-Barrera[3], Santiago Cabaleiro[4], Alexandre Campos[2], Miguel A Correa-Duarte[5], Elza Fonseca[2], Mick Mackey[6], Julie Maguire[6], Antonio Moreda-Piñeiro[3], Ivone Pinheiro[1], Mónica Quarato[1], Laura Rodriguez-Lorenzo[1], Doriane Stagnol[7], María Vázquez[4]

[1] Water Quality Group, INL-International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal
[2] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal
[3] Department of Analytical Chemistry , Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela , Santiago de Compostela , Spain
[4] Centro Tecnológico del Cluster de la Acuicultura (CETGA), A Coruña, Spain
[5] Department of Physical Chemistry, Center for Biomedical Research (CINBIO), Southern Galicia Institute of Health Research (IISGS), Vigo 36310, Spain
[6] Indigo Rock Marine Research, Gearhies, Bantry, Co. Cork, P75 AX07
[7] Pole Aquimer, 16 rue du Commandant Charcot - CS 10381, 62206 Boulogne-sur-Mer cedex, France
E-mail: begona.espina@inl.int

Engineered inorganic nanoparticles (ENPs) are emerging as a new class of pollutants with eco-toxicological impacts on marine ecosystems [1]. In addition, aquaculture is a key component of both the Common Fisheries Policy and the Blue Growth agenda and currently makes up over 50 % of the fish and seafood destined for human consumption [2]. Consequently, it is necessary for rigorous human and environmental risk assessment to consider fate, behaviour and exposure scenarios, not only related to bare nanoparticles (NPs) but also to commercialized nanomaterials throughout their life cycle [3]. Among the numerous NPs currently in production and use, silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO_2NPs) are emerging for their extensive application in consumer products, including textiles, paints or health-care products [4].

The focus of the project are the aquatic ecosystems related to aquaculture and specifically the organisms used for human consumption such as turbot, mussels, clams, seaweed, sea urchins, among others.

INTERREG Atlantic Area is an European funding programme that promotes cooperation among five European countries. NANOCULTURE is a consortium focused in aquaculture sector, which has high economic relevance in Atlantic area.

NANOCULTURE promotes a sustainable development of the aquaculture sector and will help to establish effective regulations supporting a safe and non-toxic use of ENPs through:
• The assessment of the presence, accumulation and transformation of NPs in aquaculture products.
• Sensors development for the rapid NPs identification in aquaculture facilities.
• The evaluation of the human risk of exposure posed by the presence of NPs.

We will present the main methodologies used in the NANOCULTURE project, which is in its mid-term. More information can be found in our project webpage: http://nanoculture.ciimar.up.pt/

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References
1. Matranga V, Corsi I. Toxic effects of engineered nanoparticles in the marine environment: model organisms and molecular approaches. Mar Environ Res. 2012 May; 76:32-40.
2. https://ec.europa.eu/fisheries/cfp/aquaculture/
3. Botta C, Labille J, Auffan M, Borschneck D, Miche H, Cabié M, Masion A, RoseJ, Bottero JY. TiO_2-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: structures and quantities. Environ. Pollut. 2011 Jun; 159(6):1543-50.
4. Wang H, Burgess RM, Cantwell MG, Portis LM, Perron MM, Wu F, Ho KT. Stability and aggregation of silver and titanium dioxide nanoparticles in seawater: role of salinity and dissolved organic carbon. Environ Toxicol Chem. 2014 May; 33(5):1023-9.

ATLANTIC SALMON *Salmo salar* POST-SMOLT WELFARE AND PERFORMANCE IN FISHGLOBE SEMI-CLOSED CONTAINMENT SYSTEM

Åsa Maria Espmark[1*], Kevin Stiller[1], Khurram Shahzad[1], Britt Kristin Reiten[1], Yuriy Marchenko[1], Jascha Gerwins[1], Filip Strand Radonjic[1], Bernhard Eckel[1], Carlo Lazado[1] og Arne Berge[2]

[1]Nofima AS, Breivika NO-9291 Tromsø, Norway
*e-mail: asa.espmark@nofima.no
[2]FishGLOBE AS, NO-4068 Stavanger, Norway

Introduction
Floating semi-closed containment systems for aquaculture (S-CCS) are based on systems where a physical barrier, built of either solid or flexible material, separate fish from the external environment, protecting the fish against sea lice (*Lepeophtheirus salmonis*) and escapees. Water is pumped into the rearing system from depths below the sea lice layer. Until now, most S-CCS are housing post-smolt salmon up to approximately 1kg before they are transferred to open net pens. Studies have shown that fish performance and welfare in S-CCS are equally good (Nilsen et al., 2020) or even better (Øvrebø 2020) compared to the traditional open net pens. There are different types of semi-closed containment systems available, and one of them is the 3500m^3 FishGLOBE (Figure 1), placed in Lysefjorden, Rogaland county in south-west part of Norway.

According to the producer of FishGLOBE, the use of over- and under pressure cause gentle transport of fish in and out of the globe. The large amount of water that is required is easily pumped into the tank and is exchanged two - three times per hour. This facilitates good welfare and optimal removal of faeces and uneaten food from the tank bottom.

Norwegian law obliges producers of technologies used with fish to test the equipment to ensure that they meet fish welfare requirements. The aim of the study presented here were to test fish welfare and performance in FishGLOBE tank, and to compare the results with welfare and performance both before entering the tank, and after transfer to an open net pen.

Material and Methods
One fish group (N = 200 000) was followed from a commercial RAS facility, into the FishGLOBE, and also after transfer to an open net pen at a commercial fish farm. Water quality parameters at low (13.2 kg/m^3) and maximum (62.2 kg/m^3) densities showed that O_2 > 90% at both densities and CO_2 < 1.5 mg/l at the lowest density. CO_2 was not measured at maximum density due to sensor failure. Biological data were sampled at four sampling points; S0 (November) = base line sampling in RAS; S1 (January) = three weeks after transfer to FishGLOBE; S2 (March) = 2.5 months after transfer to FishGLOBE and two weeks before transfer to open net pen; S3 (July) = three months after transfer to open net pen at commercial fish farm.

Results and Discussion
Accumulated mortality inside the FishGLOBE was 1.4%, but increased to 4.9% at S3 sampling point, three months after transfer to open net pen. This study lacked a reliable reference cage that would enable comparison between S-CCS grown fish and fish that were grown in open net pen, but the mortalities were low compared to commercial data, Nilsen et al. (2020) and Øvrebø (2020).

Fish weight at the different sampling points were 238.3 ± 57.3g (S0), 268.9 ± 114.5g (S1), 720.0 ± 291.9g (S2) and 1395.7 ± 376.7g (S3). Expressed as SGR and TGC, the best fish growth was when the fish were inside the FishGLOBE (S1 – S2) (Figure 2).

A mild increase in plasma magnesium at S2 (Figure 3) may indicate a slight lower sea water tolerance, but the lower levels at S3 shows that the fish coped well with the transfer to full strength sea water.

External welfare score did not show damages as result from the FishGLOBE. In increase in *hsp90* in the period S0 – S2 may support the findings from Mg and a slight osmotic imbalance. An increase in *Mucin* in the period S1 – S2 may also indicate stress but can also indicate strengthening of the skin. Skin histology showed an increased general appearance and surface quality in the period S1 – S2 (Figure 4).

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References
Nilsen A., Nielsen K.V., Bergheim A. (2020). https://doi.org/10.1016/j.aquaeng.2020.102124
Øvrebø T.K. (2020). Growth performance and welfare of post-smolt (*Salmo salar* L.) reared in semi closed containment systems (S-CCS) – a comparative study. Master thesis, University of Bergen, 144pp.

EFFECTS OF SUBCUTANEOUS INJECTION OF CARRAGEENIN TO GILTHEAD SEABREAM (*Sparus aurata*) SPECIMENS ON SKIN MUCUS HUMORAL IMMUNITY

J.C. Campos-Sánchez, F.A. Guardiola, J.M. García Beltrán, D. Ceballos□Francisco, 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)
aesteban@um.es

Introduction

Inflammation is a well-characterized process in mammals, but it has been poorly studied in fish (Esteban, 2012). Among the diverse methods and strategies which have been used to study and reproduce inflammation in mammals, carrageenin, a mucopolysaccharide derived from the cell walls of red algae *Chondrus crispus* has had a high-extended use for decades as a model of acute inflammation in rats and rabbits (Winter *et al*., 1962). However, it has not been studied whether this alga can trigger the inflammation process in fish, as well as, modulate its immune system (Timur *et al*. 1997). For this main reason, the present study aims to evaluate the modulation of the humoral immune response in the skin mucus of gilthead seabream (*Sparus aurata*) after a subcutaneous injection of carrageenin.

Material and methods

In this study, thirty-six specimens (10.81 ± 2.8 g, 2.78 ± 0.7 cm) of gilthead seabream (*Sparus aurata*) obtained from a local farm (Mazarrón, Spain) and climatized in the Marine Fish Facilities at the University of Murcia (Spain), were injected subcutaneously with 50 μl of phosphate-buffered saline (PBS, as control) or carrageenin solution (1%, Sigma) in PBS. Skin mucus was collected by the dorsolateral surface from the injected area and homogenized with 1 volume of sterile seawater at 1.5, 3 and 6 hours post-injection. Then, mucus samples were vigorously shaken and centrifuged, and stored at -20ºC until use (Guardiola *et al*, 2014). The following parameters were analysed: SOD activity peroxidase activity catalase activity, lysozyme activity, bactericidal activity against *Vibrio anguillarum,* and *Photobacterium damselae,* protease activity and total immunoglobulin levels (Guardiola *et al*, 2016). The results were expressed as mean ± standard error of the mean (SEM) and Data were analysed by One-way ANOVA (followed by Tukey tests) to determine differences between experimental groups and each group with respect to time, respectively. The level of significance used was $p < 0.05$ for all statistical tests.

Results and discussion

Results obtained in the skin mucus of fish demonstrate that SOD activity was increased in fish from the carrageenin group at 1.5, 3, and 6 hours post-injection, compared to the values recorded in the skin mucus of fish from the control group (injected with PBS) (Wagener *et al*, 2013). Most of the studied activities were altered after 3 hours of carrageenan administration. More concretely, injection with carrageenin increased the peroxidase, lysozyme, bactericidal activity (against *V. anguillarum* and *P. damselae*), and total immunoglobulins level in the skin mucus of fish sampled 3 hours post-injection, in comparison to the values observed in the fish injected with PBS (control). In addition, lysozyme and protease were increased and decreased, respectively, in fish injected with carrageenin and sampled 6 h post-injection, in comparison to the control group. Therefore, carrageenin seems to stimulate the release of antioxidant and bactericidal molecules to the skin mucus by the cells neighboring the injection area, as a response to the inflammation triggered (Larsen & Henson, 1983).

Conclusion

The present results suggest that carrageenin is a good stimulator of inflammation in fish. Carrageenin is able to modulate the gilthead seabream immune response. These results could be used in further studies in order to clarify the mechanism of this complex process of the immune system in fish of commercial interest, as well as its possible resolution.

(Continued on next page)

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References

Esteban, M.Á. An Overview of the Immunological Defences in Fish Skin, ISRN Immunol. 2012 (2012) 1–29.

Guardiola, F.A., Cuesta A., Arizcun, M., Meseguer J., Esteban M.A., Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*), Fish Shellfish Immunol. 36 (2014) 545–551.

Guardiola, F.A., Cuartero M., Collado-González, M.M, Díaz Baños, F.G., Cuesta, A., Moriñigo M.Á, Esteban, M.Á. Terminal carbohydrates abundance, immune related enzymes, bactericidal activity and physicochemical parameters of the Senegalese sole (*Solea senegalensis*, Kaup) skin mucus, Fish Shellfish Immunol. 60 (2016) 483–491.

Larsen, P.M., & Henson, G. L. Mediators of inflammation, Semin. Arthritis Rheum. 14 (1983) 247–262.

Timur, M., Roberts, R.J., McQueen, A. Carrageenin granuloma in the plaice (*Pleuronectes platessa*); a histopathological study of chronic inflammation in a teleost fish., J. Comp. Pathol. 87 (1977) 89-96.

Wagener, F.A.D.T.G., Carels, C.E., Lundvig, D.M.S. Targeting the redox balance in inflammatory skin conditions, Int. J. Mol. Sci. 14 (2013) 9126–9167.

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.

SUSTAINABLE PRODUCTS AND CANNING INDUSTRY BY-PRODUCTS AS INGREDIENTS IN AQUAFEEDS FOR MEAGRE JUVENILES

A.Estevez[a], M. Soula[b], M. Ferreira[b], L. Fernández[c]

a. IRTA Centre of San Carlos de la Rápita, 43540 Tarragona, Spain
b. ANFACO, 36310 Vigo, Spain
c. CARTIF, 47151 Valladolid, Spain
alicia.estevez@irta.es

Introduction
Aquafeeds are formulated to contain all the essential nutrients that farmed fish need to grow, be healthy and maintain their nutritional properties for human consumption. The use of marine products (fish meal and fish oil) have been substituted with plant-derived proteins and oils in order to reduce the fish-in:fish out (FIFO) ratio. In recent years other sustainable products and food industry by-products have been incorporated to feed formulations due to their good nutritional value and economic effects (round economy), among them insects and microalgae have been widely used, but other food industry by-products such as those generated in canning factories are considered as good candidates to be included in aquafeeds.

Material and Methods
Feed formulation of the different feeds used in the study such as insects (INS), microalgae (MICRO) and canning factory byproducts (protein and lipid fractions recuperated from tuna water coocking –CAN-) and a mix of all of them (MIX) compared to a control (CTRL) diet is included in Table 1.

Meagre juveniles (12.5 g initial weight, N=50) were distributed in triplicated 200 L tanks and fed the experimental feeds for 65 days. Two weeks before final sampling faeces were collected by abdominal stripping to evaluate the apparent digestibility coefficients (ADC) of the feeds. At the end of the trials, growth (specific –SGR- and relative –RGR- growth rates), hepatosomatic index, food conversion and protein efficiency ratios were calculated for all the treatments. At the end of the study samples of muscle and liver were also taken for biochemical analyses and fatty acid profile.

Results
Fish growth and apparent digestibility coefficients of the diet and the ingredients used in the first trial are presented in Tables 2 and 3. The proximate composition of muscle and liver of the fish at the end of the study is presented in Table 4.

All the groups showed a very high growth rate (between 6.4 and 7.5 times the initial weight) and very good FCR (lower than 1). Significant differences (ANOVA $P<0.05$) were only obtained in RGR, FCR and PER for the fish fed the mix diet, showing a relatively higher growth rate but worse results in terms of feed conversion and protein efficiency.

Feed ADC for protein and lipids was very high for all the feeds, between 70 and 86% and similar among all the groups.

Protein content of the fillet was significantly higher for the fish fed MIX diet followed by those fed insects and canning byproducts, whereas lipids were higher for the fish fed INS diet. In the case of the liver, protein was significantly higher for the fish fed CAN and lipids higher In the fish fed INS.

Conclusion
Based on the results obtained all the ingredients used: insects, microalgae and canning byproducts can be used as fish meal replacement in aquafeeds formulae for the ongrowing of marine fish. Protein content of the fillet was very high in all the cases (79-90%), especially in the fish are fed the mixture of all the ingredients. A slightly higher content of lipids was found in the liver of the fish fed insect meal.

(Continued on next page)

Table 1.- Formulation of feeds used in the study with meagre juveniles

Ingredients (%)	CTRL	INS	MICRO	CAN	MIX
Fish meal 65/67%	23.76	21.46	20.00	21.63	20.00
Squid meal					4.41
Soybean meal	14.60	11.88	4.46	11.23	
Gluten meal	15.00	8.32	15.00	10.57	1,61
Guar meal	6.00	6.00		6.00	
Soy Concentrate (60%pb)	3.00	3.00	3.00	3.00	
Krill oil	4.00				
Salmon oil	7.20	8.55	10.92		
Starch	6.68	7.97	6.32	7.14	1.14
Wheat gluten	3.57		3.54	4.23	15.00
Wheat	6.00	6.00	6.00	6.00	17.12
Wheat gluten			12.00		
Lysine	0.28	0.27	0.50	0.50	0.50
Methionine	0.07	0.14	0.05	0.18	0.09
Threonine		0.07	0.32	0.29	0.50
Taurine	0.16	0.18	0.17	0.18	0.15
Yeast	3.51	5.00	0.90	5.00	
Coline chloride	0.16	0.16	0.16	0.16	0.16
Vitamin Premix	0.10	0.10	0.10	0.10	0.10
Vitamin C	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.10	0.10	0.10	0.10	0.10
Monocalcium phosphate			0.67		0.60
Emulsifier	0.20	0.20	0.20	0.20	0.20
Other additives	5.34	5.25	5.25	5.25	2.54
Attractants	0.10	0.10	0.10	0.10	0.10
Canning Protein Fraction				7.00	2.00
Canning Lipid Fraction				10.89	9.43
Microalgae			10.00		10.00
Acheta domesticus		15.00			15.00

Table 2.- Results of the initial and final weight of the fish used in the study, specific (SGR) and relative (RGR) growth obtained. Hepatosomatic (HIS) and viscerosomatic (VSI) indices, food conversion (FCR) and protein efficiency (PER) ratios obtained at the end of the study

	Initial weight (g)		Final weight (g)		HSI		VSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	12,61	1,55	87,24	16,83 a	2,89	1,25	5,58	1,20	2,76	0,02	5,92	0,09 a	0,60	0,02 b	3,52	0,11 b
INS	12,50	1,38	79,75	17,17 b	3,26	1,52	5,78	1,46	2,65	0,01	5,38	0,03 b	0,65	0,01 b	3,77	0,04 b
MICRO	12,44	1,44	79,04	12,58 b	2,49	1,45	5,16	1,53	2,68	0,06	5,35	0,10 b	0,64	0,03 b	3,76	0,15 b
CAN	12,52	1,58	80,17	14,06 b	2,00	1,50	4,64	1,29	2,65	0,03	5,41	0,12 b	0,64	0,01 b	3,61	0,04 b
MIX	12,47	1,46	89,63	15,75 a	1,98	0,46	4,19	0,57	2,11	1,41	6,20	0,34 a	0,79	0,05 a	2,92	0,18 a
ANOVA			P<0.001		P=0.70		P=0.543		P=0.732		P<0.001		P<0.001		P<0.001	

Table 3.- Apparent digestibility coefficients of the feeds used in the study

MEAGRE	CTRL	INS	MICRO	CAN	MIX
Protein	72,76	73,76	77,07	74,67	79,96
Lipids	86,11	84,83	79,86	78,15	80,72

Feed ADC for protein and lipids was very high for all the feeds, between 70 and 86% and similar among all the groups.

Table 4.- Proximate composition of meagre muscle and liver fed the different diets used in the study

	MUSCLE						LIVER					
	Water (%)		Protein (%)		Lipids (%)		Water (%)		Protein (%)		Lipids (%)	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	77.41	0.34b	78.89	1.76c	3.75	0.06c	30.17	0.14c	19.58	0.63e	37.61	1.40b
INS	77.03	0.36b	84.11	1.22b	4.74	0.02a	59.22	0.19e	22.91	0.77c	43.06	0.72a
MICRO	77.55	0.23b	79.05	1.07c	3.63	0.04c	59.86	0.13d	24.87	0.50b	42.11	0.56a
CAN	78.64	0.14a	86.40	1.54b	3.98	0.14b	65.38	0.06a	27.27	0.27a	35.63	0.54b
MIX	77.35	0.16b	90.16	1.61a	3.29	0.07d	61.76	0.04b	20.96	0.08d	36.21	2.21b
ANOVA	P<0.001		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001	

Protein content of the fillet was significantly higher for the fish fed MIX diet followed by those fed insects and canning byproducts, whereas lipids were higher for the fish fed INS diet. In the case of the liver, protein was significantly higher for the fish fed CAN and lipids higher In the fish fed INS

BREWERY BY-PRODUCTS AS PROTEIN INGREDIENTS IN RAINBOW TROUT AQUAFEEDS

A. Estévez[a]*, B. Iñarra[b], M. Orive[b], D. San Martin[b]

a. IRTA Centre of San Carlos de la Rápita, 43540 Tarragona, Spain
b. AZTI, Food Research, Basque Research and Technology Alliance (BRTA). Derio, 48160 Vizcaya, Spain
alicia.estevez@irta.es

Introduction

Aquafeeds are formulated to contain all the essential nutrients that farmed fish need to grow, be healthy and maintain their nutritional properties for human consumption. The use of marine products (fish meal and fish oil) have been substituted with plant-derived proteins and oils in order to reduce the fish-in:fish out (FIFO) ratio. The brewing sector holds a strategic economic position in Europe with an annual production of about 396 million hectoliters, and in the process more than 7 million Tons of spent grain (SG) and spent yeast (SY, Beer statistics, 2018) are produced. Thus, the increasing demand of aquaculture derived products makes the aquafeed valorization route one of the most promising alternatives for the massive recovery of brewer's by-products. The first step of this Life project (LIFE16ENV/ES/000160) was to develop a process for mechanical dehydration to reduce humidity followed by a flash drying to reduce moisture below 10%. A hydrolysis process was also used as a pre-treatment before dehydration to increase the digestibility. In this way 4 products: spent yeast dried (DSY) and hydrolysed (HSY) and spent grain dried (DSG) and hydrolysed (HSG) were produced and used in a trial to check their acceptance by rainbow trout juveniles, used as a Mediterranean freshwater aquaculture species model.

Material and Methods

Feed formulation used in the digestibility trial is included in Table 1. A commercial yeast, also dried (DY-ABN) and hydrolysed (HY-ABN, Spain) was also included at 10 and 20% using the same formulation as in Table 1, as a reference to compare the digestibility results.

Rainbow trout juveniles (78 g initial weight, N=20) were distributed in duplicated 200 L tanks and fed the experimental feeds for 60 days. At the end of the trials, growth (specific –SGR- and relative –RGR- growth rates), hepatosomatic index (HSI), food conversion (FCR) and protein efficiency (PER) ratios were calculated for all the treatments. Two weeks prior to final sampling faeces samples were collected by stripping to analyse the apparent digestibility coefficients for proteins and lipids of the feeds and ingredients

Results

Fish growth and apparent digestibility coefficients of the diet and the ingredients used in the first trial are presented in Tables 2 and 3.

Significant differences in final weight, HIS, FCR and PER were obtained at the end of the trial, being higher for the fish fed DSY and HSY and DY-ABN and lower for the fish fed the control feed and HSG.

Feed digestibility for protein and lipids was very high for all the feeds, between 80 and 90% for all the feeds used in the study. In the case of the ingredients a higher protein ADC was obtained for the brewery's spent yeast and commercial yeast, whereas spent grain gave the lowest results. The results of fillet and liver biochemical composition and fatty acid profile will be presented in the congress

Conclusion

Based on the results obtained, brewery by products can be used as fish meal replacement (up to 15-20%) in aquafeeds formulae for the ongrowing of freshwater fish such as rainbow trout, although lower growth and conversion efficiency was obtained using spent grain, probably due to its lower digestibility.

(Continued on next page)

Table 1.- Formulation of feeds used in the digestibility trial with sea bream. D: Dried, H: Hydrolysed, SG: spent grain, SY: spent yeast

	CTRL	(D&H) SG7.5	(D&H) SG15	(D&H) SY10	(D&H) SY20
Ingredient					
Wheat gluten	8.00	7.40	3.60	4.90	1.63
Hi Pro Soy bean meal	6,00	6,00	2.52	4.06	1.00
Wheat gluten	13.78	13,00	14.8	14.62	15.15
SPC	17.00	17,00	17,00	17,00	17,00
Fish oil NA	7.75	7.74	7.72	7.50	7.38
Fish meal NA	15.00	15.00	15.00	15.00	15.00
Rapeseed oil	5.85	5.91	6.12	5.78	5.77
Lutavit C Aquastab 35%	0.01	0.01	0.01	0.01	0.01
Phosphate	0.83	0.75	0.65	0.7	0.65
Choline	0.27	0.27	0.27	0.27	0.27
Lysine HCl	0.36	0.20		0.09	
Mineral mix with iodine 2/04	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.10	0.10	0.10	0.10	0.10
Wheat	15.95	17.66	14.65	16.77	13.6
Soy lecitihin	2.00	2.00	2.00	2.00	2.00
spent grain		7.50	15.00		
Brewer's spent yeast				10.00	20.00

Table 2.- Results of the initial and final weight of the fish used in the study, specific (SGR) and relative (RGR) growth obtained, Hepatosomatic index (HIS), Feed conversion (FCR) and Protein Efficiency (PER) Ratios obtained at the end of the study

	Initial weight (g)		Final weight (g)		HSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	79.33	8.65	174.28	34.56a	2.03	0.61a	2.53	0.10	119.44	6.67	1.29	0.05a	1.19	0.07a
DSY10%	77.10	9.33	**217.88**	**48.84b**	**4.71**	**2.05b**	3.35	0.38	200.95	8.53	**1.81**	**0.05b**	**2.01**	**0.09b**
DSY20%	77.42	10.07	**219.68**	**28.75b**	3.76	0.88ab	3.37	0.17	184.13	15.25	**1.71**	**0.09b**	**1.84**	**0.15b**
HSY10%	78.82	9.60	**217.37**	**45.87b**	4.26	1.28ab	3.27	0.10	175.33	8.80	**1.66**	**0.05b**	**1.75**	**0.09b**
HSY20%	77.56	9.67	**222.80**	**31.48b**	**4.46**	**1.44b**	3.40	0.08	187.30	7.42	**1.73**	**0.04b**	**1.87**	**0.07b**
DSG7.5%	77.95	8.80	193.38	37.55ab	2.94	1.51ab	2.93	0.21	148.11	16.11	1.49	0.11ab	1.48	0.16ab
DSG15%	77.63	7.52	192.90	29.14ab	2.88	0.88ab	2.94	0.07	148.48	5.30	1.49	0.03ab	1.48	0.05ab
HSG7.5%	77.39	8.79	178.40	38.85a	2.14	0.53a	2.70	0.08	130.66	5.53	1.37	0.03a	1.31	0.06a
HSG15%	77.92	8.37	175.73	39.02a	2.32	0.53a	2.61	0.29	125.08	20.14	1.33	0.15a	1.25	0.20a
DY-ABN10%	77.41	9.55	206.19	62.02ab	3.30	0.73ab	3.16		166.37		1.61		1.66	
DY-ABN20&	48.13	10.06	**228.63**	**43.21b**	2.50	0.07ab	3.46		192.62		1.76		1.93	
HY-ABN10%	77.19	8.10	216.36	35.36ab	4.07	0.85tab	3.32		180.31		1.69		1.80	
HY-ABN20%	78.15	10.45	202.94	38.58ab	3.47	0.79ab	3.08		159.67		1.56		1.60	
ANOVA			P<0.001		P<0.001		P=0.006		P=0.007		P<0.001		P<0.001	

Table 3.- Apparent digestibility coefficients of the feeds with the highest level of inclusion (20% in the case of yeast and 15% for spent grain) and ingredients used in the study (DCY and HCY = dried and hydrolysed commercial yeast from ABN)

			Digestibility of the Feed				
RAINBOW TROUT	**CTRL**	**DSY20**	**HSY20**	**DSG15**	**HSG15**	**DCY20**	**HCY20**
Protein	89.86	86.63	86.80	87.62	86.63	87.71	87.15
Lipids	80.87	87.44	88.65	84.20	82.07	85.86	86.14

	Apparent Digestibility Coefficients of Ingredients					
RAINBOW TROUT	**D-Yeast**	**H-Yeast**	**D-Spent grain**	**H-Spent grain**	**D-Yeast ABN**	**H-Yeast ABN**
Protein	74.52	75.37	65.10	49.93	48.62	76.09

BREWERY BY-PRODUCTS AS PROTEIN INGREDIENTS IN GILTHEAD SEABREAM AQUAFEEDS

A. Estévez[a]*, B. Iñarra[b], M. Orive[b], D. San Martin[b]

a. IRTA Centre of San Carlos de la Rápita, 43540 Tarragona, Spain
b. AZTI, Food Research, Basque Research and Technology Alliance (BRTA). Derio, 48160 Vizcaya, Spain
alicia.estevez@irta.es

Introduction

Aquafeeds are formulated to contain all the essential nutrients that farmed fish need to grow, be healthy and maintain their nutritional properties for human consumption. The use of marine products (fish meal and fish oil) have been substituted with plant-derived proteins and oils in order to reduce the fish-in:fish out (FIFO) ratio. The brewing sector holds a strategic economic position in Europe with an annual production of about 396 million hectoliters, and in the process more than 7 million Tons of spent grain (SG) and spent yeast (SY, Beer statistics, 2018) are produced. Thus, the increasing demand of aquaculture derived products makes the aquafeed valorization route one of the most promising alternatives for the massive recovery of brewer's by-products. The first step of this Life project (LIFE16ENV/ES/000160) was to develop a process for mechanical dehydration to reduce humidity followed by a flash drying to reduce moisture below 10%. A hydrolysis process was also used as a pre-treatment before dehydration to increase the digestibility. In this way 4 products: spent yeast dried (DSY) and hydrolysed (HSY) and spent grain dried (DSG) and hydrolysed (HSG) were produced and used in a trial to check their acceptance by gilthead seabream juveniles, used as a Mediterranean aquaculture species model. A further step (a validation trial) using a higher inclusion level (30%) of these 4 products and a reduction of fish meal was designed in order to validate the use of these by products as protein ingredients and the results will bepresented in the Congress.

Material and Methods

Feed formulation used in the digestibility trial is included in Table 1. A commercial yeast, also dried (DY-ABN) and hydrolysed (HY-ABN, Spain) was also included at 10 and 20% using the same formulation as in Table 1, as a reference to compare the digestibility results.

Gilthead seabream fish (94 g initial weight, N=20) were distributed in duplicated 200 L tanks and fed the experimental feeds for 60 days. At the end of the trials, growth (specific –SGR- and relative –RGR- growth rates), hepatosomatic index, food conversion and protein efficiency ratios were calculated for all the treatments. Two weeks prior to the end of the trial, samples of faeces were collected by abdominal stripping to analyse apparent digestibility coefficients of feeds and ingredients.

Results

Fish growth and apparent digestibility coefficients of the diet and the ingredients used in the first trial are presented in Tables 2 and 3.

No significant differences in final weight, hepatosomatic index or growth rates were found at the end of the feeding period (60 days). Protein Efficiency Ratio (PER) was significantly higher for the fish fed dried yeast included at 20% and lower in the control group

Feed digestibility for protein and lipids was very high for all the feeds, between 85 and 95% and higher for the spent yeast obtained from brewery by-products compared to the commercial yeast. In the case of the ingredients a higher protein ADC was obtained for the brewery by-products compared to the commercial yeast. In the case of lipid ADC was negative for hydrolysed yeast and commercial yeast due to the lower lipid content of those ingredients. The results of fillet and liver biochemical composition and fatty acid profile will be presented in the congress.

Conclusion

Based on the results obtained, brewery by products can be used as fish meal replacement (up to 15-20%) in aquafeeds formulae for the ongrowing of marine fish such as gilthead seabream

(Continued on next page)

Table 1.- Formulation of feeds used in the digestibility trial with sea bream. D: Dried, H: Hydrolysed, SG: spent grain, SY: spent yeast

	CTRL	(D&H) SG7.5	(D&H) SG15	(D&H) SY10	(D&H) SY20
Ingredient					
Wheat gluten	8.00	7.40	3.60	4.90	1.63
Hi Pro Soy bean meal	6,00	6,00	2.52	4.06	1.00
Wheat gluten	13.78	13,00	14.8	14.62	15.15
SPC	17.00	17,00	17,00	17,00	17,00
Fish oil NA	7.75	7.74	7.72	7.50	7.38
Fish meal NA	15.00	15.00	15.00	15.00	15.00
Rapeseed oil	5.85	5.91	6.12	5.78	5.77
Lutavit C Aquastab 35%	0.01	0.01	0.01	0.01	0.01
Phosphate	0.83	0.75	0.65	0.7	0.65
Choline	0.27	0.27	0.27	0.27	0.27
Lysine HCl	0.36	0.20		0.09	
Mineral mix with iodine 2/04	0.10	0.10	0.10	0.10	0.10
Vitamin premix	0.10	0.10	0.10	0.10	0.10
Wheat	15.95	17.66	14.65	16.77	13.6
Soy lecitihin	2.00	2.00	2.00	2.00	2.00
spent grain		7.50	15.00		
Brewer's spent yeast				10.00	20.00

Table 2.- Results of the initial and final weight of the fish used in the study, specific (SGR) and relative (RGR) growth obtained, Hepatosomatic index (HIS), Feed conversion (FCR) and Protein Efficiency (PER) Ratios obtained at the end of the study

	Initial weight (g)		Final weight (g)		HSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	94.56	9.20	183.03	17.80	2.90	0.38	0.93	0.04	93.49	5.23	1.44	0.08	1.53	0.08b
DSY10%	94.34	7.93	187.51	18.84	3.09	0.62	0.97	0.03	98.74	4.45	1.37	0.07	1.68	0.08ab
DSY20%	94.48	8.55	190.14	20.81	2.85	0.39	0.99	0.00	101.28	0.18	1.33	0.01	1.68	0.01ab
HSY10%	94.55	8.51	183.68	20.35	3.33	0.52	0.94	0.01	94.26	0.88	1.43	0.01	1.67	0.02ab
HSY20%	94.30	9.48	190.93	19.56	3.22	0.58	0.99	0.03	102.40	4.00	1.32	0.05	1.76	0.06a
DSG7.5%	94.42	9.17	184.17	18.46	2.86	0.29	0.94	0.00	95.06	0.32	1.42	0.00	1.60	0.01ab
DSG15%	94.59	8.91	187.41	18.95	2.73	0.40	0.96	0.02	98.18	2.51	1.37	0.04	1.66	0.04ab
HSG7.5%	94.90	10.21	188.15	22.05	2.72	0.51	0.96	0.02	98.26	2.30	1.37	0.02	1.66	0.03ab
HSG15%	94.55	9.50	185.35	19.27	2.50	0.45	0.95	0.01	96.03	1.47	1.40	0.02	1.63	0.02ab
DY-ABN10%	94.25	10.57	188.37	23.35	3.06	0.32	0.96		97.74		1.35		1.70	
DY-ABN20%	94.38	10.11	184.73	21.65	2.90	0.56	0.95		95.73		1.41		1.60	
HY-ABN10%	94.64	8.30	187.13	17.78	3.13	0.52	0.96		97.74		1.38		1.69	
DY-ABN20%	94.19	9.51	192.48	16.81	3.29	0.36	1.01		104.35		1.29		1.76	
			ANOVA	P=0.681			P=0.134		P=0.133		P=0.140		P=0.049	

Table 3.- Apparent digestibility coefficients of the feeds with the highest level of inclusion (20% in the case of yeast and 15% for spent grain) and ingredients used in the study (DCY and HCY = dried and hydrolysed commercial yeast from ABN)

Apparent Digestibility Coefficients of Feeds

SEA BREAM	CTRL	DSY20	HSY20	DSG15	HSG15	DCY20	HCY20
Protein	95,54	94,01	93,62	94,04	93,10	88,48	90,46
Lipids	92,67	92,36	92,08	91,38	90,05	85,21	89,20

Apparent Digestibility Coefficients of Ingredients

SEA BREAM	D-Yeast	H-Yeast	D-Spent grain	H-Spent grain	D-Yeast ABN	H-Yeast ABN
Protein	93,22	85,53	78,66	62,19	58,65	69,71
Lipids	72,08	-78,60	73,89	45,79	-403,59	-1,99

ECO-EFICIENT AQUAFEED FORMULATIONS FOR RAINOW TROUT: EFFECTS ON PERFORMANCE, WASTE AND SENSORIAL ANALYSIS

Filippo Faccenda[1], Michele Povinelle[1], G. V. Pereira[2], Filipe Soares[2], Rodrigo Mendes[2], Jorge Dias[2], Bela Buck[3,4], Johan Johansen[5], Luís E.C. Conceição[1]

[1]Fondazione Edmund Mach, San Michele all'Adige, Italy
[2]SPAROS, Lda. Olhão, Portugal
[3]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany
[4] University of Bremerhaven, Appl. Mar. Biol., Bremerhaven, Germany
[5]Salten Havbrukspark AS, Nygårdsjøen, Norway

Email: luisconceicao@sparos.pt

Introduction

The current drive for aquaculture sustainability and circular economy principles in Europe has pushed researchers and feed formulators to design aquafeeds where alternative ingredients (e.g. insect products, by-products of aquaculture, microbial biomasses, novel vegetable protein concentrates, fisheries and terrestrial animal production, algae and algae-based products from biorefineries) have been getting more and more attention in relation to those traditionally used (e.g. fish meal, fish oil, soybean meal) due to sustainability issues but also price and future availability. However, when feed formulation become largely based on novel ingredients, formulators must ensure that all required nutrients are provided, and no negative impacts are observed, or even benefits are brought, on fish growth performance, feed conversion, environmental impacts, and fish acceptance by the consumer. This is one of the objectives of the H2020 GAIN project, which thrives to improve eco-efficiency of European aquaculture. This study aims to test novel feed formulations, based on such emerging ingredients, selected based on sustainability and circular economy principles, and evaluate how they impact fish growth, feed conversion, environmental impacts, and fish acceptance by the consumer.

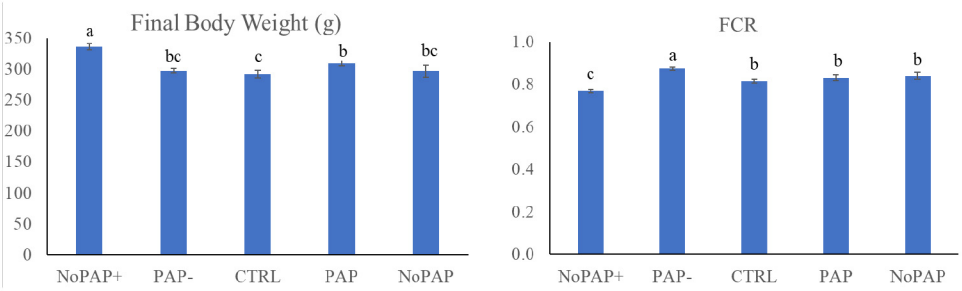

Figure 1. Final body weight and feed conversion ratio (FCR) of rainbow trout fed diets CTRL, PAP, NOPAP, PAP- and NOPAP+ for a period of 90 days. Initial body weight: 59g.

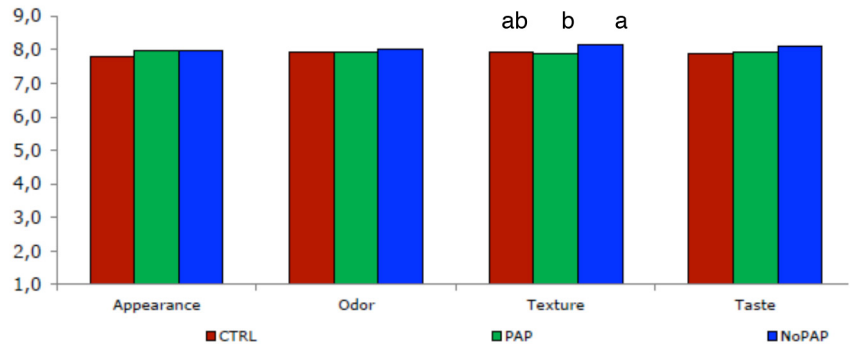

Figure 2. Evaluation by 100-persons sensory panel in a scale of 10, for appearance, odour, texture and taste of oven-baked rainbow trout fed diets CTRL, PAP and NOPAP for 90 days.

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Methods

Five extruded diets produced by SPAROS (Olhão, Portugal) where tested at the trial facility of Fondazione Edmund Mach (FEM, San Michele all'Adige, Italy): 1) a control diet, mimicking a good quality commercial diet, containing fish meal and traditional soy products (CTRL); 2) a diet rich in processed terrestrial animal proteins (PAP); 3) a diet with alternative ingredients without the inclusion of PAP (NPAP); 4) a diet similar to PAP but with a lower protein content (PAP-); and 5) a diet similar to NOPAP but with a higher protein content (NOPAP+). All diets were formulated to be isolipidic, and the 3 first diets were also isoenergetic and isoproteic. This nutritional trial was performed with four replicates tanks of 1000 L, each with 50 rainbow trout (*Oncorhynchus mykiss*) with an initial weight of 58.8g, with fish fed *ad libitum* with floating pellets for 90 days. Fish growth, feed intake, feed conversion were monitored. Environmental impacts were estimated based on feed composition, apparent digestibility coefficients and *in silico* simulations with the smart-software FEEDNETICS (ww.sparos.pt). Fish acceptance by the consumer was tested by means of a sensory panel of 100 selected consumers, at SenseTest Lda (Porto, Portugal), with in fish baked in the oven for approximately 12 min at a temperature of 170ºC, which evaluated appearance, odour, texture and taste.

Results

Rainbow trout performed very well under all feed formulations tested (Fig. 1). Still, The NOPAP+ diet was the one that performed best, with highest growth and feed conversion ratio (FCR). The PAP diet led to a better growth than the commercial-type CTRL, while PAP- diet had the worst FCR. (Fig. 2). The sensory panel evaluation (Fig. 2) shows that trout fed the five diets were very well perceived in terms of appearance, odour, texture and taste; there were only slight differences between diets, the only statistically significant being the slight preference in texture for NOPAP compared to PAP.

Discussion and Conclusions

The four novel aquafeed formulations for rainbow trout, based on emerging ingredients, selected based on sustainability and circular economy principles, were shown to be viable alternatives to current diets for trout farming in terms of performance, and with no apparent impact on fish sensorial acceptance by the consumer.

Acknowledgments

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THE SPAWNING KINETICS AND PARENTAGE CONTRIBUTION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) BROODSTOCKS AND INFLUENCE OF GNRHA-INDUCED SPAWNING

I. Fakriadis[1*], J. Superio[1,2], C. S. Tsigenopoulos[1], S.A. Lancerotto[1], A.V. Rodriguez[1,3], E. Vervelacis[1,4], and C. C. Mylonas[1]

[1] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, P.O. Box 2214, 71003, Heraklion, Crete, Greece
[2] Biology Department, University of Crete, PO Box 2208, 71409 Heraklion, Crete, Greece
[3] University of La Laguna, Calle Padre Herrera, s/n, 38200 San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain
[4] Veterinary Medical Sciences, Alma Mater – Studiorum Università di Bologna, Via Zamboni 33, 40126 Bologna, Italy
Email: fakriadis@hcmr.gr

Introduction

The European sea bass (*Dicentrarchus labrax*) together with the gilthead seabream (*Sparus aurata*) constitute the vast majority of marine aquaculture production in the Mediterranean Sea region (Chatziplis et al., 2020). Most commercial hatcheries rely on spontaneous mass spawning and communal rearing of the produced progeny for the establishment of breeding selection programs. However, not all breeders contribute to the fertilized eggs produced in a day's spawn, resulting in a small number of families and variation in the progeny, thus limiting the implementation of selection (Rhody et al., 2014)virtually no information is available on captive broodstock spawning characteristics. Understanding basic and fundamental data such as broodstock contribution of captive mass spawning snook is important, not only for the development of a successful selective breeding program for the species, but also for restocking wild fisheries and maintenance of local genetic variation. A scoping study was undertaken to explore the potential of DNA profiling for monitoring mating outcomes in captive snook. Spawning success was monitored among wild harvested broodstock that were undergoing hormonal treatment to induce spawning. The broodstock were maintained in three separate tanks (Tank A: 18 males and 15 females; Tank B: 22 males and 11 females; Tank C: 40 males and 16 females. The use of gonadotropin-releasing hormone agonists (GnRHa) have been shown to overcome reproductive dysfunctions of captive-bred fish and increase parental participation in other species (Setiawan et al., 2016). In the present study, two broodstocks were evaluated over two consecutive reproductive seasons to examine (a) the spawning kinetics, egg production parameters and parentage contribution in spontaneous spawning broodstocks, and (b) the potential of a hormonal therapy to synchronize spawning and increase parentage contribution.

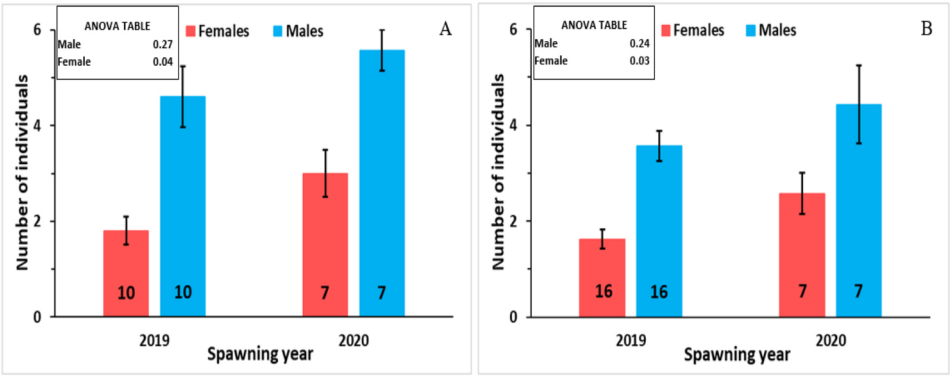

Fig.1. Mean (± S.E.M) number of female and male European sea bass *Dicentrarchus labrax* participating in each spawn in broodstock G1 (A) and G2 (B). In broodstock G1, the fish spawned spontaneously in 2019, while they were induced with a GnRHa implant in 2020. In broodstock G2, fish spawned spontaneously in both years. The numbers inside the bars indicate the n values of the means (daily spawns).

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Materials and methods

Two broodstocks (G1 and G2) of reproductively mature (>6 years old) hatchery-produced European sea bass kept at the broodstock facilities (15m^3, 2 m deep tanks) of HCMR were utilized during the spawning seasons (January to March) of 2019 (Year 1) and 2020 (Year 2). In Year 1, both groups were allowed to spawn spontaneously. The following year, the broodstock G1 was induced to spawn with GnRHa with a mean dosage (± SD) of 121 ± 28 μg GnRHa kg^{-1} BW (females) and 45 ± 11 μg GnRHa kg^{-1} BW (males) loaded in controlled release implants given after the first spawn was observed (16th of January), while broodstock G2 was again allowed to spawn spontaneously. The eggs were collected after each spawning for egg quantity (fecundity) and quality (fertilization, hatching, 24-h embryo survival, 7-d larval survival) evaluation as well as for parentage analysis.

Results and discussion

Over the two years of the study, spontaneous spawns were obtained with variable periodicity, ranging from 10 days to every day for up to 4 consecutive days. Between 12 and 21 spontaneous spawns were obtained during the two reproductive seasons, with no trend in daily fecundity or fertilization success in either broodstocks. Parentage analysis showed limited female contribution in each day's spawn (1-3 breeders), a slightly better male contribution (3-7 breeders), but the resulting progeny was not distributed equally among families, with one dominant family producing ≥50% the progeny.

GnRHa treatment was not very effective in increasing overall parentage participation, compared to the spontaneous spawning broodstock (Fig.1), but it increased parentage participation and fecundity in the first spawn obtained after treatment (3 days) and resulted in the production of more families of similar contribution to the obtained progeny. No significant differences were observed in overall egg production/quality between spontaneous and GnRHa-induced spawns *(i.e.* fecundity, embryo survival and hatching), but 7-d larval survival increased significantly between 2019 and 2020 in the GnRHa-implanted G1 broodstock, whereas it was reduced in the spontaneous spawning G2 broodstock.

Conclusion

The present study showed that GnRHa implants administration in European sea bass increased the number of breeders participating in the first spawn after treatment and enhanced the relative fecundity and 7-d larval survival. With more careful female breeder selection, GnRHa administration may "maximize" the parental participation in the first spawn resulting in the production of a higher number of families with equal contribution to the produced progeny, thus enhancing the implementation of breeding selection programs relying on mass spawning.

References

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/10.3390/ani10091668

Rhody, N.R., Puchulutegui, C., Taggart, J.B., Main, K.L., Migaud, H., 2014. Parental contribution and spawning performance in captive common snook *Centropomus undecimalis* broodstock. Aquaculture 432, 144–153. https://doi.org/10.1016/j.aquaculture.2014.04.022

Setiawan, A.N., Muncaster, S., Pether, S., King, A., Irvine, G.W., Lokman, P.M., Symonds, J.E., 2016. The effects of gonadotropin-releasing hormone analog on yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833) spawning and egg quality. Aquac. Rep. 4, 1–9. https://doi.org/10.1016/j.aqrep.2016.05.001

IMTA AS A MARINE EXAMPLE OF CIRCULAR AQUACULTURE

Fang Jianguang

Yellow Dea Fisheries Research Institute, CAFS
106, Nanjing Road, Qingdao, China. 266071
fangjg@ysfri.ac.cn

China's traditional fishery economy could hardly support the coastal rural revitalization. In a bid to tackle this issue, Chinese Government serves as a role model by implementing conservational development and sustainable fisheries to restore the provisioning services of marine ecosystems. Based on the management theory of coastal ecosystem, different models of integrated multi-trophic aquaculture (IMTA) has carried out in Sungo Bay for several decades for achieving a comprehensive improvement in the yield per unit area of sea, the quality of aquatic products and the marine environment. Such practices have helped Sungo Bay maintain a green environment, carry forward traditional culture, and develop recreational fisheries. Through the combination of multiple measures and the joint efforts of government, industry and institutes, a rural revitalization model of mariculture industry that improved villagers' income, social equality and environment has been created, realizing goals of sustainable development.

Since the end of the 1990s, the Eco-farming aquaculture of non-feeding species, including filter-feeding shellfish, kelp, and sea cucumber, has developed to protect the ocean environment and help the local fisherman to gain higher economic benefit. Since 2005, the various IMTA, such as the longline aquaculture of Seaweeds +bivalves, Seaweeds +bivalves+abalone, Seaweeds +bivalves+fish, combination of longline and bottom aquaculture of Sea weeds +bivalves+sea cucumber, eelgrass+ manila clam + sea cucumber etc have innovated and well practiced in commercial scale in the bay, The IMTA model changes the traditional one-species, high-density aquaculture method, and improves the resilience of aquaculture farmers.

IMTA utilized the ecological characteristics of trophic at different levels to realize the recycling of biological nutrients, which can not only reduce the pollution of aquaculture itself, but also improve the output efficiency per unit area. IMTA has promoted the diversification of aquaculture species and the industry's risk-tolerance capability, significantly promoting ecological and economic benefits. In accordance with the development trend of the aquaculture industry, Sungo Bay is the first to undertake the demonstration and training of a raft-based standardized ecological aquaculture model in China. Against the background of rising labor costs in marine aquaculture, this model ensures the sustainable development of the aquaculture industry.

EVALUATION OF GROWTH PERFORMANCE, OXIDATIVE STRESS AND IMMUNE RESPONSE IN GILTHEAD SEABREAM FED WITH NOVEL FEED FORMULATIONS

A.M. Fernandes[*1,2], L.E.C. Conceição[1], J.A. Calduch-Giner[3], B. Silva[1], G.V. Pereira[1], B. Costas[4], F. Naya-Català[3], C. Piazzon[3], J.M.O. Fernandes[2], A. Sitjà-Bobadilla[3] and J. Pérez-Sánchez[3]

[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]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal
E-mail: anafernandes@sparos.pt

Introduction
As the aquaculture sector continues to expand while being more environmentally conscious, the development of sustainable aquafeeds is becoming increasingly important (FAO, 2020). Tolerance to the replacement of fishmeal and fish oil in feeds has been largely studied in gilthead seabream (Sparus aurata) (Gasco et al., 2018; Karapanagiotidis, Psofakis, Mente, Malandrakis, & Golomazou, 2019), and many products emerge now as potential alternatives to ingredients used in conventional formulations. A main goal of GAIN EU project is to evaluate emerging ingredients, already commercially available, using different formulation concepts that consider all fish nutritional requirements. GAIN diets are based on circularity principles, maximizing resource efficiency, while contributing towards zero waste in the agro-food value chain, feed cost-effectiveness, and having good social acceptance. The present study aims to understand the real impacts of these novel feed formulations on growth performance, nutritional condition, immunity, and oxidative status using biomarkers.

Methods
Quadruplicate groups of gilthead seabream (Sparus aurata) were fed ad libitum with four different diets. Three of them have been designed to facilitate aquaculture eco-intensification through increased circularity and resource utilization: NOPAP - formula without terrestrial animal by-products processed animal protein; PAP - formula with terrestrial animal by-products processed animal protein; and MIX - a mixture of NOPAP and PAP. The fourth feed followed a standard commercial formulation and was used as a control diet. After a 77-day feeding trial, plasma samples were collected to evaluate humoral parameters (protease, anti-protease, bactericidal activity and IgM). Liver and head kidney tissues were collected for the simultaneous profiling of a panel of 42 (liver) or 29 (head kidney) genes, as markers of growth performance, lipid and energy metabolism, and immune and antioxidant activities by qPCR. Liver samples were also used to analyse oxidative biomarker (Lipid peroxidation and catalase).

Results
Tested feed formulations did not affect growth performance or feed intake. However, fish fed PAP and MIX diets had a higher feed conversion ratio (FCR) and protein efficiency ratio than control and NOPAP groups. This impairment was accompanied by a decreased hepatic expression of *igf-i* and *ghr1*. NOPAP diet slightly increased innate immunity parameters, showing better results on bactericidal, IgM, and anti-protease activity, as well as a significant up-regulation of *il-8* in head kidney. Fish fed with PAP diet displayed an up-regulation of pro-inflammatory genes, namely *il-8* and other cytokines (*il-1β*, *tnf-α*), chemokines (*ck8*), and chemokine receptors (*ccr3*). The same pattern was found for the T-cell markers *cd3x*, *cd4*, and *cd8a*. The activity of the antioxidant enzyme catalase was significantly lower in fish fed with PAP and MIX diet, being a possible indication of decreased antioxidant defences. This is supported by the observed regulation of antioxidant genes (*mn-sod*/*sod2*, *gpr-170*, *gpr-94*, and *gpr-75*), although not statistically significant.

Discussion
The similar performance of novel formulations and the control diet indicates that they can be considered as viable options for seabream feeds. Differences in FCR suggest that NOPAP can promote a better bioavailability and/or increased absorption of key nutrients than PAP and MIX diets. Indeed, this impairment was also evidenced by their hepatic expression pattern of markers of growth performance. In general, PAP exhibited an opposite response to the NOPAP group. NOPAP was closer to the control diet, and MIX showed intermediate values between PAP and NOPAP in almost all parameters. The markedly pro-inflammatory head kidney expression profile in PAP fish may be also indicative of an impaired response at the mucosal level. In any case, the low proportion of differentially expressed genes between the experimental diets and control (18 out of 71) constitutes an additional and indirect confirmation of their suitability.

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Conclusions

Novel feed formulations for gilthead seabream seem to be viable options for a 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 from 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 SA (Portugal)".

References

FAO. (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in action*. https://doi.org/https://doi.org/10.4060/ca9229en

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., & Caruso, G. (2018). *Fishmeal Alternative Protein Sources for Aquaculture Feeds*. https://doi.org/10.1007/978-3-319-77941-6_1

Karapanagiotidis, I. T., Psofakis, P., Mente, E., Malandrakis, E., & Golomazou, E. (2019). Effect of fishmeal replacement by poultry by□product meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and gene expression of gilthead seabream (*Sparus aurata*). *Aquaculture Nutrition*, *25*(1), 3–14.

EFFECTS OF PRE-TREATED MACROALGA, *Ulva rigida,* IN DIGESTIVE ENZYMES AND OXIDATIVE STATUS OF EUROPEAN SEABASS *Dicentrarchus labrax*

Fernandes, H.[1,2*]; Martins, N.[1,2]; Ferreira, P.[2]; Castro, C.[1,2]; Salgado, J.[3]; Couto, A[1,2]; Oliva-Teles, A.[1,2]; Peres, H.[1,2]; Belo, I.[3]

[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

Corresponding author: helenafernandes012@gmail.com

Introduction

Macroalgae are a sustainable nutrient-rich bioresource with potential application in food and feed industries (Jung *et al*., 2013). However, macroalgae nutrients bioavailability can be an issue due to cell-wall components and intercellular polysaccharides.

Following a biorefinery approach, different processing methods can be applied as a strategy to valorize macroalgae biomass (Torres *et al*., 2019) by modifying its cell-wall composition and enhance its nutrient availability (Ben Yahmed *et al*., 2017).

A preliminary study already demonstrated that inclusion of 5% SSF-treated *U. rigida* did not compromised growth and improved feed utilization of European seabass, while higher phenolic compounds were found in fish fed untreated *U. rigida* (Fernandes *et al*., 2019). The present study aimed to evaluate the effect of dietary inclusion of untreated and pre-treated *U. rigida* on gut digestive function and oxidative status of European seabass (*Dicentrarchus labrax*).

Materials and Methods

Dry micronized *U. rigida* was pre-treated following two approaches: a physical treatment, using ultra-sounds (US) for 1 hour in closed containers, with a high-intensity ultrasonic processor operating at 50-60 Hz; and a biological treatment, using solid-state fermentation (SSF) with *Aspergillus ibericus* (MUM 03.49) for 7 days at 25 °C, using tray-type bioreactors. Four isoproteic (45%) and isolipidic (18%) diets were formulated: a control diet containing 25% fishmeal (FM); and three other diets replacing 5% (w/w) of FM with untreated *U. rigida*, US-treated *U. rigida* and SSF-treated *U. rigida*. All diets were tested in triplicate with European seabass juveniles with initial body weight of 108 g. Fish were fed until apparent satiation for 64 days, six days-a-week. After the experimental period, gut lipid peroxidation (LP), and digestive and antioxidant enzyme activities were assessed.

Results and Discussion

After the growth experiment, fish achieved an average final body weight (FBW) of 240 g and fish fed SSF and US-treated *U. rigida* achieved similar FBW than those fed control diet. SSF-treated macroalgae promoted an increase of feed efficiency in comparison to the control. Untreated and both pre-treated macroalgae also resulted in higher protein efficiency ratio than the control group.

In this study, the lipase activity was significantly lower in fish fed diets with untreated or pre-treated macroalgae than with the control diet. Protease activity of fish fed the untreated macroalgae-based diet was higher than that fed the pre-treated macroalgae diets. Amylase activity was not affected by the dietary treatments.

Regarding the oxidative damage markers, the dietary FM replacement by untreated macroalgae increased gut LP, while these values were partially restored with the inclusion of US or SSF pre-treated macroalgae. However, the activity of antioxidant enzymes was similar among diets. The SSF pre-treatment contributed for a successful cell-wall disruption and structure alteration of *U. rigida,* which may have contributed for the reduction of LP in fish fed this diet.

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Table 1: Gut digestive (U mg^{-1} protein) and antioxidant (mU mg^{-1} protein) enzyme activities and LP levels of European seabass fed the experimental diets.

	Control	*U. rigida*	US	SSF	SEM
Digestive enzymes					
Amylase	68.9	49.0	56.6	55.9	3.70
Lipase	9.8^{b}	6.0^{a}	6.2^{a}	5.7^{a}	0.53
Protease	39.0^{ab}	48.6^{b}	32.8^{a}	34.6^{a}	1.93
Antioxidant enzymes					
SOD (U mg^{-1})[1]	309.4	346.8	293.4	198.1	22.9
CAT (U mg^{-1})[2]	242.5	275.9	251.7	243.9	11.9
G6PD[3]	50.2	36.0	36.6	48.2	4.6
GPX[4]	5.45	7.45	7.45	6.42	0.54
GR[5]	42.5	42.1	40.2	37.4	1.9
LP (nmols MDA g^{-1})[6]	41.2^{a}	90.1^{c}	67.7^{bc}	61.4^{ab}	4.3

Values are present as mean ± standard error of mean (SEM).
Letter in the same row with different superscript letters are significantly different (Duncan's test; $p<0.05$).
[1]Superoxide dismutase; [2]Catalase; [3]Glucose-6-phosphate dehydrogenase; [4]Glutathione peroxidase; [5]Glutathione reductase; [6]Lipid peroxidation

Conclusions

The dietary FM replacement by non-treated macroalgae resulted in higher gut protease activity and increased LP in European seabass. On the other hand, the inclusion of pretreated macroalgae resulted in lower lipase activities than those observed in the control group, as the SSF-treated macroalgae also lowered the LP to values closer to those obtained when fish were fed the control diet.

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References

Ben Yahmed, N., Carrere, H., Marzouki, M. N., & Smaali, I. (2017). Enhancement of biogas production from *Ulva* sp. by using solid-state fermentation as biological pretreatment. *Algal Research*, *27*(April), 206–214. https://doi.org/10.1016/j.algal.2017.09.005

Fernandes, H., Salgado, J. M., Martins, N., Peres, H., Oliva-Teles, A., & Belo, I. (2019). Sequential bioprocessing of *Ulva rigida* to produce lignocellulolytic enzymes and to improve its nutritional value as aquaculture feed. *Bioresource Technology*, *281*, 277–285. https://doi.org/10.1016/j.biortech.2019.02.068

Jung, K. A., Lim, S. R., Kim, Y., & Park, J. M. (2013). Potentials of macroalgae as feedstocks for biorefinery. *Bioresource Technology*, *135*, 182–190. https://doi.org/10.1016/j.biortech.2012.10.025

Torres, M. D., Kraan, S., & Domínguez, H. (2019). *Seaweed biorefinery*. *Reviews in Environmental Science and Biotechnology* (Vol. 18). https://doi.org/10.1007/s11157-019-09496-y

GENE EXPRESSION ANALYSIS AS AN INDICATOR OF MICROPLASTIC EFFECTS IN MATURING BROODSTOCK ATLANTIC COD *Gadus morhua*

M. Fernández-Míguez[1]*, V. Puvanendran[2], P. Presa[1], M. Pérez[3], E. Burgerhout[2], H. Tveiten[2], K. Vorkamp[4], Ø. J. Hansen[2], G. Johansen [2], A. Bogevik[5]

[1] Laboratory of Marine Genetic Resources, CIM-Universidade de Vigo, 36310 Spain
[2] Nofima AS, Tromsø, Norway
[3] AquaCOV, Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, 36390, Spain
[4] Aarhus University, Department of Environmental Science, Roskilde, Denmark.
[5] Nofima AS, Fyllingsdalen, Norway
E-mail: mariafernandezm@uvigo.es

Introduction

Plastic litter has become a major global problem with the increase in use and production of plastics over the last decades. Over time, plastic waste breaks down to fragments smaller than 5 mm, called microplastics (MP) (Teuten et al. 2009). Despite interactions of large plastic items with seabirds and marine mammals have been observed (Moore, 2008), the effects of degraded smaller plastic particles on marine biota, are not well known. As MP have a high surface area to volume ratio and a large potential to absorb/adsorb toxicants, plastics can accumulate hydrophobic persistent organic pollutants (POPs) from the environment (Goldstein et al 2013). Exposure to MP could affect the physiological function including disrupted enzyme production and function, reduction of feeding or reproductive failure (Oehlmann et al. 2009). Atlantic cod (*Gadus morhua*) is an important commercial finfish species in the North Atlantic region and an emerging species in aquaculture. Studies on the occurrence of MP in cod tissues are lacking, but its presence cannot be ruled out, e.g. Lusher et al. (2016) reported the presence of MP in 11% of the mesopelagic fishes sampled in the North Atlantic. Our study opens up the possibility of examining the effects of feed containing microplastics contaminated with POP's on digestion, endocrine cycle and gonadal development in Atlantic cod broodstock.

Materials and methods

In order to study the effects of MP-POP in feed on broodstock performance and to develop molecular criteria to assess these effects, cultured adult cod broodstock were transferred from sea to six indoors tanks in 2017. Three tanks were designated as control group (formulated dry diet, C-diet) and other three tanks as experimental group (1% MP added to feed, MP-diet) containing industrial polyethylene powder (0.3 - 0.6 mm). Sampling was performed 5 times during the experiment: 1) June 2017, after the initial control spawning and the post-spawning recovery; 2) September 2017, prior to vitellogenesis; 3) December 2017, mid/late vitellogenesis; 4) February 2018, gonadal maturation; 5) May 2018, post spawning. Ovarian and testis tissue samples from different stages of the reproductive cycle were taken to analyse histopathological changes. Faecal samples were also collected for digestibility of nutrients. For toxicological contamination and nutritional analysis, bile, muscle, liver and gonads were also sampled. Conventional egg quality determinants such as fertilization rate, normal cleavage and egg mortality were also recorded. Pituitary, brain, gonads and liver were sampled to analyse genes related to gonadal development and hormone production. Sixteen out of twenty-nine genes were selected upon their efficiency values for expression analysis to explore the transcriptome activity during maturation (Table 1). Three reference genes were included to normalise mRNA levels.

Results and discussion

No major differences were observed in fish biometrics or in spawning biometrics between dietary groups, e.g., in the spawning volume or in larvae length. Gene expression analyses showed different expression levels among treatments and among samplings. For instance, FSHβ gene (Follicle Stimulating Hormone subunit beta) showed an increase of expression in control pituitary samples (CTRL) in February (gonadal maturation) that was reduced in brain samples of MP-treated groups (Fig. 1). Gene FSHβ induces egg and sperm production in coordination with the follicle-stimulating hormone. Therefore, the MP treatment could negatively affect gonadal development and fish reproduction. The modelling of such differential expression in transcripts playing an active role in cod reproduction, could allow the detection of unacceptable levels of MP contaminants in marine waters.

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Table 1. Names, abbreviations, efficiency (E) and reference of the analysed genes.

Gene	Code	E	Reference
Reference genes			
Translation elongation factor 1 alpha	ef1a	108.32	Dale et al., 2019
b-Actin	actb	101.68	Yadetie et al., 2018
18S	18S	94.43	Seppola et al., 2009
Target genes			
Luteinizing hormone subunit beta	LHB	112.73	Hodne er al., 2010
Follicle stimulating hormone subunit beta	FSHb	107.39	Hodne er al., 2010
Follicle stimulating hormone receptor	FSHr	100.11	Mittelholzer et al., 2009
Estrogen receptor 1	esr1	103.64	Yadetie et al., 2018
20β-hydroxysteroid dehydrogenase	20B-hsd	104.27	Dale et al., 2019
Fatty acid syntase	FASN	79.39	Dale et al., 2019
Cyp1a	Cyp1a	113.70	Yadetie et al., 2018
Fatty acid binding protein 7	FABP7	99.01	Dale et al., 2019
Catalase	CAT	97.44	Skjærven et al 2013
Glutathione S-transferase	GSTA3b	91.34	Dale et al., 2019
ATP citrate lyase	ACLY	99.07	Dale et al., 2019
Lipoprotein Lipase	LPL	140.53	GFIX01044573.1
Lipase E_2	lipe2	122.12	GFIX01017304.1
Fatty Acid Desaturase 1	FADS1	90.00	Olsvik et al., 2015
Vitellogenin 1	VTG1	84.46	Bratberg et al., 2013
Gonadotropin-Releasing Hormone 2	GnRH2	90.03	Hildahl et al., 2011

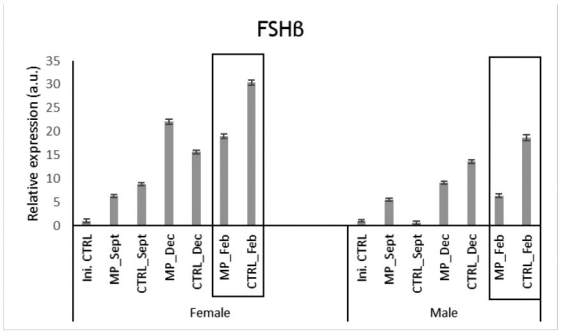

Fig. 1. Relative expression of FSHβ gene among MP and CTRL pituitary samples of Atlantic cod.

References

Goldstein, M. C., Titmus, A. J. and Ford, M., 2013. Scales of spatial heterogeneity of plastic marine debris in the northeast Pacific Ocean. PloS one, 8(11), p.e80020.

Lusher, A.L., O'Donnell, C., Officer, R. and O'Connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES Journal of Marine Science, 73(4), pp.1214-1225.

Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environmental research, 108(2), pp.131-139.

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J. and Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), pp.2047-2062.

Teuten, E. L., Saquing, J. M., Knappe, D. R., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R. and Ochi, D., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), pp.2027-2045.

SEARCHING FOR ANTIMICROBIAL PEPTIDES FROM SEA URCHIN *Paracentrotus lividus* COELOMIC FLUID

S. Fernandéz-Boo[1]*, R. A. Santos[1,2], J. Azevedo[1], P. N. Leão[1], B. Costas[1,3], C. R. Serra[1,2], A. Garcia[1,3]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR). Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal.
[2] Universidade do Porto, Faculdade de Ciências, Departamento de Biologia, Porto, Portugal.
[3] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Porto, Portugal.
*Email: sboo@ciimar.up.pt

Introduction

Antimicrobial peptides from natural sources are being explored as an alternative to conventional antibiotics in order to fight the ongoing problem of microbial resistance. Sea urchins are constantly exposed to challenging environments, where they have to face changes in temperature, UV radiation, metals and pathogens like virus and bacteria. As so, they have an effective innate immune system, which relies on a varied population of immune cells (coelomocytes) and extracellular products [1]. These cells are found on the coelomic fluid, which baths the internal organs of these animals. This study aimed to search for antimicrobial peptides (AMPs) from coelomocytes and coelomic fluid of the sea urchin *Paracentrotus lividus* by testing extracts against bacterial strains known as etiological agents responsible for diseases in aquaculture.

Material and Methods

Adult sea urchins were sampled from the coast of Vila Chã, Porto. Coelomic fluid was collected and centrifuged and both supernatant (perivisceral fluid) and pellet (coelomocytes) were lyophilized. A liquid-liquid extraction was performed with 60% acetonitrile (ACN), in water. The aqueous phase was further subjected to solid phase extraction (SPE) on a reverse phase C18 cartridge and eluted with growing ACN concentrations: 10%, 40%, 80% and 100%. All fractions were diluted in H_20 and saved for HPLC extraction or in 0.9% NaCl and tested against five bacterial strains: *Aeromonas hydrophyla, Vibrio anguillarum, Vibrio parahaemolyticus, Photobacterium damselae* and *Tenacibaculum maritimum*. The fractions which showed a higher antimicrobial activity were fractionated by reverse phase high-performance liquid chromatography (RP-HPLC) with a continuous growing gradient of ACN and the main peaks were collected and tested again against the previous five bacterial strains.

The peptides of the most promising fractions were characterized by shotgun proteomic, as described by [2], and data were analysed with Software Thermo Proteome Discoverer 2.5.0.400.

Results

Antibacterial activities of perivisceral fluid and coelomocytes fractions were assessed by measuring the inhibition of growth of the five selected bacterial strains, at OD 600 nm, during 24 hours. Of all fractions, the higher inhibition rates were observed for the fractions obtained after elution of the SPE column with 10% and 40% ACN (fig. 1a). These eluates were fractionated by RP-HPLC and the main peaks were screened for antimicrobial activity. Growth inhibition proprieties, previously found before fractionation were considerably diminished on the purified fractions. These new fractions, containing one or more peaks, were analysed by shotgun proteomics to identify possible antimicrobial compounds. For instance, for the 40% ACN eluate, the peak corresponding to 13 minutes (fig. 1b) indicated the presence of several proteins and peptides, including toposome and metallothionein (fig. 1c).

Discussion

Antimicrobial peptides have been previously found on the coelomic fluid of several echinoderms [3,4]. The main goal of this study was to search for antimicrobial peptides on the coelomic fluid of the sea urchin *Paracentrotus lividus*.

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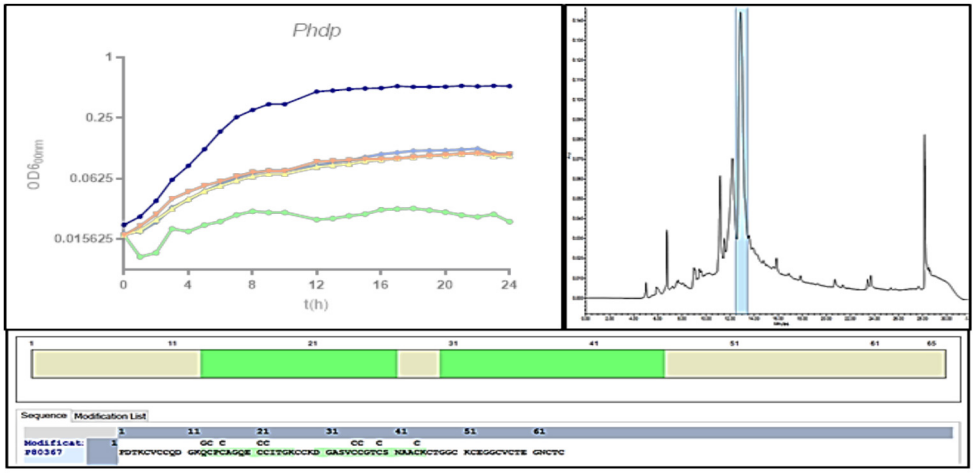

Figure 1: a) bacteriostatic activity assay of *Paracentrotus lividus* extracts: ACN-rich phase (orange), H_2O-rich phase (yellow), 10% ACN eluate (light blue), 40% ACN eluate (green), tested at 1 mg/ml against *Photobacterium damselae*, measured at OD 600 nm, for 24h. A negative control was used (dark blue). **b)** RP-HPLC chromatogram of the 40% ACN eluate, obtained using linear gradients, increasing the ACN concentration, from 5% to 99%. **c)** Sequence of a Metallothionein, obtained after a shotgun proteomic analysis of the peak represented in b).

Several extracts obtained after liquid-liquid extraction, SPE and RP-HPLC were tested for antimicrobial activity. Some of these extracts negatively affected the growth of the selected bacteria. Of all, the 40% ACN eluate and also the 10% ACN eluate showed the higher inhibition of growth. These data are consistent with previous studies performed by [5], on sea urchin *Echinus esculentus*, which found antimicrobial peptides on equivalent fractions. When extracts are fractionated by RP-HPLC, the new fractions seem to loss the inhibitory effect on bacterial growth, what seem to indicate that the extracts lose their activity or, on the other hand, may also indicate that the activity previously observed is the result of the interaction of several compounds. The shotgun proteomics data indicate the presence of several AMPs that may be the responsible for the antimicrobial activity of sea urchin extracts as the Toposome, that was recently described involved in antimicrobial activity in the sea urchin *Lytechinus variegatus* [6]. This study supports the idea that marine organisms can be a valuable source of antimicrobial compounds, with sea urchin as a promising candidate.

Bibliography

[1] Pinsino, A. & Matranga (2015). doi:10.1016/j.dci.2014.11.013.
[2] Matos A, et al. (2020). doi.org/10.3390/ md18070362.
[3] Beauregard KA, et al. (2001). doi: 10.1007/978-1-4615-1291-2_5.
[4] Stabili, L. et al. (1996). doi: 10.1016/0305-0491(95)02080-2.
[5] Solstad, R. G. et al. (2016). doi.org/10.1371/ journal.pone.0151820.

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF MICRO- AND MACROALGAE, SINGLE OR BLENDED, UNRAVEL THEIR POTENTIAL USE FOR AQUAFEEDS

Mariana Ferreira[1, 2*], Carla Teixeira[1, 2], Helena Abreu[3], Joana Silva[4], Benjamin Costas[1, 2], 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]ALGAplus, 3830-196 IÏlhavo, Portugal
[4] Allmicroalgae, Microalgae Production Plant, 2445-411 Pataias, Portugal
E-mail: marianaipf@gmail.com

Introduction

As the world population growths, the demand for fish increases as well. In this context, the importance of aquaculture as a food-supply sector has been steadily increasing over the past years. The optimization of aquafeeds is a subject of particular interest for the aquaculture sector, and efforts have been made to produce novel functional diets capable of promoting fish growth, disease resistance and overall health, while being economicaly and environmentaly sustainable. Algae are added-valued natural products rich in bioactive compounds with antibacterial and antioxidant activities, being promising products to improve fish health. The main goals of this work were to determine the nutritional value and the *in vitro* antimicrobial and antioxidant activities of different micro- and macroalgae, single or blended, to unravel the potential of these natural products to be included in aquafeeds.

Materials and Methods

Two microalgae (*Nannochloropsis oceanica* and *Chlorella vulgaris*) and two macroalgae (*Gracilaria gracilis* and *Ulva rigida*) produced under commercial conditions, as well as a commercial blend of these algae (Algaessence™ – Allmicroalgae and ALGAplus) were analysed for dry matter, crude protein, gross energy and total lipids. Their fatty acid and total amino acid profiles were also determined. The *in vitro* bactericidal and bacteriostatic activities of the algae were tested against main pathogenic bacteria for farmed fish and shrimp (i.e. *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Photobacterium damselae* subsp. *piscicida* – Phdp and *Tenacibaculum maritimum*). The radical-scavenging potential of the algae was determined through the ABTS radical cation (ABTS+ •) and DPPH radical (DPPH•) assays.

Results and Discussion

Results showed that, in terms of dry matter basis, the highest protein content was found in *C. vulgaris* (57.7 %), followed by the blend (36.7 %). The gross energy ranged from 11.0 kJ g^{-1} (*G. gracilis*) to 21.1 kJ g^{-1} (*C. vulgaris*), with the blend presenting an intermediate value of 15.7 kJ g^{-1}. The microalgae presented the highest total lipid content: 6.8 and 6.4 % for *N. oceanica* and *C. vulgaris*, respectively, while the blend had an intermediate level of lipid content (4.8 %). *N. oceanica* and the blend had a more balanced fatty acid composition compared to the other single algae: high levels of polyunsaturated fatty acids (28 and 34 %, respectively), eicosapentaenoic acid (EPA, 16 and 7 %, respectively), and arachidonic acid (ARA, 6 and 7 %, respetively). Likewise, the blend also appeared as good source of essential amino acids (18 %), second best after *C. vulgaris* (28 %).

The single algae and the blend displayed bactericidal and bacteriostatic activities against many pathogenic bacteria for farmed fish and shrimp (except for *V. anguillarum* and *A. hydrophila*), with the most promising results being observed against *T. maritimum* (40-45 % bactericidal activity). In some cases (e.g. *V. harveyi*) the micro- or macroalgae presented no bactericidal and bacteriostatic activities, respectively, while the blend was able to both kill and inhibit the growth of these bacteria, which suggests that the inclusion of the algae in a blend potentiate synergetic effects among distinct species (Figure 1). The algae had also some antioxidant activity, as determined by their ABTS and DPPH scavenging capacities, with *G. gracilis* and the blend presenting the highest values. Overall, the *in vitro* studies showcased the blend as a promising ingredient to be included in aquafeeds, with an added-value potential in comparison with the individual algae.

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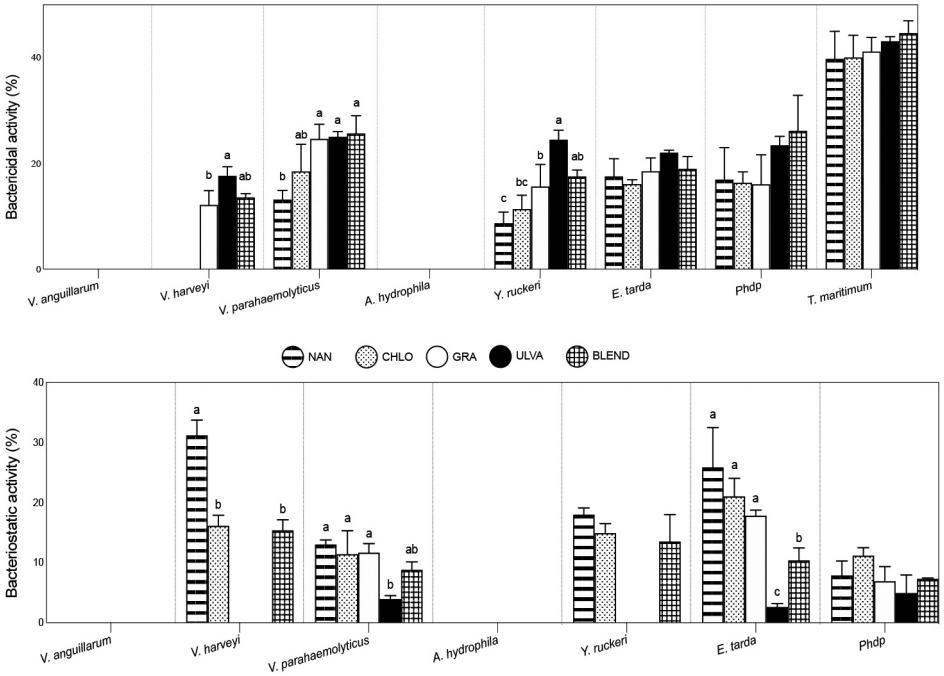

Figure 1. *In vitro* bactericidal and bacteriostatic activities of the tested algae against main pathogenic bacteria for farmed fish and shrimp. NAN - *Nannochloropsis oceanica*; CHLO - *Chlorella vulgaris*; GRA - *Gracilaria gracilis*; ULVA - *Ulva rigida*; BLEND – blend of the four algae.

Acknowledgements

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ENHANCING CORN DISTILLER'S DRIED GRAINS WITH SOLUBLES THROUGH SOLID-STATE FERMENTATION FOR USE IN EUROPEAN SEA BASS DIETS

D. Filipe[1*], M. Dias[1], J. Salgado[3], I. Belo[3], A. Oliva-Teles[1,2] H. Peres[1,2].

1- Faculty of Sciences, University of Porto. 2- CIIMAR- Interdisciplinary Centre of Marine and Environmental Research. 3- CEB- Centre of Biological Engineering, University of Minho
Email: diogomoreirafilipe123@gmail.com

Introduction

Aquaculture has an ever-growing need for more sustainable protein sources. Replacing traditional protein sources like fish meal and some agriculture feedstuffs, as soybean meal, with more economic and eco-friendly feedstuffs would help assure the future of sustainable aquaculture. Under this context, the reutilization of abundant and low-cost agro-industrial by-products as new potential feedstuffs would promote the circular economy and reduce the carbon footprint of the global aquaculture sector. Corn distillers dried grains with solubles (DDGS) is one of these by-products, originating in the corn ethanol industries. The use of DDGS is hindered mostly by high indigestible fiber content and moderate protein content. Through biotechnological processes, as solid-state fermentation (SSF), the nutritional value of DDGS may be improved. SSF is an accessible and ecological bioprocess that may be applied to agriculture by-products, like DDGS, using it as a substrate for microbial growth which in turn will produce desirable effects like reducing indigestible fiber, produce valuable bioactive compounds and increase protein content.

This trial aimed to optimize the SSF of DDGS to increase the nutritional profile and nutrients bioavailability of DDGS for European seabass.

Materials and method

Tree species of *Aspergillus* fungi (*Aspergillus niger* MUM-01.183; *Aspergillus ibericus* MUM-01.29; *Aspergillus uvarum* MUM-01.128.) from the Micoteca da Universidade do Minho (MUM) were tested in 7 day fermentations at 30º C. After fermentation, DDGS's composition and enzymatic activity were analyzed and compared. The fungi species that promoted the highest enrichment of DDGS content on bioactive compounds and protein was chosen and the SSF was scaled up to produce enough quantity of fermented DDGS to assess the in vivo digestibility of fermented and unfermented DDGS in European sea bass (*Dicentrarchus labrax*) juveniles. For this purpose, a control diet (48% protein; 15% lipids) and two test diets (70% of the control diet and 30% of either fermented or unfermented DDGS) were formulated. Digestibility trials were performed in a RAS system equipped with 9 tanks with a feces settling column connected to the outlet of each tank. Feces were collected daily, after an acclimatization period of 7 days. Apparent digestibility coefficients (ADCs) of dry and organic matter, protein, lipid, energy, and starch of the experimental diets and non-fermented and fermented DDGS were determined, according to Bureau et al. (1999).

Results

Irrespective of the fungi species, DDGS cellulose content decreased after the SSF, along with a general increase in protein, total phenolic compounds, and reducing sugars content. *Aspergillus ibericus* yielded the highest amount of nitrogen, the highest decrease in lignin levels, and the highest cellulase (44.63±1.58 U/g fermented DDGS) and xylanase (67.95±2.05 U/g fermented DDGS) activities, being chosen to be used for the *in vivo* digestibility trial.

The ADC of the control diet had the highest ADC of dry matter, organic matter, lipids, and starch while the fermented-DDGS diet had the highest protein digestibility. Comparing test diets, ADC of lipid and energy were higher with the fermented-DDGS diet than with the non-fermented-DDGS diet, while no differences were observed ADC of dry matter, organic matter, protein, starch, and phosphorus.

Regarding the digestibility of the ingredient, ADC of protein, lipids and energy was higher for the fermented-DDGS than with the non-fermented-DDGS.

Conclusion

The SSF of DDGS was successful in improving its composition. SSF of DDGS reduced indigestible fiber content, like cellulose and lignin, increased protein and reduced sugar content and added cellulolytic enzymatic activity. SSF of DDGS enhanced digestibility of protein, lipids, and energy, increasing the potential of DDGS as a feedstuff for aquaculture.

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IMPACT OF ELEVATED CO_2 ON LUMPFISH (*Cyclopterus lumpus*) GROWTH

J. Finlay*[1], C. Porteus[2], D. Phillips[3], and R.W. Wilson[1]

[1]Biosciences, Geoffrey Pope building, University of Exeter, Stocker Road, Exeter, EX4 4QD, U.K.
[2]Biology Department, University of Toronto, Mississauga, ON L5L 1C6, Canada
[3]Ocean Matters, Ltd., Black Point, Penmon, Anglesey, LL58 8RR, U.K.
Email: jf536@exeter.ac.uk

Introduction

Lumpfish (*Cyclopterus lumpus*) are a relatively new aquaculture species that is increasingly being farmed within recirculating aquaculture systems (RAS). These cleaner fish are deployed in salmon pens as biocontrol against sea lice (Powell et al., 2017), a parasite that causes substantial losses (Costello, 2009). Lumpfish are under-researched, so it is impossible to assess what impacts intensive farming are having on growth parameters. Within RAS, due to the technical difficulty of removing respiratory CO_2, it builds to levels which can detrimentally impact growth (e.g. Mota et al., 2019). Because CO_2 acidifies water, an alkali, typically sodium bicarbonate in marine systems, is often added to compensate pH. However a return to the original pH is rarely achieved, and CO_2 levels always remain well above atmospheric equilibrium. Here we used water chemistry data from the U.K.'s largest lumpfish RAS facility to inform an experimental growth trial.

Materials and methods

The study was performed at the Aquatic Resources Centre (ARC), University of Exeter. The 32 mixed sex juvenile lumpfish used were obtained from Ocean Matters Ltd., Anglesey, where they were raised from eggs. After acclimation to the ARC, lumpfish were randomly distributed into 10 L capacity isolation tanks, allowed 21 days tank-acclimation, then were exposed to one of four treatments for 3 weeks, each within which their own 200 L volume mini-RAS. A multifactorial design was implemented to examine the combined and sole impacts on growth of high (4,491 ± 265 μatm) and low CO_2 (564 ± 37 μatm, controlled by Aalborg gas flow controllers) and high (6966 ± 699 µM/kg seawater) and low alkalinity (2,338 ± 70.5 µM/kg seawater, controlled by $NaHCO_3$ addition). Fish were fed 1.5 % of their body mass daily, split over two feeds, for the duration of the study. Body mass was measured once per week and, using consumption data, food conversion rate (FCR, feed input / mass gain) was also calculated.

Results and discussion

Lumpfish growth over the whole 3 weeks was not significantly impacted by any of the experimental treatments (figure 1, ANOVA: DF = 3, F = 0.656, p = 0.206). Elevated alkalinity with control CO_2 treatment reduced growth after the first week alone (figure 1, ANOVA: DF = 3, F = 6.197, p = 0.00258), however, by the end of the 3 week study, there was no significant effect of any treatment on body mass (g). Fish exposed to the high alkalinity treatment were noted to have eaten fewer pellets throughout the experiment, and so the associated high water pH (mean pH = 8.61 ± 0.01 SE) may have been a stressor causing reduced appetite that took > 1 week to acclimate to; this has been noted in other species, such as the Amazon catfish (Lemos et al., 2018). However, elevated alkalinity is unlikely to occur in the absence of high CO_2 within a commercial RAS, so this result may be of little relevance to farmers.

There was no significant effect of treatment on FCR (ANOVA: DF: 3, F = 0.5227, p > 0.05). However, this at least partly due to high variability in both the high alkalinity treatments. This may be of economic interest, as fish exposed to the RAS-relevant combination of high CO_2 and alkalinity required 35 % more feed to obtain the same growth as control fish. Interestingly, fish exposed to high CO_2 with control alkalinity had an 8 % lower FCR than the control treatment. This suggests that high alkalinity may play a role in reducing conversion of feed to mass which would obviously translate to a greater economic cost to the industry.

Future research

Further studies are required to understand the impact of elevated CO_2 and alkalinity on lumpfish physiology and behaviour. Disturbances in vision and activity (Ferrari et al., 2011 & 2012), have been observed in fish exposed to elevated CO_2. These results are concerning as lumpfish require good vision to detect and graze on sea lice. Currently, there is research investigating lumpfish 'learning' to improve sea lice consumption, however, if the high CO_2 environment in which lumpfish are being grown is not taken into consideration, this research may be less effective.

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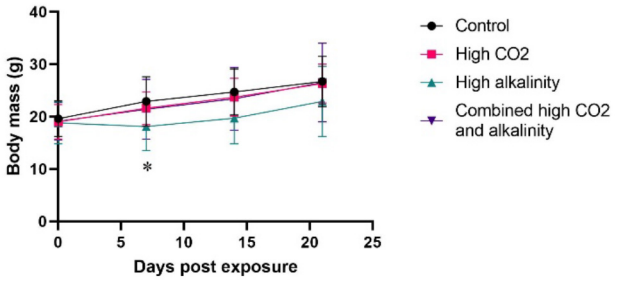

Figure 1. Body mass (g) of fish (± SD) over the duration of the growth trial. Significant ($p < 0.05$) differences between treatments are marked (*). Treatments are: Control (control CO_2 (486 ± 14.59 μatm) and control alkalinity (2,290.4 ± 53.30 μM/kg)), High CO_2 (elevated CO_2 (4,376 ± 177.49 μatm) and control alkalinity (2,386 ± 88.25 μM/kg)), High alkalinity (control CO_2 (641 ± 59.39 μatm) and elevated alkalinity (7044 ± 715.81 μM/kg), and Combined high CO_2 and alkalinity (Elevated CO2 (4606 ± 352.39 μatm) and elevated alkalinity (6887 ± 682.54 μM/kg)).

References

Costello, M. J. 1993. Review of methods to control sea-lice (Caligidae, Crustacea) infestations on salmon farms. In Pathogens of wild and farmed fish: sea lice (eds G. A. Boxshall & D. Defaye), pp. 219 –252. London, UK: Ellis Horwood.

Ferrari, M.C., et al., 2011. Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. Global Change Biology, 17(9), 2980-2986.

Ferrari, M.C., et al., 2012. Effects of ocean acidification on visual risk assessment in coral reef fishes. Functional Ecology, 26(3), 553-558.

Lemos, C., Chung, S., Ribeiro, C., Copatti, C., 2019. Growth and biochemical variables in Amazon catfish (Pseudoplatystoma reticulatum ♀ x Leiarius marmoratus ♂) under different water pH. Marine Pollution Bulletin, 48, 108-110. DOI: http://dx.doi.org/10.1590/0001-3765201820180241.

Mota, V., et al., 2019. The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (Salmo salar) in recirculating aquaculture systems. Aquaculture, 498, 578-586.

Powell, A., et al., 2017. Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture, doi: 10.1111/raq.12194.

THE IMPORTANCE OF FEEDING STRATEGY AND WATER MONITORING TO OBTAIN HIGH QUALITY MEAT IN RAINBOW TROUT PRODUCTION

E. Fiordelmondo*, F. Mariotti, G.E. Magi, A. Roncarati

School of Biosciences and Veterinary Medicine, Camerino University, Italy
*Email: elisa.fiordelmondo@unicam.it

Introduction

In Europe, Rainbow trout is one of the main freshwater fish farm that requires more studies to improve the relationship between feeding management and water quality. The correct balance of the protein component in the diet directly influences the nitrogen concentration into the water tanks, which affects fish wellness. Also, the quality of feedstuffs is directly related to the quality of fish meat, in particular on the composition and the balance of amino-acids, that determinate the optimization of growth and the conversion index.

In this paper, we focused our attention to underline the relationship between feed quality and water quality, mastered the ammonia levels in terms of protein in feed and Total Ammonia Nitrogen (TAN) in water. Two feeding strategies were compared considering the pellet one, adopted in past decades (2009), and the extruded one. The study also considered the feed conversion rate, to show the influence of feed quality on fish growth.

Materials and methods

The study was performed on a Rainbow trout farm located in Sefro (MC), in the central Apennine area of Italy. The productive cycle started from young Rainbow trout with a mean body weight of 90 ± 2 g, that are grown until the market size of 350 g. Fish received pellet feed until the year 2011, and after that time only extruded feed, both with a close formula edited by the same Company. In order to monitor the quality of the outlet water, one sample was obtained monthly from 2009 to 2019 at the lagoon basin (outlet water). TAN was determined using a spectrophotometer (Hach mod-2005, Hach Company, Loveland, USA) following the American Water Works Association and Water Pollution Control Federation of American Public Health Association (APHA) standard methods (1995). To evaluate the meat quality, samples of fillets were taken monthly from random trout during slaughter, and then processed at the laboratory of the University of Camerino (UNICAM). Each time a portion of about 50 g of skinless dorsal muscle was collected from a pool of specimens, homogenized, and subjected to analysis (moisture, protein, lipid and ash content). In addiction the amount of n-3PUFA and n-6PUFA were calculated. The analysis was determined in duplicate according to the AOAC procedure (1990) and data were expressed on wet weight basis. The protein content was determined using the standard Kjeldahl. Total lipids were measured using a modification of the chloroform:methanol procedure (Folch et al., 1957). After determining the total lipid content, fatty acids were converted to methyl esters following Sukhija and Palmquist (1988). Final mean body weight was recorded and feed conversion ratio (FCR) was calculated from the amount of diet consumed (kg) and the total biomass (kg) gained:

FCR = kg diet consumed/kg final biomass – (kg initial biomass + kg sampled fish) + mortalities.

All data were subjected to one-way analysis of variance (ANOVA) using the General Model Procedure of SPSS 25.

Results and discussion

The proximate composition of the two feeding types (Pellet, Extruded) employed to feed Rainbow trout is reported in Tab. J

In order to show the mass balance of nutrients released into the outlet water, Fig. 1 reports the amount of TAN derived from the administered feed and the amount retained by Rainbow trout, expressed as the mean in the first and in the last year of the considered period (2009-2019).

Concerning the meat characteristic of Rainbow trout fillets, the proximate composition showed a good protein content (mean of 19.72 ± 0.38 g/100g) and a low lipid level (mean of 2.14 ± 0.08 g/100g).

Analysing the fatty acid profile, the fillets of Rainbow trout exhibited a mean of n-3PUFA content at 29.5 ± 0.82 mg/100g and n-6PUFA content at 17.67 ± 3.42 mg/100g, with a ratio of 1.71 ± 0.41 mg/100g.

The significant reduction of the TAN load is a proof of the more efficiency of the extruded diet than the pellet one. Also, after the adoption of extruded feed in 2011 a more favourable feed conversion rate was showed respect to the first time when pellet feed was administrated, determining better growing performances exhibited by trout saving less than 40% of feed. Data concerning meat quality shows a high quality of that product, rich in protein and PUFA, that reflects a good health status of trout.

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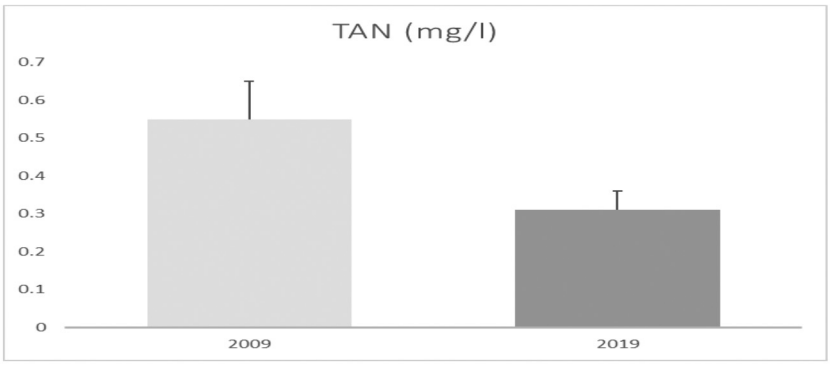
Tab. 1- Proximate composition of the feeds employed for the Rainbow trout growing in the decade 2009 – 2019

Feed	Pellet	Extruded
Chemical composition (%)		
Moisture	6.8	5.5
Crude protein	45.7	44.8
Crude lipid	16.0	21.0
Ash	6.7	8.4
Gross energy (MJ kg)	21.16	18.38

Fig.1 - Total ammonia nitrogen (TAN) budget in the lagoon basin outlet water.



References

AOAC. 1975. Official Methods of Analysis. AOAC (ed), Washington D.C., USA.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater, A.P.H.A., Washington, DC.

Folch J., Lees M., and Sloane Stanley G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. and Chem. 60:497-509.

Sukija P.S., and Palmquist D.L. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and faces. J. Agr. Food Chem. 36:1202-1206.

SELECTIVELY BRED OYSTERS CAN ALTER THEIR BIOMINERALISATION PATHWAYS, PROMOTING RESILIENCE TO ENVIRONMENTAL ACIDIFICATION

S. C. Fitzer[1], R. A. R. McGill[2], S. Torres Gabarda[3], B. Hughes[4], M. Dove[5], W. O'Connor[5], and M. Byrne[3]

[1] Institute of Aquaculture, Pathfoot Building, University of Stirling, Stirling, FK9 4LA, UK
[2] Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, G75 0QF, UK
[3] School of Life and Environmental Sciences, University of Sydney, NSW 2006, Australia
[4] Hunter Local Land Services, 98 Victoria St, PO Box 440, Taree 2430, Australia
[5] New South Wales Department of Primary Industries, Fisheries NSW, Port Stephens Fisheries Institute, Taylors Beach Rd, Taylors Beach NSW 2316, Australia
Email: susan.fitzer@stir.ac.uk

Abstract
Commercial shellfish aquaculture is vulnerable to the impacts of ocean acidification driven by increasing CO_2 absorption by the ocean as well as to coastal acidification driven by land run off. These drivers of environmental acidification have deleterious effects on shell growth. We investigated shell biomineralisation of selectively bred and wild-type lines of the Sydney rock oyster *Saccostrea glomerata* in a study of oysters being farmed in estuaries at aquaculture leases differing in environmental acidification. The contrasting estuarine pH regimes enabled us to determine the mechanisms of shell growth and the vulnerability of this species to environmental acidification. Determination of the source of carbon, the mechanism of carbon uptake and use of carbon in biomineral formation are key to understanding the vulnerability of shellfish aquaculture to future environmental acidification. We characterised the crystallography and carbon uptake in the shells of *S. glomerata*, resident in habitats subjected to coastal acidification, using high resolution electron back scatter diffraction and carbon isotope analyses (as $\delta^{13}C$). We show that oyster families selectively bred for fast growth and families selected for disease resistance can alter their mechanisms of calcite crystal biomineralisation, promoting resilience to acidification. For Scottish aquaculture acidification is less of an imminent threat, but as coastal acidification is made worse by climate change, in particular freshwater run-off from increased rainfall, this could have a serious effect on commercial shellfisheries all over the world. Changes in seawater chemistry associated with freshwater run-off include lowered salinity and pH, and carbonate availability. This, coupled with increasing temperatures, adds pressures to shellfish farming and oyster restoration. The responses of *S. glomerata* to acidification in their estuarine habitat provides key insights into mechanisms of mollusc shell growth under future climate change conditions. The Sydney rock oyster breeding programme has been immensely successful especially for restoring the populations after invasion of the Pacific oyster. Importantly, we show that selective breeding in oysters is likely to be an important global mitigation strategy for sustainable shellfish aquaculture to withstand future climate driven change to habitat acidification.

Introduction

Oysters are a major component of a $19 billion mollusc aquaculture industry, and are vulnerable to climate change-driven acidification on shell growth due to global (e.g. atmospheric CO2 uptake) and local (e.g. land run-off) stressors. We investigated the impact of coastal acidification on shell growth mechanisms in the Sydney rock oyster, a valuable specie to the south-eastern Australian aquaculture industry known to be impacted by sulphate soil runoff (O'Connor & Dove, 2009). In these areas, the production of *S. glomerata* has declined over recent decades attributed to water quality issues, including acidification from land run-off and freshwater input (Dove & Sammut, 2013; Fitzer et al., 2018) as well as disease. Oysters growing at these acidified sites regularly experience low pH (~pH 7.4–7.5; Fitzer et al., 2018) and there has been a decline in production of larger, higher value, 'plate' grade oysters and an increase in the smaller 'bistro' and 'bottle' grade oysters (O'Connor & Dove, 2009).

Material and methods

S. glomerata from families bred for QX disease resistance (F15), fast growth on the basis of whole oyster weight (F30) and wild-type (F31) were generated by the Sydney rock oyster breeding programme were obtained from commercial leases in Wallis Lake (Upper Wallamba latitude –32.174205, longitude 152.469004) and Port Stephens (Tilligerry Creek latitude –32.76852, longitude 151.965973) (Fitzer et al., 2019). Oysters were dissected, and shells sampled for $\delta^{13}C$ isotopes analyses and embedded in epoxy resin, sectioned and polished for scanning electron microscopy (Fitzer et al., 2019).

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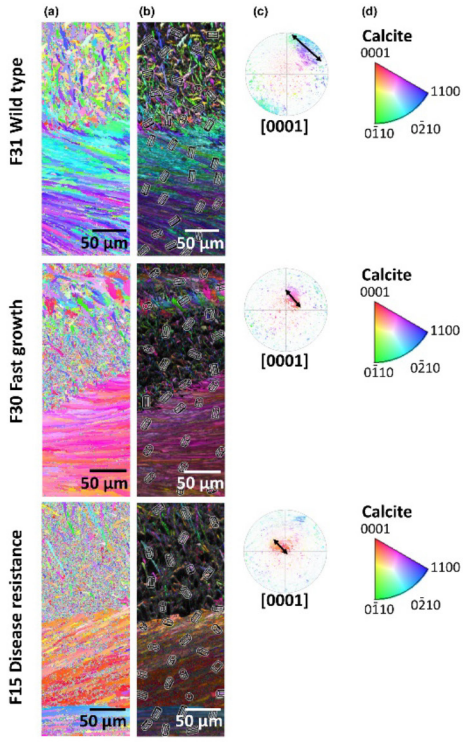
Results



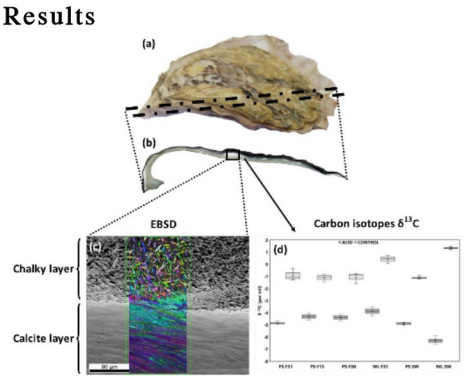


Figure 1. Oyster shell crystallography by electron back scatter diffraction (EBSD) (c) and carbon uptake (d) carbon isotope analyses (δ13C) (right image) of shells being farmed in habitats subjected to acidification. Crystallographic orientation maps (left image) show how shell growth changes in families bred for fast growth and disease resistance compared to wild oysters. The colour maps and spread of colour (c) show that wild oysters shells are more disordered in their shell growth.

Conclusions

We show that selectively bred oyster families can alter their mechanisms of biomineralisation, promoting resilience to coastal acidification. Selective breeding in oysters is likely to be an important global mitigation strategy for shellfish aquaculture and restoration to withstand future climate driven change to habitat acidification.

References

Fitzer, S. C., *et al*., (2019) Selectively bred oysters can alter their biomineralization pathways, promoting resilience to environmental acidification. Global Change Biology, 25: 4105-4115. https://doi.org/10.1111/gcb.14818

Fitzer, S. C., *et al*., (2018). Coastal acidification impacts on shell mineral structure of bivalve mollusks. Ecology and Evolution, 8(17), 8973–8984.

O'Connor, W., and Dove, M. C. (2009) The changing fate of oyster culture in New South Wales, Australia. *Journal of Shellfish Research,* 28(4): 803-811.

"NET9": A STEP-CHANGE IN MARINE FIN-FISH PRODUCTION COSTS AT OFFSHORE LOCATIONS, THROUGH THE APPLICATION OF INNOVATIVE FLEXIBLE STRUCTURAL TECHNOLOGY

John Fitzgerald*[1] and Michael O'Shea[2]

[1]Impact-9 Energy and Marine Ltd., Skerries, Dublin, Ireland K34F751, [2]University College Cork, Cork, Ireland
Email: [1]john@impact-9.com
[2]michaeloshea@ucc.ie

Impact-9 has conducted a detailed techno-economic assessment for the deployment of a novel offshore aquaculture farm system called Net9, which is designed to facilitate the production of salmon at higher energy sites than is currently possible. The assessment is undertaken as part of a feasibility study considering deployments in the UK and Ireland, including potential integration at future floating offshore wind project sites.

The objective is to out-perform incumbent salmon production models prevalent in Norway and Chile, where 75% of salmon is currently produced in sheltered fjord locations. However, production licences at these locations are increasingly constrained for environmental reasons and the cost of licences has increased greatly in Norway, where such licences are tradeable and issued in price-setting auctions. The Net9 objective is to produce a farm system that overcomes the geographic constraints in such a way that any increase in capital cost is more than compensated by reduced production licence costs as well as improved biological performance. Net9's technology strategy is to use its experience in flexible materials, synthetic fibre structures and mooring analysis, to greatly reduce the cost of containment structures. Current technology trends in semi-closed or onshore closed RAS systems mean that the cost-effective supply of larger post-smolt salmon of approximately 500g for offshore stocking is possible. The Net9 concept could provide for late stage grow-out of salmon from 0.5kg to 5kg live weight in offshore, high-energy environments. Growth at sea is far more economic for later stage grow-out. This analysis addresses the economic assessment of a 3000-tonne annual production project, which would involve the deployment of a single Net9 structure that would run in 12 month grow-out cycles. High level results of the economic analysis are outlined below.

The results of the analysis show that if the cost and performance metrics can be met, fish production costs of €4 per kg (gutted weight) can be achieved, even when generous smolt costs of €4 / 500g smolt and a significant licence cost of €13.5 million per pen is included. The economic model is calibrated to the production cost structure reported by MOWI for 2019 costs of Norwegian salmon production. The model is used to identify the cost and performance metrics that can out-compete incumbent fish producers at locations away from fjords.

Ongoing technical analysis demonstrating the technical feasibility is progressing well. Numerical analysis has been used to optimise design and inform cost projections. Experimental work at 1:30 scale in a laboratory concluded at University College Cork in March 2021 provides further technical understandings for the novel structure. The potential to increase UK / Ireland salmon production from current levels (210,000 tonnes per year) is essentially limited only by market demand. However, in this respect maintaining cost-competitiveness will be important as once production is more profitable outside fjords, there will be no geographic limitations to increasing supply in various regions around the globe.

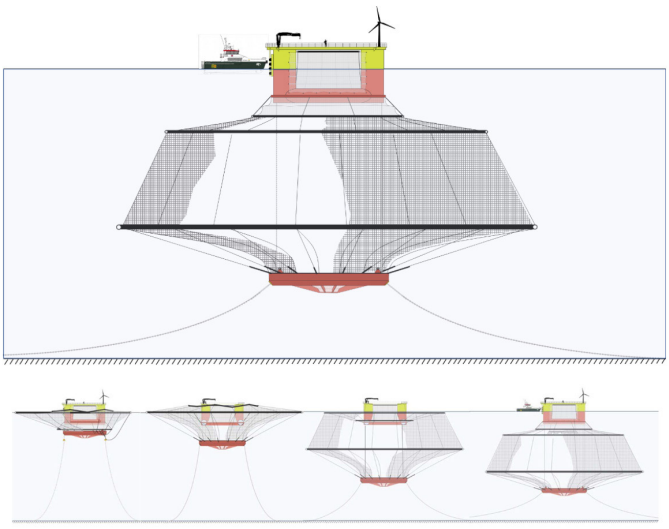


Fig 1: General Arrangement of patented Net9 farm system solution

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KEY ECONOMIC PERFORMANCE	
INITIAL INVESTMENT	€19.7 million (of which €13.5 million production licence)
PAYBACK PERIOD	4 years
IRR	21%
PRODUCTION COST	€4.00 /kg (HOG)
EBIT	€2.50 @ €6.50 /kg market price
DISCOUNT RATE	10% per annum applied to all future cost / revenues
PROJECT LIFE	16 years

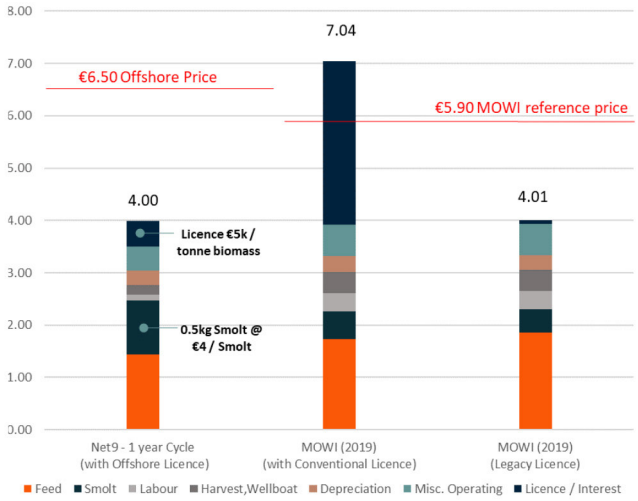
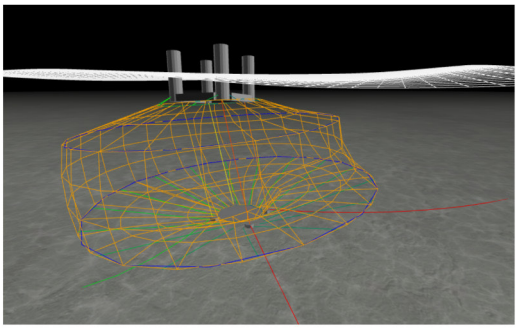


Fig 2: Net9 production cost in comparison to costs published by MOWI, presented both with (new entrant cost) and without (legacy operator) licence costs included



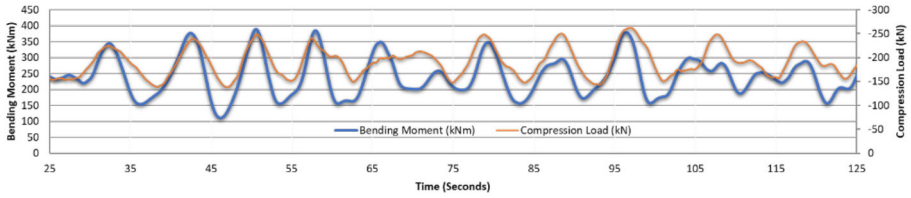


Fig 3: Net9 Numerical Model: Sample results giving the bending moment and compressive loads applied to the collar structure, which is used to determine structure size and cost.

TOWARD A SELECTION TO IMPROVE TURBOT (*Scophthalmus maximus*) RESISTANCE TO EDWARDSIELLOSIS

Y. François*1, M. Villa3, B. Peyrou3, P. Haffray1, T. Morin2, A. Bestin1

1SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), INRAE-LPGP, Rennes, France
2French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Ploufragan-Plouzané-Niort Laboratory, Unit Virology, immunology and ecotoxicology of fish (VIMEP), Plouzané, France
3France Turbot Ichtus, L'épine, France
Email : yoannah.francois@anses.fr

Introduction

First identified on catfish in 1969 (Meyer and Bullock, 1973), edwardsiellosis has been described on turbot in 1993 (Nougayrede et al. 1994). This disease is caused by an enterobacteria called *Edwardsiella tarda (E. tarda)* which can affect a wide range of animals from fishes to mammals, inducing septicemia. Responsible of important economic losses in farmed fish, edwardsiellosis has become a disease of concern these past years (ANSES 2015). Numerous studies showed the possibility of using genetic selection to improve fish resistance to a disease on various fish species (Ødegård et al. 2011) and on various pathogens including *E. tarda* in olive flounder (Li et al. 2019).

The Turboost project supported by the EMFF aims at estimating the possibility of using genetic selection to improve the resistance of turbot to *E. tarda*. The first step of the work presented here is dedicated to the development of an infectious challenge in controlled conditions in order to phenotype the trait "resistance to edwardsiellosis".

Material and Methods

All experiments were carried out at the ANSES-SYSAAF FORTIOR Genetics Platform, within the facilities of the ANSES VIMEP unit, in accordance with the European guidelines and the French legislation. Procedure was approved by the ethic committee on animal experimentation ANSES/ENVA/UPC N°16, and authorized by the French Ministry of Higher Education, Research and Innovation.

Fish (mean weight of 50g) were provided by the breeding farm France Turbot Ichtus. They were maintained in seven tanks supplied with filtered seawater (open circuit) at a temperature of 15°C ±2 prior to infectious challenges. Bacteria were grown on TSA (Triptic Soy Agar) plates for 24h at 24°C and were then suspended in sterile saline solution.

Challenge development tests used 460 fish divided in tanks of 30 fish. For bath challenges, turbot were contaminated in hyperoxygenated static seawater during 3 hours with a fresh bacteria suspension. Inoculation dose were adjusted at $1x10^{11}$ or 10^{10} Colony Forming Unit/ml (CFU/ml). Intra-peritoneal (IP) injections were done on fish anaesthetized using 100μl of a fresh bacteria suspension, with inoculation doses adjusted to 10^{10}, 10^{9}, 10^{8} and 10^{7} CFU/ml. Tests were performed at two different temperatures (15 and 18°C).

For the phenotyping challenge, 1220 fish distributed in 7 tanks were IP injected with 100μl of a fresh bacteria suspension at $3x10^{12}$ CFU/ml. In parallel, a negative control group (n=80) received 100μl of a sterile saline solution. During the challenge, fish were maintained in filtered seawater at 19°C and fed twice a day. Observations occurred daily and the challenge was carried out until mortality stops. Bacteriological analyzes were performed on dead fishes to assess the presence of *E. tarda*.

Results and discussion

Development tests clearly showed that turbots were not sensitive to *E. tarda* at a temperature of 15°C since no clinical signs nor mortality was recorded. At 18°C, fish infected by bath started to show clinical signs 3 weeks after contamination and mortality was recorded during 12 days. A dose effect clearly appeared with IP injection with faster mortalities for the highest doses (figure 1). These results were in accordance with Qin et al. (2014) who showed that high bacteria concentrations lead to high mortality rates. Cumulative mortalities were also more important with IP injection (80-100%) compare to bath (35-80%).

Conclusion

These first trials of experimental infection of turbots with *E. tarda* in controlled conditions highlighted the parameters to optimize (injection routes, temperature) in order to improve challenges efficiency and to strengthen protocols. In this study we demonstrated the feasibility of phenotyping edwardsiellosis resistance, which will enable estimation of genetic parameters and genetic correlations with production traits.

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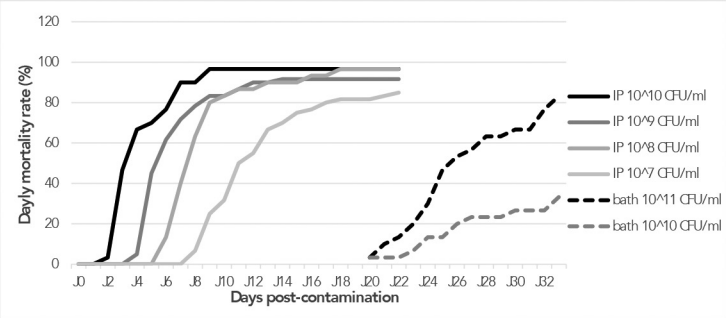

Figure 1: Mortality kinetics obtained during the development of IP and bath challenges. The kinetic of mortality recorded during the phenotyping challenge was similar to that observed during the preliminary tests, with a cumulative mortality of 90%. Acquisition of genetic parameters of the trait "resistance to edwardsiellosis" is in progress.

Bibliography

ANSES, 2015. Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif à la « hiérarchisation des dangers sanitaires exotiques ou présents en France métropolitaine chez les poissons d'élevage ». Saisine n°2013-SA-0049 C. 125 pp

Li, Y., Wang, L., Lu, S., Wang, S., Zhang, H., Yang, Y., Li, M., Chen, S., 2019. Heritability of disease resistance to Edwardsiella tarda in olive flounder (Paralichthys olivaceus). Aquaculture 519. https://doi.org/10.1016/j.aquaculture.2019.734750

Meyer, F.P., Bullock, G.L., 1973. Edwardsiella tarda, a New Pathogen of Channel Catfish (Ictalurus punctatus). Appl Microbiol 25, 155–156.

Nougayrede, P., Vuillaume, A., Vigneulle, M., Faivre, B., Luengo, S., Delprat, J. (Laboratoire departmental des L., 1994. First isolation of Edwardsiella tarda from diseased turbot (Scophthalmus maximus) reared in a sea farm in the Bay of Biscay. Bulletin of the European Association of Fish Pathologists (United Kingdom).

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquaculture Research 42, 103–114

Qin, L., Xu, J., Wang, Y.G., 2014. Edwardsiellosis in farmed turbot, Scophthalmus maximus (L.), associated with an unusual variant of Edwardsiella tarda: a clinical, aetiological and histopathological study. Journal of Fish diseases 37, 103-111

DEALING WITH A TOUGH CHILDHOOD - MORPHOMETRIC AND VIDEO ANALYSIS OF EMBRYONIC TO LARVAL PIKEPERCH (*Sander lucioperca*)

G.P. Franz [1,2*], L. Lewerentz[1,3], B. Grunow[1]

[1]Institute for Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[2]Department of Cytology and Evolutionary Biology, University of Greifswald, Zoological Institute and Museum, Soldmannstraße 23, 17489 Greifswald, Germany
[3]Institute of Physics, University Greifswald, Felix-Hausdorff-Straße 6, 17489 Greifswald, Germany
E-Mail: franz@fbn-dummerstorf.de

Introduction
In the course of the diversification of the range of farmed species, pikeperch, *S. lucioperca* has become a species of growing interest for the aquaculture industry, as it can provide food products of high quality and value (Policar *et al*., 2019). The overall high demand for animals for stock and further rearing can hardly be met, so that still most specimens are collected from the wild (Zakęś & Demska-Zakęś, 2009). Presently, many obstacles prevent breeding and raising in captivity. A major reason is the tedious rearing process, with low survival rates in the early developmental phases between hatching and the larval stages. Named reasons for mortality are problems with organ development as well as cannibalism (Kestemont *et al*., 2015; Ostaszewska, 2005). Therefore, our aim was two-fold: In a first project, we described the morphometric and morphological changes of pikeperches obtained from a wild population from embryonic to larval stages to gain insights into the basic developmental scheme. In a second project, we counted and tracked the movements of specimen, to be able to study the onset and extent of cannibalism. For this, we set up a video system that targets a later application in the industry, to increase the survival rate of larvae.

Materials and methods
Specimens were raised under close to natural conditions using a flow-through system, that provided freshwater from the nearby lake. 162 specimens were collected over the course of 10 age stages. On these, we analysed a total of 21 morphometric parameters including volumes of endogenous resources and postanal muscle tissue calculated from these. Additionally, morphological changes were noted. Based on the measurements, a PCA was conducted to gain insight into overall growth tendencies. Following, the parameters were analysed separately.

For video tracking, hatchlings were transferred to an aquarium with steady temperate lake water. A prototype structure using GoPro cameras was placed around the tank, providing videos from the three perspectives. Gained video sequences were then analysed using an OpenCV-software (Bradski, 2000) and improved by training a neuronal net based on the software Tensorflow (Abadi *et al*., 2016). Based on image comparison, the moving specimens could be tracked and counted.

Results
The morphometric analysis indicate three different phases of growth. During embryonic growth, most parameters developed with a linear trend. Shortly after hatch, an intermediate threshold phase started, showing stagnation of growth and a focus on internal development. After the finalised change to exogenous feeding, the larval stage began. Larvae were characterised by an increase in growth with high size variations and a developmental focus on tissues for locomotion.

The video tracking showed a decrease in the number of specimens over time, with some very substantial decreases between days.

Discussion
Our study showed the severity of changes occurring in the embryonic-larval shift, as strong depletion of internal resources were combined with a stop of external growth. We see this as the result of a change in energy expenditures, which are necessary due to developmental and environmental constraints (Rombough, 2011; Wieser, 1995). Additionally, this is followed by a phase in which a strong cannibalistic pressure is formed by increasing growth rates and size variations between the specimens. The digital monitoring of hatchling stocks thereby showed promising results for possible implementation as a rearing aid. Being able to count specimen numbers in real-time can show time points for necessary size-selection to prevent cannibalism. With better cameras and optics, additional information on locomotion/activity shifts and thus the health of the specimens (Pikarsky *et al*., 2004) will be obtainable from by tracking locomotion patterns.

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Sources

Abadi, M., Agarwal, A., Barham, P., Brevdo, E., Chen, Z., Citro, C., Corrado, G. S., Davis, A., Dean, J., & Devin, M. (2016). Tensorflow: Large-scale machine learning on heterogeneous distributed systems. *arXiv:1603.04467*.

Bradski, G., (2000). The OpenCV Library. *Dr. Dobb's Journal of Software Tools*.

Kestemont, P., Dabrowski, K., & Summerfelt, R. C. (2015). *Biology and culture of percid fishes: principles and practices*. New York: Springer.

Ostaszewska, T. (2005). Developmental changes of digestive system structures in pike-perch (*Sander lucioperca* L.). *Electronic journal of ichthyology,* 2, 65-78.

Pikarsky, E., Ronen, A., Abramowitz, J., Levavi-Sivan, B., Hutoran, M., Shapira, Y., Steinitz, M., Perelberg, A., Soffer, D., & Kotler, M. (2004). Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. *Journal of virology,* 78 (17), 9544-9551.

Policar, T., Schaefer, F. J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., & Zarski, D. (2019). Recent progress in European percid fish culture production technology-tackling bottlenecks. *Aquaculture International,* 27 (5), 1151-1174.

Rombough, P. (2011). The energetics of embryonic growth. *Respiratory Physiology & Neurobiology,* 178 (1), 22-29.

Wieser, W. (1995). Energetics of fish larvae, the smallest vertebrates. *Acta Physiologica Scandinavica,* 154 (3), 279-290.

Zakęś, Z., & Demska-Zakęś, K. (2009). Controlled reproduction of pikeperch Sander lucioperca (L.): a review. *Archives of Polish Fisheries,* 17 (4), 153-170.

GENOMIC BASIS OF RESISTANCE TO *Flavobacterium columnare* IN RAINBOW TROUT

C. Fraslin[1]*, A. Nousiainen[2], H. Koskinen[2], R.D. Houston[1], A. Kause[2]

[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 (Declercq et al., 2013). 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). The objectives of this study were first to estimate genetic parameters and detect quantitative trait loci (QTL) associated with resistance to *F. columnare* in a large rainbow trout population from a Finnish breeding programme, and then assess the efficiency of genomic selection compared to standard pedigree-based selection 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 vet for a diagnostics. 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 peace of tail was sampled from those 1,531 fish for later DNA extraction and genotyping. Thereafter, the fish rearing continued in the tanks until October 2019, when 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.

Resistance was analysed as a binary trait (0=alive; 1=dead) with the rearing tank as a fixed effect in the statistical model. A Genome-Wide Association Study (GWAS) was performed to detect QTL associated with resistance using the Mixed Linear Model Association implemented in GCTA software (Yang et al., 2011) with the "Leave-One-Chromosome-Out" option (MLMA-LOCO). The additive and dominance effects of the top SNP of the significant QTLs were estimated using the three genotypes of a SNP fitted as fixed effects along with the tank effect in ASReml (Gilmour et al., 2015). The (genomic) estimated breeding values [(G)EBV] of fish were obtained using pedigree-based BLUP, genomic BLUP (GBLUP), and GBLUP weighted with the allele effects of SNPs (wGBLUP, 1 iteration) (BLUPF90 software; Misztal et al., 2002). The efficiency of pedigree-based and 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 pedigree based heritability.

Results and discussion

After quality controls, 27,907 SNPs and 2,874 challenged fish (1,403 dead fish and 1,471 alive fish) and 78 parents remained and were used to recover the pedigree of 98.6% of the challenged fish. Pedigree based heritability was estimated to be 0.18 (± 0.038) on the observed scale, and genomic heritability was estimated to be 0.32 (± 0.045) on the underlying scale and 0.21 (± 0.029) on the observed scale. Genomic and phenotypic variances were 0.05 (± 0.009) and 0.25 (± 0,008), respectively. The GWAS detected one QTL significant at the genome-wide level, one QTL significant at the chromosome-wide level on chromosome Omy15, along with several suggestive QTLs (Fig. 1). The additive and dominance effects of the peak SNP from Omy3 were estimated to be 0.11 (0.018 se) and -0.03 (0.02 se). Pedigree-based prediction accuracy was 0.64 (± 0.01) and the use of genomic evaluation increased the prediction accuracy by 14% for the GBLUP (0.73 ± 0.014), and by 17% for the wGBLUP (0.75 ± 0.017) after one iteration.

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Conclusion

These results suggest that, in this rainbow trout population, resistance to CD is moderately heritable. While resistance to CD is polygenic, there was a notable and significant QTL detected on Chromosome 3, with several other minor putative QTLs detected. Genomic prediction accuracy was shown to be 15% higher than pedigree-based prediction accuracy. Therefore, resistance is a suitable target trait for genetic improvement by selective breeding, and genomic selection is a useful approach to speed up this process.

Acknowledgment

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References

Declercq, A.M., Haesebrouck, F., Van den Broeck, W., Bossier, P., Decostere, A., 2013. Columnaris disease in fish: a review with emphasis on bacterium-host interactions. Vet. Res. 44, 27.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J., Thompson, R., 2015. ASReml User Guide Release 4.1 Functional Specification. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

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.

Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: A Tool for Genome-wide Complex Trait Analysis. Am. J. Hum. Genet. 88, 76–82.

ORGANIC AQUAPONICS IN THE EUROPEAN UNION: TOWARDS SUSTAINABLE FARMING PRACTICES

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. It has been identified by the European Commission as one of the ten technologies that will change our lives. Whilst there are a number of successful commercial aquaponic ventures, mainly in the USA, it is speculated that organic certification could help with its marketability and commercialisation. However, organic certification is currently unachievable, given the several rules EU organic regulation that prevent this. The newly published Council Regulation (EU) 848/2018, which entered into force in January 2021 has introduced stricter rules that further hinder the certification of aquaponic products. Regarding organic production and certification, the main regulatory committees are the Expert Group for Technical Advice on Organic Production (EGTOP), the Committee on Organic Production, and the Civil Dialogue Group (CDG). The 2013 report by EGTOP titled 'Final Report on Greenhouse Production (Protected Cropping)', was very influential in shaping the rules of the new Commission Regulation (EU) 2018/848.

This paper investigates the current status quo with regard to aquaponics and organic status in the EU, both in terms of the plants as well as aquatic organisms and discusses the regulatory anomalies that prevent organic recognition.

The 'Organicness' of Aquaponics

Essentially, aquaponic produce can be seen to be environmentally sustainable, respecting natural cycles, employing high standards of health and welfare for the farmed organisms; it is also safe to eat, and can support rural and social development. This means that aquaponics embodies the true spirit of the principles of organic production, and thus should be considered as an organic mean of food production.

Organic Rules Preventing Organic Aquaponic Production

There are several rules that prevent the organic certification of aquaponic products in Council Regulation (EU) 848/2018; the main ones are:

- The mandatory use of a living soil as a growing medium for crops in connection with the subsoil and bedrock;
- The prohibition of hydroponic technologies;
- The mandatory maintenance and increase of soil fertility through the use of terrestrial animals' manure whilst fish waste is not allowed;
- The prohibition of recirculating aquaculture systems (RAS);
- The prohibition of the artificial heating and cooling of water in aquatic animal production; and
- The mandatory presence of a bottom type that is as close as possible to natural conditions for the farming of freshwater fish species.

EU Policies in Support of Aquaponics and Potential ones for the Development of Organic Aquaponics

Although no policies or regulations are in place directly for aquaponics in the EU, some existing policies and strategies from related fields can provide opportunities and support. Since aquaponics involves both fish and plant production, relevant EU policies that apply include the Common Agriculture Policy (CAP), the Common Fisheries Policy (CFP) which has established the Aquaculture Advisory Council (AAC), the EU Food Safety and Nutrition Policy, and the EU Environmental Policy. The goals of these policies include promoting innovation, improving access to space and water, increasing sustainability and competitiveness, preventing the generation of waste, improving the welfare of animals including fish, developing a low-carbon economy, promoting the efficiency of resource use (thus directly relating to organic aquaponics and its low water and nutrient use), promoting the use of areas unfit for other food production systems, and employing local food production approaches.

The Way Forward: Policies and Potential Advances in Aquaponic Technology

Several policies are suggested for the development of organic aquaponics. The concept behind the policies and the rules is to maintain the tripartite goals of 'environmentally, socially and economically sustainable production', maintaining ethical, circular economy and nature-based aspects that should be part of organic production and certification, leaving behind those aspects which are now entrenched in aquaponic certification but that are not scientifically based. Regarding technological advances, 'soil-based' aquaponics and environmental enrichment in RAS are two innovations that are being investigated at the University of Greenwich and which could bring aquaponics one step closer to organic certification in addition to the regulatory challenge of the status quo which is based on non-scientific criteria.

BIOLOGICAL CARBON BUDGET OF MUSSELS CULTURED IN THE GALICIAN RÍAS (NW SPAIN): CO_2 STORAGE OR SEQUESTRATION?

Isabel. Fuentes-Santos[1,]*, Mª José Fernández-Reiriz[1], Uxío Labarta[1], Xosé Antón Álvarez-Salgado[1]

[(1)]CSIC Instituto de Investigaciones Marinas, C/Eduardo Cabello 6, 36208 Vigo, Spain.
*E-mail: isafusa@iim.csic.es

Introduction

Bivalve aquaculture is a low carbon footprint food production system that also provides key ecosystem services such as nutrients removal in eutrophicated areas or CO_2 storage in bivalve shells, which represent 50% to 90% of the total bivalve weight. The large volume of marine bivalve aquaculture, more than 17.7 million tons in 2018, has revitalized the interest on the implications of CO_2 storage and eventual sequestration in bivalve shells, and on applications to inert shell calcium carbonate (Alonso et al., 2021).

In this work we calculate the biological carbon budget of the *Mytilus galloprovincialis* cultured in the Galician rias (NW Spain), where 15% of the world mussel production takes place. The contribution of the biological processes that remove or release CO_2 and affect the total alkalinity (TA) of the surrounding waters are evaluated using local environmental data, and the biochemical composition and physiological rates of mussels cultured in the Galician rias.

Methods

Following the culture practices in Galicia (Labarta and Fernández-Reiriz, 2019), we have estimated the CO_2 biological budget for mussels growing from 15 mm to 50 mm (legal minimum) and 75 mm (medium commercial size). Given that the time to reach the harvesting size and, consequently, the CO_2 budget, depends on the seeding time, we have considered April and September as extreme seeding times (Fuentes-Santos et al., 2019). The biological processes that remove or release CO_2 to the surrounding water during mussel growth are, 1) CO_2 removal for shell calcium carbonate ($CaCO_3$), shell protein matrix, and mussel flesh synthesis, 2) CO_2 release during bio-calcification, 3) CO_2 release by respiration to support the cost of synthesis and maintenance of flesh and shell synthesis, 4) CO_2 removal by refractory dissolved organic matter (RDOM) excretion. 5) CO_2 removal by NH_4^+ excretion and subsequent phytoplankton uptake, and 6) CO_2 removal – release balance of faeces egestion and the subsequent microbial degradation.

Site-specific physiological and biochemical data (Fernández-Reiriz and Labarta, mussel lab database), experimental allometric rules for respiration and ammonia excretion by Arranz et al. (2016) and a growth model by Fuentes-Santos et al. (2019) based on are used to estimate the CO_2 removal or released by each biological process.

The CO_2 biological budget, which determines the environmental carbon footprint of cultured mussel is given by:
CO_2 biological budget = – (CO_2 removed by $CaCO_3$ + shell protein matrix + flesh
+ RDOC + faeces egestion + phytoplankton growth from NH_4^+ excretion)
+ (CO_2 released by biocalcification + respiration + faeces microbial degradation)

	Seeding time	April		September	
	Harvesting size	**50 mm**	**75 mm**	**50 mm**	**75 mm**
	Culture length	**120 d**	**180 d**	**300 d**	**390 d**
CO_2 biological budget: -$CaCO_3$ + biocalcification +	– (shell protein + flesh) + respiration	**-0.90**	**-3.17**	**-0.17**	**-1.38**
	– (shell protein + flesh + RDOC) + respiration	**-0.95**	**-3.31**	**-0.36**	**-1.68**
	– (shell protein + flesh + RDOC + NH_4^+ exc.) + respiration	**-1.31**	**-4.43**	**-1.27**	**-4.10**
CO_2 storage budget: -$CaCO_3$+ biocalcification +	+ respiration	**0.01**	**-0.11**	0.71	1.62
	+ respiration – RDOC	**-0.04**	**-0.25**	0.6	1.32
	shell respiration	**-0.23**	**-0.77**	**-0.2**	**-0.68**
	shell respiration – RDOC	**-0.27**	**-0.91**	**-0.31**	**-0.98**

Table 1: Individual CO_2 budget estimates (in g CO_2 $indv^{-1}$) for the four scenarios tested. Negative budgets (favourable to carbon sequestration) in bold.

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The CO_2 storage budget, which includes those biological processes with potential of carbon sequestration (associated to shell $CaCO_3$), is:
CO_2 storage budget = – (CO_2 removal by $CaCO_3$ + RDOC) +
+ (biocalcification + respiration to cover shell maintenance)

The opposite effects of faeces egestion and subsequent microbial degradation to the CO_2 budget generally result in a null effect of these processes. RDOC is suggested here for the first time and the partition of the CO_2 released by respiration between shell and flesh has been already suggested by Filgueira et al., (2015).

Results and discussion

Table 1 summarizes the CO_2 biological and storage budgets for mussels in the Galician rias under the four culture scenarios considered in this work (two seeding times, two harvesting sizes). We obtain a negative CO_2 biological budget for the four scenarios, but the CO_2 fixation decreases as the length of the culture cycle increases. When CO_2 fixation associated to phytoplankton uptake of the excreted NH_4 is considered, the effect of the culture cycle is minimized.

The CO_2 storage budget shows that the sequestration capacity reduces as the culture length increases, highlighting once again the role of the culture practices. When only the respiration associated to the synthesis and maintenance of the shell structure is considered, the culture is favourable to carbon sequestration under the four culture scenarios

References

Alonso, A.A., Álvarez-Salgado, X.A., Antelo, L., 2021. Assessing the impact of bivalve aquaculture on the carbon circular economy. J. Clean. Prod. 279, 123873.

Arranz, K., Labarta, U., Fernández-Reiriz, M.J., Navarro, E., 2016. Allometric size-scaling of biometric growth parameters and metabolic and excretion rates. A comparative study of intertidal and subtidal populations of mussels (Mytilus galloprovincialis). Hydrobiologia 772, 261–275.

Filgueira, R., Byron, C., Comeau, L., Costa-Pierce, B., Cranford, P.J., Ferreira, J., Grant, J., Guyondet, T., Jansen, H., Landry, T., others, 2015. An integrated ecosystem approach for assessing the potential role of cultivated bivalve shells as part of the carbon trading system. Mar. Ecol. Prog. Ser. 518, 281–287.

Fuentes-Santos, I., Labarta, U., Álvarez-Salgado, X.A., 2019. Modelling mussel shell and flesh growth using a dynamic net production approach. Aquaculture 506, 84–93.

Labarta, U., Fernández-Reiriz, M.J., 2019. The Galician mussel industry: Innovation and changes in the last forty years. Ocean Coast. Manag. 167, 208–218.

BIOANALYTICAL DEVICES FOR THE DETECTION OFCIGUATOXINS AND DNA FROM THE CIGUATOXIN-PRODUCING GENERA *Gambierdiscus* AND *Fukuyoa*

G. Gaiani[1]*, Anna Toldrà[1], Sandra Leonardo[1], Àngels Tudó[1], Karl B. Andree[1], María Rey[1], Takeshi Tsumuraya[2], Masahiro Hirama[2], Jorge Diogène[1], Carles Alcaraz[1], Ciara K. O'Sullivan[3,4], Mònica Campàs[1]

[1]IRTA, Ctra. Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Spain
[2]Department of Biological Science, Graduate School of Science, Osaka Prefecture University, Osaka 599-8570, Japan
[3]Departament d'Enginyeria Química, URV, Av. Països Catalans 26, 43007 Tarragona, Spain
[4]ICREA, Pg. Lluís Companys 23, 08010 Barcelona, Spain
Email: greta.gaiani@irta.cat

Introduction

Ciguatera fish poisoning (CFP) is one of the most relevant seafood-borne diseases worldwide. It is caused by the ingestion of fish containing ciguatoxins (CTXs), lipophilic marine toxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa* (Litaker et al. 2017) that accumulate into fish and through the food webs. CFP is characterized by severe neurological, gastrointestinal, and cardiovascular disorders and affects approximately between 50,000 and 500,000 consumers annually worldwide (Friedman et al. 2017).

Here, the first electrochemical immunosensor for the detection of CTXs is presented. Three different monoclonal antibodies (mAbs), two capture (3G8, 10C9) (Tsumuraya et al. 2006, Tsumuraya et al. 2012) and a detector (8H4) (Tsumuraya et al. 2006), were merged in a sandwich configuration for the combined detection of two main groups of CTX congeners (CTX1B and CTX3C). Additionally, the development of three molecular assays for the detection of the *Gambierdiscus* and *Fukuyoa* genera and for *G. australes* and *G. excentricus* species, based on the isothermal recombinase polymerase amplification with detection via hybridization, is described.

Materials and methods

The immunosensing technique involves the use of monoclonal antibodies (mAbs) showing high specificity and sensitivity for their CTX targets, and their exploitation in a sandwich colorimetric immunoassay and electrochemical immunosensor on magnetic beads (MBs). Specifically, the 3G8 mAb has affinity for the left wing of CTX1B and 54-deoxyCTX1B, the 10C9 mAb for the left wing of CTX3C and 51-hydroxyCTX3C, and the 8H4 mAb for the right wing of the four congeners. At first, the immunosensor has been used to screen fishes (nine *Variola louti*, six *Lutjanus bohar*, one *Thyrsitoides marleyi*) naturally contaminated with ciguatoxins. Then, it has been exploited to investigate the CTXs production of nine *Gambierdiscus* strains belonging to three species (*G. australes, G. excentricus* and *G. caribaeus)* and four *Fukuyoa paulensis* strains.

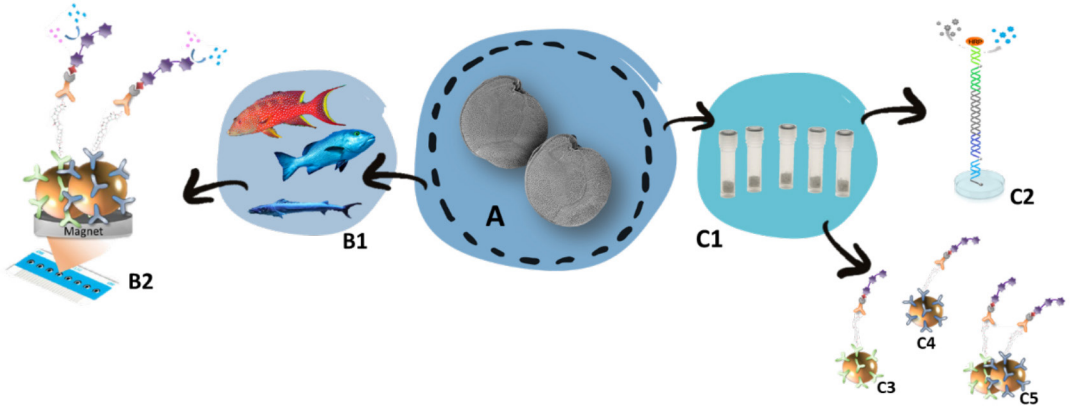

Figure 1. Scheme of the study. *Gambierdiscus* and *Fukuyoa* (A) can produce CTXs and accumulate in fishes. On one hand, extracts of fishes naturally contaminated (B1) were analyzed with the immunosensor (B2) On the other hand, *Gambierdiscus* and *Fukuyoa* cell pellets (C1) were prepared and either DNA or CTXs were extracted and analyzed respectively with RPA-SHA (C2) or with the immunosensor with separate (C3, C4) or merged (C5) antibodies.

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Among isothermal DNA amplification techniques, recombinase polymerase amplification (RPA) is very convenient as it does not require any initial denaturation step and it is carried out at a constant temperature (in this work, 37 °C). We exploited the use of primers modified with short oligonucleotide tails, which result in double-stranded DNA (dsDNA) amplicons fringed with single-stranded DNA (ssDNA) tails. The detection was achieved using a sandwich hybridization assay (SHA), where specific surface-anchored thiolated capture probes are complementary to one of the amplicon tails and an enzyme-labelled reporter probe is complementary to the tail in the other extreme.

Results

The applicability of the immunosensor to the analysis of fish samples is demonstrated, attaining detection of CTX1B at contents as low as 0.01 μg/kg and providing results in correlation with those previously obtained using mouse bioassay (MBA) and cell-based assay (CBA), and confirmed by liquid chromatography coupled to high-resolution mass spectrometry (LC-ESI-HRMS). Furthermore, the analysis performed on the nine *Gambierdiscus* strains and the four *Fukuyoa paulensis* strains allowed to detect CTXs presence at very low cell concentrations. The particularity of this study is the ability to discriminate between two series of CTX congeners, giving more information on the toxic profile of *Gambierdiscus* and *Fukuyoa* species.

The development and application of the RPA-SHA system to the detection of microalgae of the genera *Gambierdiscus* and *Fukuyoa,* and the discrimination between *G. australes* and *G. excentricus* species, is successfully reported. The method showed a high specificity for the target species/genera and even DNA extracted from a single cell was detected. Furthermore, the ability of the *Gambierdiscus* & *Fukuyoa* primer set to amplify target DNA in the presence of different species was demonstrated, together with the discriminable capacity of the species-specific primer sets (*G. australes* and *G. excentricus*).

Conclusion

The immunosensor provides robustness, specificity, simplicity, and rapidity. Therefore, it can be included in the group of methods ready to be used for ciguatera management (such as CBA, LC-MS/MS and RBA). Furthermore, it provided a better understanding of CTXs production in the genera *Gambierdiscus* and *Fukuyoa*. The use of the immunosensing tools can open the way for regional and international comparative studies, and on ciguatera as an expanding phenomenon. Moreover, the inclusion of the RPA-SHA system in monitoring programs would be useful to assess the risk of ciguatera, to predict possible outbreaks and consequently to preserve human health.

References

Friedman, M. A., M. Fernandez, L. C. Backer, R. W. Dickey, J. Bernstein, K. Schrank, S. Kibler, W. Stephan, M. O. Gribble, and P. Bienfang. 2017. An updated review of ciguatera fish poisoning: clinical, epidemiological, environmental, and public health management. Marine Drugs 15:72.

Litaker, R. W., W. C. Holland, D. R. Hardison, F. Pisapia, P. Hess, S. R. Kibler, and P. A. Tester. 2017. Ciguatoxicity of Gambierdiscus and Fukuyoa species from the Caribbean and Gulf of Mexico. PLoS One 12.

Tsumuraya, T., I. Fujii, M. Inoue, A. Tatami, K. Miyazaki, and M. Hirama. 2006. Production of monoclonal antibodies for sandwich immunoassay detection of ciguatoxin 51-hydroxyCTX3C. Toxicon 48:287-294.

Tsumuraya, T., K. Takeuchi, S. Yamashita, I. Fujii, and M. Hirama. 2012. Development of a monoclonal antibody against the left wing of ciguatoxin CTX1B: Thiol strategy and detection using a sandwich ELISA. Toxicon 60:348-357.

FISH MEAL REPLACEMENT BY INSECT MEALS (*Tenebrio molitor* AND *Hermetia illucens)* IN DIETS FOR TENCH (*Tinca tinca*). GROWTH AND LIVER REDOX STATUS

J. F. Galera[1]*, G. Cardenete[1], A.E. Morales[1], H. Pula[1], F. Melenchón[2], D. Fabrikov[3] and M.C. Hidalgo[1]

[1]Dpt. of Zoology. University of Granada. Campus Fuente Nueva. 18071 Granada (Spain)
[2]Research Aquaculture Center. Agro-Technological Institute of Castilla **y León.** Ctra. Ar**évalo** s/n, 40196, Segovia-Spain
[3] Dpt. Biology and Geology. University of Almería, 04120, Almería-Spain
Email: janirafernandezga@correo.ugr.es

Introduction

Sustainability of intensive fish farming requires limiting fishmeal in feed and therefore using alternative protein sources. Insect meal is a promising protein source recognized by FAO and provides an opportunity to replace, at least partially, fish meal protein in fish feed. The inclusion of two insect meals (*Tenebrio molitor* and *Hermetia illucens*) in diets for a freshwater fish, *Tinca tinca*, were assessed paying attention not only to growth, but also to the effect of the inclusion of these raw materials on the redox state of the fish. Both the decreased levels of dietary fish meal (rich in histamine), and the presence of chitin in insect meal can improve both aspects.

Material and methods

Juveniles of tench (24.1 ± 0.7 g) were kept in polyester circular tanks by triplicate during 8 weeks. Fish were fed at satiation twice a day with 5 isoproteic (42.2 ± 0.1%) and isolipidic (18.0 ± 0.1%) diets randomly assigned. The fish meal of the diets (36% in control diet) was replaced by *Hermetia illucens* and *Tenebrio molitor meals* at levels of 30 and 50% of substitution. Thus, experimental diets were: Control (100% fishmeal), H30 (70% fishmeal-30% *H. illucens*), H50 (50% fishmeal-50% *H. illucens*) H50M (50% fishmeal-50% *H. illucens* enriched in EPA and DHA), and T50 (50% fishmeal–50% *T. molitor*). At the end of experiment 90 fishes of each treatment were sampled to determine biometric indices and 9 livers of each to analyze redox parameters (Hidalgo *et al*., 2017): SOD, CAT, GPx, GR and GP6DH activities and MDA content.

Results and discussion

T. molitor meal at 18 % dmb of diet (replacing 50% of fish meal) significantly improves the Instant Growth Rate of tench (Fig.1 IGR); nevertheless diets including *H*. meal promotes in general lesser growth than control diet. These effects can be also observed in the Condition Factor and Hepatosomatic Index, although the differences are not always statistically significant.

In addition, the T50- diet includes higher antioxidant enzyme activities in liver than the *H. illucens* diets In (Table 1). These results are statistically significant for CAT and GPx; the activity of the latter was also higher than of fish feed on the control diet. On the other hand, no significant effect of PUFA enrichment of *H.illucens* (H50M) on the antioxidant enzymatic activities was observed.

Oxidative damage to liver lipids (Fig. 1 MDA), measured by malondialdehyde content, is reduced by the presence of insect meal, especially in the case of the H50M and T50 diets. This protective effect could be due to components of the insect exoskeleton, in addition to the increase in antioxidant defenses in the case of the *T. molitor* diet, as pointed out by Henry et al. (2018). Indeed, these authors detail very similar effects on MDA content and antioxidant enzymes activities in the intestine of rainbow trout fed a diet with a 67% replacement of fish meal by *T. molitor* meal.

In conclusion, based on results of growth and redox status, *Tenebrio molitor* meal appears to be a promising source of protein to replace in high proportions the fish meal present in tench diets.

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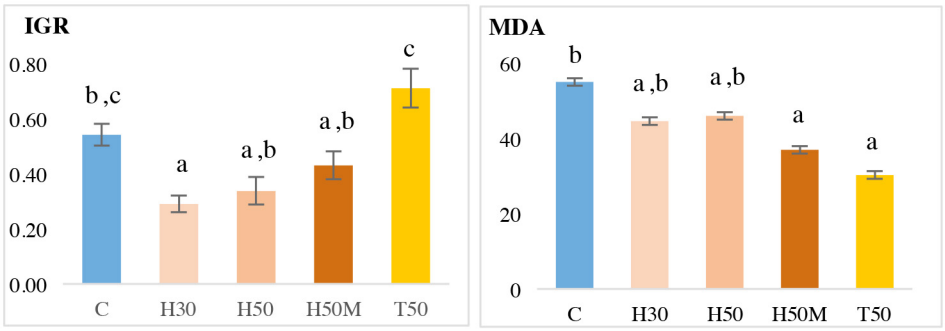

Fig. 1. IGR: Instant growth rate (%). MDA: Malondialdehyde content in liver (nmol/g tissue) C: control diet; H30, H50: fish meal replacement with *H. illucens* meal at 30% and 50% respectively; H50M: 50% of fish meal replaced with *H.illucens* enriched in PUFAs and T50: *T. molitor* meal replacing 50% of fish meal in diet. [a,b,c] Different letters identify statistically significant differences between diets ($p < 0.05$).

	C	H30	H50	H50M	T50	SEM
SOD	$198{,}7^{b}$	$173{,}7^{ab}$	$133{,}5^{a}$	$182{,}9^{ab}$	$185{,}2^{ab}$	5.8
CAT	248.0^{ab}	199.0^{ab}	230.2^{ab}	195.2^{a}	271.0^{b}	8.4
GPx*	35.5^{a}	28.0^{a}	32.3^{a}	33.0^{a}	58.2^{b}	2.2
GR*	6.98	5.47	6.74	6.91	7.55	0.3
G6PDH	45.4^{b}	26.0^{a}	34.0^{ab}	31.5^{ab}	39.3^{ab}	3.7

Table 1. Specific activity of antioxidant enzymes. Results expressed as U/mg protein; * mU/mg protein. U: unit of enzyme activity, defined as the amount of enzyme required to transform 1 µmol of substrate per minute. C: control diet; H30, H50: fish meal replacement with *H. illucens* meal at 30% and 50% respectively; H50M: 50% of fish meal replaced with *H.illucens* enriched in PUFAs and T50: *T. molitor* meal replacing 50% of fish meal in diet. [a,b,c] Different letters identify statistically significant differences between diets ($p < 0.05$), n=9.

Bibliography

Henry, M.A., F. Gai, P. Enes, A. Pérez-Jiménez, L. Gasco. (2018). *Fish and Shellfish immunology* 83: 308-313. http://dx.doi.org/10.1016/j.redox.2017.01.022

Hidalgo, M.C., A.E. Morales, M. Arizcun, E. Abellán, G. Cardenete. (2017). *Redox Biology* 11: 682–687. https://doi.org/10.1016/j.fsi.2018.09.040

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INCLUSION OF SEAWEED WRACKS IN DIETS FOR GRASS CARP *Ctenopharyngodon idella*

Ana Galindo[1*], José A. Pérez[1], David Barrios[1], Diana B. Reis[1], Manuel Marrero[1], Jaime de Urioste[2], Marianna Venuleo[3], Covadonga Rodríguez[1]

[1]Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, Spain
[2]Centro de Investigación y Conservación de la Biodiversidad. Fundación Neotrópico. Spain
[3]Departamento de Biotecnología División de Investigación y Desarrollo Tecnológico, Instituto Tecnológico de Canarias (ITC), Spain
*Email: agalindg@ull.es

Introduction

Stranding of macroalgal wracks that regularly appear in coasts from offshore seaweed beds play a key role in beach ecosystems. However, this clumping natural litter is often interpreted as an indicator of beach poor quality by tourists. Thus, algae biomass is usually removed, causing an increased pressure on the handling and management of beach wracks (Mossbauer et al., 2012). Inclusion of algae in fish feed has been recently described to have several physiological benefits such as an improvement in growth performance and lipid metabolism (Moutinho et al., 2018). Grass carp (*Ctenopharyngodon idella*) is a freshwater species with rapid growth and easy adaption to captivity that was the major fish species produced in the world in 2016 with more than 6 million tons (FAO, 2018). The use of Macaronesian macroalgal wracks as a supplement in aquafeeds from a feasible ecological and economical perspective is proposed in the present study.

Material and methods

Two complementary experiments were carried out in 1 m^3 fish tanks under recirculating aquaculture system (RAS). In the first experiment (E1), individuals of *C. idella* (8.6 ± **1.9 g)** were fed with an extruded diet for tilapia (Skretting) (control group) or with the same tilapia diet supplemented with a 15% of a wind dried powder (1 mm) product of multispecific macroalgae wrack (33.8% *Asparagopsis taxiformis*; 28.6% *Lobophora* sp.; 22.6% *Dictyota* sp.; 14.5% *Cymopolia barbata*, and 0.5% *Laurencia* sp.), as the experimental treatment. After 133 days, 4 individuals of each treatment were slaughtered, while the remaining individuals were used for the second experiment (E2). Thus, in E2, fish were fed with the tilapia diet (control group) or with the same diet supplemented with either a 7% of the multispecific macroalgae (experimental treatment 1) or a 7% of monospecific macroalgae wrack of *Lobophora* sp. (experimental treatment 2). After 99 days, 15 individuals of each treatment were slaughtered. Fish growth parameters, hepatosomatic (HSI), viscerosomatic (VSI), and visceral-fat index (VFI) were determined in both experiments. Muscle samples were also collected for the analysis of total lipid (TL) contents, fatty acid (FA) profiles, peroxides index (PI) and antioxidant enzymes. Finally, gut was removed in order to analyze the activity of digestive enzymes.

Table I. Survival, growth and fat indexes of *C. idella*.

	E1		E2		
	Control diet	Exp. treatment (15% multispecific wrack)	Control diet	Exp. treatment 1 (7% multispecific wrack)	Exp. treatment 2 (7% monospecific wrack)
Survival (%)	100.0	100.0	83.3	85.7	95.5
Weight increment (g)	30.7	16.1	15.7	23.2	18.2
WG (%)	345.0	194.5	41.5	95.6	47.3
SGR (% day^{-1})	1.1	0.8	0.4	0.7	0.4
HSI (%)	1.7 ± 0.2	0.8 ± 0.2*	1.5 ± 0.4	1.3 ± 0.5	1.5 ± 0.6
VSI (%)	7.3 ± 0.8	5.6 ± 1.6	8.0 ± 1.3^{b}	6.9 ± 0.9^{a}	7.7 ± 1.4^{ab}
VFI	2.7 ± 0.5	1.0 ± 0.0*	2.8 ± 0.4^{b}	2.1 ± 0.5^{a}	2.7 ± 0.5^{b}

VFI was calculated depending on visible fat of organs: 1 (low), 2 (medium) or 3 (high). *, and different superscript letters denote significant differences (P<0.05) in E1 and E2, respectively.

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Results

Although survival was not affected by the experimental treatment in E1, fish growth, specific growth rate (SGR) and weight gain (WG) were lower with the 15% of algae dietary inclusion of multispecific wrack than in the control group. Digestive enzymes activities were also negatively affected by the experimental treatment. On the other hand, fish fed the experimental diet showed better values of both VFI and HSI (Table I-E1). Overall, muscle lipid composition (TL and FA profile) remained unchanged regardless of dietary treatment. Finally, a better oxidation status of muscle (PI) was detected in the experimental fish. In E2, weight increment, WG and SGR were higher with a 7% of multispecific seaweed inclusion, compared to the control diet or a 7% of monospecific wrack inclusion. Digestive enzymes were not affected by any of the experimental treatments, while both VFI and VSI were lower in the multispecific wrack treatment (Table I-E2). By contrast, main lipid composition and PI were not modified by the experimental diets.

Discussion and conclusions

As reported in other feeding studies using >10% of dietary algae inclusion, the lower digestive activity of *C. idella* when fed the diet with the 15% of seaweed inclusion (E1) may be related to the presence of anti-nutrients in macroalgae that reduced digestibility and nutrient absorption, and, consequently, gave rise to a lower growth and fat deposition (VFI) in fish. By contrast, and similar to what occurs in our present experiment, lower seaweed supplementation (2.5-10%) improved growth performance in several species (Moutinho et al., 2018). Furthermore, the inclusion of up to 15% of algae in fish diets led to a lower liver lipid content (Xuan et al., 2019) and HSI, probably due to the lipolitic action of some brown algae (Bourgougnon, 2014) or even to a fat depletion due to fish undernourishment. On the contrary, a 7% of inclusion of this same multispecific macroalgal wrack displayed better fish growth also reducing the VFI and VSI, confirming the reported lipolitic action of the algae. Finally, muscle of fish supplemented with a 15% of multispecific wrack presented a better oxidative status than that of the control ones, a trend which was also observed with a 7% of inclusion. In summary, the inclusion of the multispecific macroalgae in *C. idella* diet reduced both fish perivisceral fat and liver deposition regardless of the percentage of inclusion, without affecting fish muscle TL and FA profiles. A 15% of inclusion reduced fish growth and digestive activity, whereas a lower supplementation of 7% did not significantly affect these parameters. The use of a 7% of multispecific seaweed as a feed additive seems to have beneficial effects in terms of both growth and visceral fat deposition in *C. idella*.

References

FAO. 2018. FAO yearbook. Fishery and Aquaculture Statistics 2016. Roma, 104 pp.

Bourgougnon, N. 2014. Advances in Botanical Research. Sea Plants. Elsevier Ed. Vol. 71. 561pp.

Mossbauer et al., 2012. Management of stranded eelgrass and macroalgae along the German Baltic coastline. Ocean Coast Manag., 57, 1-9.

Moutinho et al., 2018. Inclusion of 10% seaweed meal in diets for juvenile and on-growing life stages of *Senegalese sole* (*Solea senegalensis*). J. Appl. Phycol., 30(6), 3589-3601.

Xuan et al., 2019. Effects of different levels of macroalga *Gracilaria lemaneiformis* on growth performance and feed utilization on the red sea bream, *Pagrosomus major*. J. Appl. Phycol., 31(5), 3213-3222.

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SCREENING OF POTENTIAL PROBIOTIC BACTERIA FROM GILTHEAD SEABREAM (*Sparus aurata*) GASTROINTESTINAL TRACT: AN ENZYMATIC PRODUCTION APPROACH

J. García-Márquez[(1)], M. Domínguez-Maqueda[(1)], I.M. Cerezo[(1)], F.J. Alarcón[(2)], J.M. Mancera[(3)], M.A. Moriñigo[(1)], M.C. Balebona*[(1)]

(1) Departamento de Microbiología, Universidad de Málaga, Andalucía Tech, Campus de Excelencia Internacional del Mar (CEIMAR), 29071 Málaga, Spain. Email: j.garcia@uma.es
(2) Departamento de Biología y Geología, CEIMAR-Universidad Almería, 04120 Almería, Spain.
(3) Departamento de Biología, Instituto Universitario de Investigación Marina (INMAR), CEI-MAR-Universidad de Cádiz, 11510, Puerto Real, Cádiz, Spain.

Introduction

Fish aquaculture is greatly dependent on fish meal (FM). This is particularly obvious in carnivorous fish species due to their high dietary protein requirement (40–50 %), which is mainly provided by FM. Interest has focused in macro- and microalgae as a source of nutrients and functional ingredients and sustainable alternatives to FM. However, the nutritive value of these algae is often limited by the presence of several anti-nutritional factors. Bacteria that are capable of producing several enzymes can help to digest major feed constituents included in the diet of the host organism (Wanka et al., 2018). It has been suggested that an increase in the level of digestive enzymes could influence the digestion of feed contents (Midhun et al., 2016). Therefore, this study focuses on identifying potential probiotics among isolates from the gastrointestinal tract of *Sparus aurata* specimens fed with a diet containing a blend of microalgae. Thus, isolation and characterization of *S. aurata* intestinal bacteria producing enzymes able to hydrolyze nutritional and antinutritional factors present in algae supplemented-aquafeeds has been carried out.

Materials and methods

Potential probiotic bacteria were isolated from the gastrointestinal tract (GIT) of juvenile *S. aurata* fed (at Servicio Central de Investigación en Cultivos Marinos, University of Cadiz, Spain; Operational Code REGA ES1102800031) an experimental diet formulated with partial replacement of fishmeal, soybean concentrate and wheat meal by 25% of a blend of microalgae (*Chlorella*, *Isochrysis*, *Nannochloropsis* and *Spirulina*). The experimental diet was manufactured at the Servicio de Dietas Experimentales (Universidad de Almería) (http://www.ual.es/stecnicos_spe). After 15 weeks of feeding, nine specimens (146.8 ± 16.4 g) were euthanized by overdose of 2-phenoxyethanol (1mL L-1) followed by spine severing. Whole intestines were aseptically excised. Serial 10-fold dilutions were prepared from central intestinal contents in Phosphate Buffer Saline (PBS) and 100µL aliquots spread on two non-selective media (tryptic soy agar supplemented with 2% NaCl, TSA 2%; and minimum media (M9) supplemented with 2% NaCl and 25% microalgae mix, MMA). Marine lactic acid bacteria were selectively cultured on De Man, Rogosa and Sharpe agar (MRS) supplemented with 2% NaCl (Wanka et al., 2018) and spore-formers were selected after heat treatment and culture on TSA 2% according to Nicholson & Setlow (1990). Plates were incubated at 22 ºC (37 °C for lactic acid bacteria) in aerobic conditions for up to 2-3 days. The isolated bacteria were screened for different enzymatic activities. Proteolytic, collagenolytic, lipolytic and amylolytic activities were assayed according to Chabrillon et al. (2005). Phytic, tannic and cellulose hydrolysis were assayed according to Kumar et al. (2010).

Table 1. Hydrolytic activity (% of isolates) of culturable bacterial strains isolated from *S. aurata* GIT.

Medium	*Isolates (N)*	*Hydrolytic activity (% of isolates)*						
		Protease	Collagenase	Lipase	Amylase	Phytase	Tannase	Cellulase
TSA 2%	50	56	80	44	32	42	10	66
MMA	26	46	81	46	23	73	8	39
MRS	11	36	64	36	9	55	0	55
Sporeformers	30	40	73	33	40	27	7	57
Total	117	48	77	41	30	46	8	57

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Results and discussion

Altogether, 117 strains were isolated (50 from TSA 2 %, 26 from MMA, 11 from MRS and 30 spore-formers) from *S. aurata* GIT and screened for hydrolytic enzyme activities (Table 1). Results showed that 48 %, 41 %, 77 % and 30 % of strains isolated bacteria were able to hydrolyze protein, lipids, collagen and starch, respectively. Moreover, 46 %, 8 % and 57 % of isolates exhibited ability to degrade phytate, tannins and cellulose, respectively (Table 1).

A total of 17 % of the isolated strains showed five or more enzymatic activities and, although none of them were able to hydrolyze all nutritional and anti-nutritional factors evaluated, 3 strains were capable of hydrolyzing six of them (the 3 strains did not degrade tannins). The results confirmed the enzymatic-producing abilities of selected bacterial strains. Characterization of some enzyme-producing bacteria from fish GIT has been reported previously (Wanka et al., 2018; Serra et al., 2019). According to Serra et al. (2019), enzyme-producing bacteria in fish gut exert positive effects to the host digestive processes which can be considered as a potential probiotic character. Further analysis regarding inhibitory activity against fish pathogens and a safety evaluation of the selected probiotic strains need to be carried out in order to enclose and identify the strains of interest and, finally determine its applications in aquaculture.

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References

Chabrillón, M., Rico, R.M., Arijo, S., Díaz-Rosales, P., Balebona, M.C. & Moriñigo, M.A. (2005). Interactions of microorganisms isolated from gilthead sea bream, *Sparus aurata* L., on *Vibrio harveyi*, a pathogen of farmed Senegalese sole, *Solea senegalensis* (Kaup). J Fish Dis, 28, 531–537.

Kumar, R., Kumar, A., Nagpal, R., Sharma, J. & Kumari, A. (2010). A novel and sensitive plate assay for screening of tannase-producing bacteria. Annals of Microbiology, 60(1), 177-179.

Midhun, S.J., Arun, D., Edatt, L., Sruthi, M.V., Thushara, V.V., Oommen, O.V., Kumar, V.B.S. & Divya, L. (2016). Modulation of digestive enzymes, GH, IGF-1 and IGF-2 genes in the teleost, Tilapia (*Oreochromis mossambicus*) by dietary curcumin, Aquac. Int. 24, 1277–1286.

Nicholson, W.L. & Setlow, P. (1990). In Molecular Biological Methods for *Bacillus* (eds Harwood, C. R. & Cutting, S. M.), 391–450 (John Wiley & Sons Ltd.).

Serra, C.R., Almeida, E.M., Guerreiro, I. Santos, R., Merrifield, D.L., Tavares, F., Oliva-Teles, A. & Enes, P. (2019). Selection of carbohydrate-active probiotics from the gut of carnivorous fish fed plant-based diets. Sci Rep. 9: 6384 (2019).

Wanka, K.M., Damerau, T., Costas, B., Krueger, A., Schulz, C., & Wuertz, S. (2018). Isolation and characterization of native probiotics for fish farming. BMC Microbiol., 18, 1–13.

IS THERE ROOM IN THE FISH MARKET FOR THE DEVELOPMENT OF NEW AQUACULTURE PRODUCTS?

S. García Muñoz, R. Llorente, I. Peral

AZTI, Basque Research and Technology Alliance (BRTA), Spain
E-mail: sgarcia@azti.es

Introduction:
Aquaculture production is growing year by year representing in 2018 the 46% of the total fish production and 52% of fishes consumed by humans (FAO, 2020). However, new food products based on aquaculture are still scarce on the market. Thus, to assist in bridging the gap between producers and end users, Azti BRTA (Spain) has successfully developed four new food products.

The new fish food products were developed based on two premises: (i) considering specific Mediterranean aquaculture fish species: sea bass, sea bream and meagre, and (ii) the consumers needs and expectation identified by our research group in Spain, France and Germany. The consumer-centred approach during the developing of new food products could improve their degree of market success. Finally, the new products were tasted by Spanish fish consumers.

The aims of this study were (i) to analyse the consumers acceptability and preferences about new food products developed in the frame of H2020 MedAID project and based on aquaculture fish species and (ii) to describe the consumers willing to pay for them.

Material and methods:
In this study, four products (Grilled sea bass with lemon, Sea and mountain burger, Sea bream breaded bites and Organic sea bream with couscous) were tasted at home environment in 2020 by 75 Spanish fish consumers (60% female, 40% males) from 18 to 67 years old (mean age: 43.64 ± 1.49) and from to geographic areas (North and Middle East).

All the participants were provided with information about the products, how to prepare them at home and how to complete the online questionnaire. Afterwards, they completed an online questionnaire regarding sensorial attributes of the new products and purchase intention. Consumers' acceptance for various product attributes was measured through hedonic scale (Liker scale).

Results
The consumers evaluated positively the new food products. The results indicate that Grilled sea bass with lemon was preferred by consumers in terms of general taste and juiciness. By contrast, Organic sea bream with couscous was the least preferred in terms of general taste and juiciness. In terms of preference, the consumers preferred the most the grilled sea bass with lemon and sea bream breaded bites. Surprising, the Sea and mountain burger was chosen by consumers in the third position. The consumers purchase intention was aligned with the sensorial evaluation, as much they liked the product, higher purchase intention was obtained. It is remarkable that when a brief product description information is given to consumers, their purchase intention increased.

Conclusions
Considering the obtained results, the developed new products could be available on the market. It seems that all the new developed products are likely to be accepted by Spanish consumers and Grilled sea bass with lemon are likely to succeed more as future aquaculture product in Spain.

Information given to consumers about new fish products could change consumers purchase intention. Thus, this information should be considered.

Reference
FAO. (2020). The state of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

DIFFERENCES IN FATTY ACID COMPOSITION BETWEEN REARED VS. WILD THICK-LIPPED GREY MULLET (*Chelon labrosus*)

Jorge García-Márquez*[(1)], Félix L. Figueroa[(2)], Francisco Javier Alarcón[(3)], S. Arijo Andrade[(1)], R.T. Abdala-Díaz[(2)]

(1) Microbiology Department, Faculty of Science, University of Málaga, CEI-MAR, 29071 Málaga, Spain. Email: j.garcia@uma.es
(2) University of Malaga, Institute of Blue Biotechnology and Development (IBYDA), Ecology Department, Faculty of Sciences, Campus de Teatinos s/n, 29071, Malaga, Spain.
(3) Department of Biology and Geology, Ceimar-University of Almería, Almería, Spain

Introduction

The need to diversify marine aquaculture products has stimulated the farming of new species. Thick-lipped grey mullet (*Chelon labrosus*) has several characteristics that make it an interesting candidate for diversification and sustainability of aquaculture production (Ben Khemis et al., 2013). First, it has omnivorous/detritivorous feeding habits and therefore feeds on the lowest trophic levels (Ben Khemis et al., 2013). Thick-lipped grey mullet larvae and post-larvae mainly feed on zooplankton and small crustaceans, while the natural diet in adults is based on benthic diatoms, epiphytic algae, small invertebrates, and detritus (Fernández-Delgado et al., 2000). Besides, as other mullets, *C. labrosus* has a high osmoregulatory capacity, allowing it to inhabit a broad range of salinities without negative effects on growth rate (Pujante et al., 2018).

Fish is the major readily available and edible source of health-promoting omega-3 polyunsaturated fatty acids (n-3 PUFA) for human consumption (Calder, 2014). Muscle metabolic profile and the ratio of n-3/n-6 fatty acids of wild and farmed fish may vary according to their species, genetic profile, habitat, season, and nutrition. As such, the lipid composition of aquaculture feed can influence the fatty acid content of farmed fish flesh (Sargent et al., 2002), resulting in a reduced amount of PUFA. In this sense, the aim of this study was to compare the liver and muscle fatty acid profiles of cultured and wild thick-lipped grey mullet.

Materials and methods

Wild thick-lipped grey mullet, *Chelon labrosus*, (n = 5; average weight and length: 60.2 ± 1.6 g and 16.7 ± 1.5 cm, respectively) were caught in an estuary in San Fernando, Cádiz, Spain. Farmed thick-lipped grey mullet (n = 5; average weight and length: 62.5 ± 3.1 g and 17.1 ± 2.0 cm, respectively) were collected from the C.I.F.P. Marítimo Zaporito facilities in San Fernando (Cádiz). Farmed specimens were fed with a commercial feed (Tilapia TI-3 feed, Skretting Co., Trouw, France) containing 32% protein and 6% fat. Wild and cultures animals were euthanized by overdose of 2-phenoxyethanol (1 mL L^{-1}) followed by spine severing. Specimens were then individually weighted and the liver and muscle were dissected out of the fish. Samples were immediately frozen and kept under – 80 °C individually until they were analyzed for lipid and fatty acid profile. The lipid content of the tissue was measured after extraction with chloroform/metanol (2:1 v/v) according to the method described by Folch et al., (1957). The lipids were dissolved in toluene and the fatty acids methyl esters (FAME) were obtained by transesterification with sulfuric acid (1%) in methanol (Christie, 2003).

Results and discussion

The liver of cultured thick-lipped grey mullet contained significantly ($p < 0.05$) higher proportions of C16:0, C18:00, C16:1, C18:1n9, C18:2n6 and C22:5n3, and statistically ($p < 0.05$) lower proportions of C14:00, C20:3n6, C20:4n-6 (arachidonic acid, ARA), C20:4n3, C20:5n-3 (eicosapentaenoic acid, EPA) and C22:6n-3 (docosahexaenoic acid, DHA) fatty acids than wild thick-lipped grey mullet. The percentages of total polyunsaturated fatty acids, total n3, total n6, as well as the n-3/n-6 and EPA/DHA ratio were significantly higher ($p < 0.05$) in wild than in cultured thick-lipped grey mullet, whereas the corresponding total saturated, total monounsaturated and total n9 fatty acid content was statistically lower ($p < 0.05$).

The muscle of cultured thick-lipped grey mullet contained significantly ($p < 0.05$) higher proportions of C14:0, C16:0, C20:0, C16:1, C18:1n9, C20:1, C18:2n6 and C20:2, and lower proportions of C18:3n6, C20:3n6, C20:4n6, C20:5n3, C22:6n3 and C22:2 fatty acid residues than wild thick-lipped grey mullet. The percentages of total polyunsaturated fatty acids, total n3, as well as the n-3/n-6 ratio were significantly higher ($p < 0.05$) in the wild than in cultured thick-lipped grey mullet, whereas the corresponding total saturated, total monounsaturated and total n9 fatty acid content was lower.

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Thus, the observed results indicate that cultured and wild thick-lipped grey mullet may be differentiated using total lipid content and fatty acid proportions and these differences may be attributed to the constituents of the diet of the fish. The lower proportion of n-3 PUFA in cultured fish may reduce the nutritional quality of their lipid components. Dietary manipulation of the fatty acid profile of farmed fish should be used to meet the standards and expectations of consumers and to maximise the nutritional benefits by consuming farmed fish according to the recommendations of clinical and nutritional scientists.

References

Ben Khemis, I., Gisbert, E., Alcaraz, C., Zouiten, D., Besbes, R., Zouiten, A., Masmoudi, A.S. & Cahu, C. (2013). Allometric growth patterns and development in larvae and juveniles of thick-lipped grey mullet Chelon labrosus reared in mesocosm conditions. Aquac. Res., 44, 1872–1888

Calder, P. C. (2014). Very long chain omega-3 (n-3) fatty acids and human health. European Journal of Lipid Science and Technology, 116, 1280–1300.

Christie, W.W. (2003) Lipid analysis. Pergamon, Oxford, UK, 3th edition.

Folch, J., Lees, M., & Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological chemistry 226, 497-509.

Fernández-Delgado, C.; Drake, P.; Arias, A.; García, D. (2000). Peces de Doñana y su Entorno; Organismo Autónomo Parques Nacionales—Ministerio de Medio Ambiente: Madrid, Spain.

Pujante, I. M., Moyano, F. J., Martos-Sitcha, J. A., Mancera, J. M., & Martínez-Rodríguez, G. (2018). Effect of different salinities on gene expression and activity of digestive enzymes in the thick-lipped grey mullet (Chelon labrosus). In Fish Physiology and Biochemistry (Vol. 44, Issue 1, pp. 349–373). Springer Netherlands.

Sargent, J. R., Tocher, D. R., & Bell, G. J. (2002). The lipids. In J. E. Halver, & R. Hardy (Eds.), Fish nutrition (pp. 181–257). San Diego, CA: Academic Press.

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UNVEILING THE TRANSCRIPTIONAL REGULATORY PATHWAYS ASSOCIATED WITH SHAPE QUALITY IN SENEGALESE SOLE, *Solea senegalensis*

Gayo, P.[1]*, Zerolo, R.[2], Claros, M.G.[3] and Manchado, M.[1]

[1]IFAPA Centro El Toruño, Junta de Andalucía, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
[2]CUPIMAR, Ctra. Carraca, s/n, Salina San Juan Bautista, San Fernando, Cádiz, Spain
[3]Department of Molecular Biology and Biochemistry, Universidad de Málaga, 29071, Málaga, Spain
Email: patricia.gayo@juntadeandalucia.es

Introduction

The Senegalese sole is an economically important species whose aquaculture is rapidly growing in Southern European. They are cultivated in recirculation aquaculture systems (RAS) that provide a stable environment to grow juveniles until harvest size in a competitive way. Nevertheless, there are still some aspects that need to be optimized and improved such as the morphology quality (Manchado, et al., 2019). Several studies have reported the high plasticity of the skeletal components in this species that accumulates high rates of skeletal malformations, most of them remaining unnoticed or with a moderate effect on external shape (de Azevedo, et al., 2017). However, studies dealing with shape quality as defined by ellipticity, the major indicator by consumers of high-quality sole morphology (Blonk, et al., 2010), are still lacking. The aim of this study was to investigate the meristic and molecular regulatory pathways that determine ellipticity in a fast-growing family. Two groups of high (HE) and low ellipticity (LE) were identified and main biometric traits were determined *in vivo* and by digital image analysis (DIA). Malformations were evaluated by X-ray analysis and expression patterns were investigated by RNA-seq deep sequencing.

Materials and methods

The family selected for the analysis was obtained from the breeding program that is currently ongoing by the company CUPIMAR (San Fernando, Spain). Animals were genotyped and pedigree reconstructed by using a SSR Multiplex PCR. For each animal, weight was in vivo measured and standard length (SL), body maximum height (MH), aspect ratio (AR) and roundness were determined by DIA using the Fiji 2.1.0/1.53c. Ellipticity (E) was determined as previously described (Blonk, et al., 2010). Corrected breeding values using the tank as fixed factor were estimated using restricted maximum likelihood adjusted linear mixed models (REML). After analysis, two groups of siblings (n_{HE} =10 and n_{LE}=14) with HE (residues between +1.2 and +17.4) and LE (residues between -7.4 and -24.4) containing approximately half of females and males were selected. For gene expression analysis, animals were euthanized and samples of muscle were collected, fixed in liquid nitrogen and stored at -80°C until analysis. Moreover, X- rays were also taken to evaluate skeletal anomalies. RNA purification and Illumina sequencing were carried out as described in Cordoba-Caballero, et al. (2020). ANOVA analysis, with gender and E group (HE and LE) as fixed factors and the weight as covariate, was carried out using the package SPSS. Principal component analysis (PCA) was performed in R. RNA-seq differential expression analysis (n =4) for each group was carried out using edgeR and DESeq with a false discovery rate (FDR) cut-off of 0.05 as implemented in the DEgenesHunter package. Functional analysis was also carried out with this package using the RefSeq information from orthologous zebrafish (*Danio rerio*) genes. Enrichment results were considered significant at FDR $p< 0.05$.

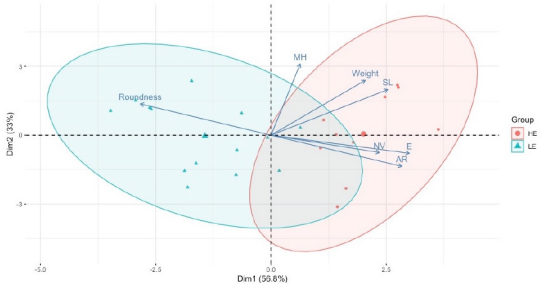

Figure 1. PCA biplot for morphological variables analyzed in this study for HE and LE. The confidence ellipses (95%) are shown. AR: Aspect ratio, E: Ellipticity, MH: Maximum height, NV: Number of vertebrae, SL: Standard length).

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Results and conclusions

Two sole groups from the same family with different ellipticity (HE=0.4170.004 and LE=0.3810.003) were identified. Statistical analysis indicated that soles of the HE group were as average significantly longer (35.050.24 cm) and with a lower height (14.590.14 cm) than LE (33.740.20 cm and 15.010.11 cm), respectively. The AR and roundness were also significantly different between groups and show correlation >0.90 with ellipticity. PC1 in the PCA analysis explained 56.8% of variation and it was associated with SL, AR, roundness and E. The PC2 explained 33% of variation and it was mainly associated with MH (Figure 1). X-ray analysis showed clear differences in the number of vertebrae between groups. Most of the soles in the HE group (81.8%) had 37 abdominal and caudal vertebrae without any vertebral fusions. In contrast, LE showed a greater incidence of vertebral fusions (21.4 %) and a lower number of vertebrae (36; 42.9%). PCA results indicated that the number of vertebrae and fusions were associated with E and AR indicating a major role on elliptical shape.

To investigate the expression profiles associated with E in muscle, RNA-seq analysis was carried out using both males and females classified as HE and LE. A high number of differentially expressed genes (DEGs) associated with gender (4,996 DEGs) and only 1 associated to E were identified. When this analysis was carried out by sex, a total of 105 DEGs were found in males and 45 DEGs in females. Main pathways enriched for DEGs were proteasome and cell adhesion molecules in males and the phagosome and adherens junction in females. All these results indicated that AR and roundness are also good predictors of ellipticity. Moreover, the RNA-seq analysis indicated structural pathways modified associated with E. All these are relevant to understand the mechanisms that determine shape quality in sole.

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References

Manchado et al. (2019) The Biology of Sole, 375-394.
de Azevedo, et al (2017). Dis Aquat Organ, 124, 117-129.
Blonk, R J. et al (2010) Aquaculture, 307, 6-11.
Cordoba-Caballero, J. et al., (2020) Sci Rep, 10, 20654.

TRACKING AND ANALYSIS OF THE MOVEMENT BEHAVIOUR OF EUROPEAN SEABASS *Dicentrarchus larbax* IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

Dimitra G. Georgopoulou[1] *, Orestis Stavrakidis-Zachou[1,2], Nikos Mitrizakis[1], Vaggelis Chalkiadakis[1], Nikos Papandroulakis[1]

1. Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, AquaLabs, Thalassocosmos 71500, Gournes, Heraklion, Greece
2. Department of Biology, University of Crete, 71003 Heraklion, Greece
E-mail: d.georgopoulou@hcmr.gr

Introduction

Animal welfare and ecosystem health can be achieved by increasing efficiency in the aquaculture procedures. Feeding is the primary factor determining efficiency and cost, so it is important to improve feeding management to maximize efficiency. Until now, fish feeding has been mostly based on manual practices by fish farmers, which is usually time-consuming and laborious. In recent years, intelligent feeding control according to changes in behaviour and growth status has gained increasing attention. In the current study we developed an automated routine that enables us to extract fish trajectories (of short time length) in RAS systems and we present its prediction accuracy and its potential use.

Materials and methods

Fifty individuals of European seabass *(Dicentrarchus larbax)* were recorded for a period of 2 months (December and January 2019) in Recirculating Aquaculture Systems (RAS) from 8.00 in the morning till 19.30 in the afternoon. Fish were fed twice in week days, once on Saturdays and no feeding was realized on Sundays. An automated routine (using OPENCV/Python) has been developed that automatically tracks the fish for a short time interval. The instantaneous speed is extracted and compared against the groundtruth values to assess the accuracy of the automated routine.

Results

Manually (1a) and automatically (1b) extracted instantaneous speeds measured in body-length/frame are shown in figure 1. The systems' instantaneous speed shows higher variations and more extreme values as opposed to that of the groundtruth. Overall, the temporal pattern of the speeds is successfully captured, but the system tends to overestimate the median instantaneous speed.

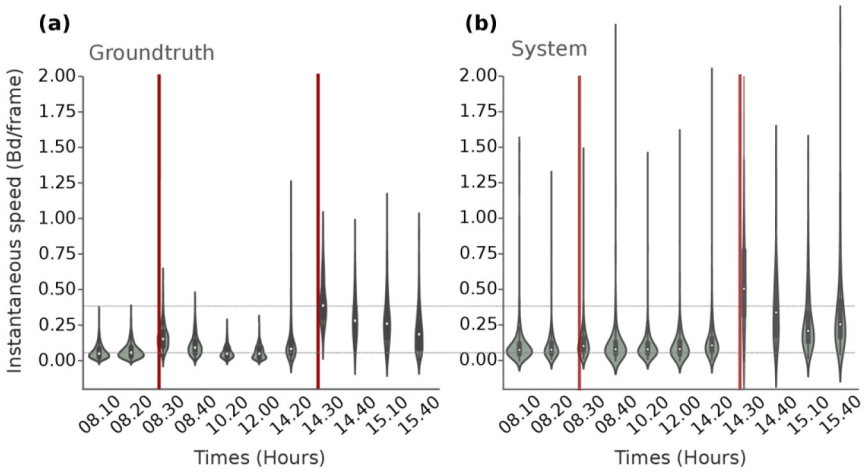

Figure 1. Violin plots of the instantaneous speed for different times, extracted (a) manually and (b) automatically from our system. Red lines indicate feeding times.

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Conclusion

The routine can successfully capture abrupt speed changes, but tends to estimate higher absolute values. A reason for this could lie on the low contrast videos (i.e. low contrast between fish and background) that result in imperfect fish detection and smaller size estimation. We expect that improving fish detection will increase the accuracy of speed estimation. As abrupt changes in speed can successfully be captured, the system can be used for detecting fish behavioural responses to feeding and/or threats.

Acknowledgments

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References

Zhou, C., Xu, D., Lin, K., Sun, C. and Yang, X. 2018. Intelligent feeding control methods in aquaculture with an emphasis on fish: a review. Rev Aquacult, 10: 975-993. https://doi.org/10.1111/raq.12218

Walther D., Edgington D. R. and Koch C.. 2004. "Detection and tracking of objects in underwater video," Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition. CVPR 2004., Washington, DC, USA, 2004, pp. I-I, doi: 10.1109/CVPR.2004.1315079.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B. and Sih, A. 2011, Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. Journal of Fish Biology, 78: 395-435. https://doi.org/10.1111/j.1095-8649.2010.02874.x

Bradski, G., & Kaehler, A. 2008. Learning OpenCV: Computer vision with the OpenCV library. " O'Reilly Media, Inc."

EFFECTS OF FISHMEAL REPLACEMENT BY *Chlorella vulgaris* AND FISH OIL REPLACEMENT BY *Microchloropsis gaditana* AND *Schizochytrium* sp. BLEND ON GROWTH AND FEED EFFICIENCY OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

E.Z. Gkalogianni[1]*, P. Psofakis[1], A. Asimaki[1], A. Moustogianni[1], S. Bouras[2], D. Antoniadis[2], I. Zarkadas[3], G. Papapolymerou[4], N. Katsoulas[2], I.T. Karapanagiotidis[1]

[1] Department of Ichthyology and Aquatic Environment, University of Thessaly, Fytoko Street, 38446, Volos, Greece, egkalogianni@uth.gr
[2] Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Fytokou Street, 38446 Volos, Greece
[3] BioMar Hellenic SA, 2nd Industrial Zone of Volos, Velestino 37500, Greece
[4] General Department, University of Thessaly, Larissa 41110, Greece

Introduction

The need for dietary replacement of marine-origin resources by suitable alternatives still remains a major issue for aquaculture. Microalgae are regarded as promising alternatives that could potentially reduce dependence on conventional raw materials, thus ensuring sustainability standards in aquaculture (Shah et al. 2018). *Microchloropsis gaditana* and *Schizochytrium* sp. are rich sources of n-3 essential fatty acids, while *Chlorella vulgaris* is a rich in protein microalgae species. The present study evaluated the effects of fishmeal substitution by *C. vulgaris* and fish oil substitution by a blend of *M. gaditana* and *Schizochytrium* sp. on the diet of European seabass (*Dicentrarchus labrax*).

Materials and Methods

Juvenile seabass of 2.85±0.01 g initial mean weight were obtained from a commercial fish hatchery, transferred to our departmental facilities and then distributed after an acclimatization period of 10 days in triplicate to 18 closed seawater circulation system tanks (120L) (35 individuals/tank, 3 reps/dietary group). The groups were fed six different isoenergetic (21 MJ/Kg) and isonitrogenous (52% CP) diets, at which fishmeal protein of the Control diet was replaced by *C. vulgaris* meal at 10% (CM10), 20% (CM20) and 30% (CM30) and fish oil by a *M. gaditana* and *Schizochytrium* sp. blend at 50% (SM50) and 100% (SM100). Fish were hand-fed to apparent satiation twice a day for 11 weeks.

Table 1. Growth performance and feed utilization of *D. labrax* fed with the experimental diets.

Parameters / dietary groups	Control	CM10	CM20	CM30	SM50	SM100
FBW (g/fish)	24.0±2.6^{a}	24.8±1.6^{a}	23.8±2.1^{a}	22.9±0.8^{a}	31.2±1.2^{b}	28.9±0.6^{b}
Feed intake (g/fish)	30.3±1.1^{a}	29.6±1.4^{a}	28.9±1.1^{a}	28.2±0.2^{a}	36.6±1.4^{b}	34.5±1.2^{b}
WG (g/fish)	21.2±2.6^{a}	21.9±1.6^{a}	21.0±2.1^{a}	20.1±0.8^{a}	28.3±1.2^{b}	26.0±0.6^{b}
SGR (%/day)	2.8±0.1^{a}	2.8±0.1^{a}	2.8±0.1^{a}	2.7±0.1^{a}	3.2±0.1^{b}	3.0±0.0^{b}
FCR	1.4±0.1	1.3±0.1	1.4±0.1	1.4±0.1	1.3±0.1	1.3±0.1
Survival (%)	87.6±5.9^{ab}	92.4±3.3^{a}	95.2±3.3^{a}	94.3±2.9^{a}	98.1±3.3^{c}	97.1±0.0^{bc}
HIS (%)	1.7±0.0	1.4±0.2	1.5±0.1	1.6±0.0	1.7±0.1	1.8±0.1
VSI (%)	12.5±1.0	12.3±0.5	11.7±0.3	12.3±0.8	11.1±0.5	12.4±1.4
CF	0.8±0.1^{a}	1.0±0.2^{a}	0.7±0.1^{a}	0.8±0.1^{a}	0.9±0.1^{b}	1.0±0.2^{b}
PER	1.3±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.5±0.1	1.5±0.1

Note. Values represent means ± standard deviation of triplicates. Values within each row not sharing a common superscript letter are significantly different ($P < 0.05$).

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Results and Discussion

Both SM-fed groups had significantly ($P<0.05$) higher feed intake, final body weight, weight gain, SGR and survival, and a lower FCR, although not significant, compared to the Control group (Table 1). This indicated that the dietary fish oil can be replaced totally by the *M. gaditana* and *Schizochytrium* sp. blend in the diet of juvenile seabass, leading to even better growth performance and feed utilization. *M. gaditana* and *Schizochytrium* sp. have not been tested comprehensively in sea bass. Haas et al. (2016) found that fish oil can be successfully replaced not more than 50% by *Microchloropsis* sp. in the diet of juvenile *D. labrax*. Studies with other microalgae species, such as *Isochrysis* sp., *Tisochrysis lutea* and *Tetraselmis suecica* have shown that 36% of fish oil replacement is achievable in the diet of seabass without adversely affecting fish growth performance (Tibaldi et al. 2015, Cardinaletti et al. 2018). *Schizochytrium* sp. has been tested with great success fully replacing fish oils in the diets of other fish species such as the carnivore *Salmo salar* (Tibbetts et al. 2020) and the omnivore *Oreochromis niloticus* (Sarker et al. 2016). It is worth mentioning that a blend of *M. gaditana* and *Schizochytrium* sp. that leads to a balanced EPA and DHA content, as used in the present study, have also been proved successful in *Sparus aurata* (Metsoviti et al. 2018) and *Paralichthys olivaceus* (Qiao et al. 2014).

As far as the use of *C. vulgaris* is concerned, our study showed that all CM-fed groups had similar ($P>0.05$) survival, feed intake, growth performance and feed utilization with the Control group (Table 1), suggesting that up to 30% fishmeal protein substitution by *C. vulgaris* is attainable for a carnivorous species such as *D. labrax*. The protein quality of *C. vulgaris* has been proved to be high also in the carnivore *Salmo salar* (Tibbetts et al. 2017). Nevertheless, *C. vulgaris* has been proved that could be used in higher dietary inclusion levels in omnivorous species such as *O. niloticus* (Badwy et al. 2008). The study suggests that *C. vulgaris*, *M. gaditana* and *Schizochytrium* sp. are promising alternatives for fishmeal and fish oil replacement in the diet of *D. labrax*.

Acknowledgements

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References

Badwy, T. M., Ibrahim, E. M., & Zeinhom, M. M. (2008, October). In 8th International Symposium on Tilapia in Aquaculture (pp. 801-811).

Cardinaletti, G., Messina, M., Bruno, M., Tulli, F., Poli, B. M., Giorgi, G., & Tibaldi, E. (2018). Aquaculture, 485, 173-182.

Haas, S., Bauer, J. L., Adakli, A., Meyer, S., Lippemeier, S., Schwarz, K., & Schulz, C. (2016). Journal of applied phycology, 28(2), 1011-1021.

Metsoviti M.N., Gkalogianni E.Z., Katouni A.M., Rougkas G., Savvaki E., Psofakis P., Katsoulas N., Papapolymerou G., Karapanagiotidis I.T. (2018). Proceedings of the European Aquaculture Society and World Aquaculture Society (AQUA 2018), August 25-29, Montpellier, France, p. 508

Qiao, H., Wang, H., Song, Z., Ma, J., Li, B., Liu, X., & Zhang, L. (2014). Aquaculture nutrition, 20(6), 646-653.

Sarker, P. K., Gamble, M. M., Kelson, S., & Kapuscinski, A. R. (2016). Aquaculture nutrition, 22(1), 109-119.

Shah, M. R., Lutzu, G. A., Alam, A., Sarker, P., Chowdhury, M. K., Parsaeimehr, A., & Daroch, M. (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of applied phycology, 30(1), 197-213.

Tibaldi, E., Zittelli, G. C., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., & Poli, B. M. (2015). Aquaculture, 440, 60-68.

Tibbetts, S. M., Mann, J., & Dumas, A. (2017). Aquaculture, 481, 25-39.

Tibbetts, S. M., Scaife, M. A., & Armenta, R. E. (2020). Aquaculture, 520, 735003.

THE THEORY OF RELATIVITY – AS APPLIED TO ESSENTIAL FATTY ACID REQUIREMENTS IN ATLANTIC SALMON (*Salmo salar*)

David Huyben, Chessor Matthew, Marta Bou, Bente Ruyter, Douglas Tocher,
Brett Glencross*

Institute of Aquaculture, University of Stirling, FK9 4LA, Stirling, Scotland, United Kingdom
*Presenting author: b.d.glencross@stir.ac.uk

Requirements for omega-3 (n-3) polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for Atlantic salmon are typically represented as an absolute level in the diet (e.g. g/kg or % of diet). Data for other species suggests that requirements for n-3 PUFA are actually relative to dietary lipid (e.g. % of total fatty acids). A 2 x 2 factorial design of dietary lipid level x n-3 PUFA level was designed to examine this question. Atlantic salmon post-smolts of 187 ± 4 g were fed one of four diets for 116 days that either had a low (LL) or high lipid (HL) level (180 or 230 g/kg) and a low (Ln-3) or high n-3 (Hn-3) PUFA level (7 or 14 g/kg). Fish fed the diet with HL+Hn-3 had greater final weight and weight gain than the HL+Ln-3 diet, but no differences were noted between the two LL diets. Significant effects of n-3 and a lipid*n-3 interaction were observed. However, no effects on feed intake, FCR and survival were found. Feeding high n-3 diets generally increased n-3 PUFA levels and retention in the whole body, especially EPA and DHA. Relative expression of lipid metabolism genes in the liver showed that fish fed HL+Hn-3 diet had lower levels of expression of fatty acid synthesis genes (*fads2d5*, *fads2d6* and *elovl2*). Upregulation of lipid transcription factor (*srebp2* and *lxr*) and fatty acid beta-oxidation (*hoad* and *aco*) genes in fish fed LL+Hn-3 further suggest that the proportion of dietary n-3 PUFA and energy level in those diets were lower than the HL+Hn-3 treatment. In conclusion, the significant interaction between lipid and n-3 levels on growth clearly shows that n-3 PUFA requirements are relative to the lipid level in diets for Atlantic salmon. These results support the notion that requirements for this species should be defined based on a percent of total fatty acid content, implying that the absolute amount of n-3 PUFA needs to increase as lipid content of the diet increases.

PARTIAL REPLACEMENT OF FISH MEAL WITH SHRIMP WASTE MEAL IN PRACTICAL DIETS FOR EUROPEAN LOBSTER (*Homarus gammarus*, L.) JUVENILES

R. Goncalves*, I. Lund, D. Sousa, P.V. Skov

Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, DK-9850 Hirtshals, Denmark
E-mail: rego@aqua.dtu.dk

Introduction

The combined effect of high market price and wild stocks decline makes the European lobster (*Homarus gammarus*) an excellent candidate species for commercial aquaculture. European lobster hatcheries experience high mortalities often related to moulting problems and knowledge on nutritional requirements and diet formulation for larvae and early juvenile stages is essential. While previous studies have focused on the substitution of fresh natural diets by dry pelleted feeds [(1)], the use of more sustainable ingredients in the formulation of lobster feeds has shown limited success [(2)]. The use of sustainable feeds is necessary to support the future commercialization and a more viable production. Cold-water shrimp waste meal has been identified as an animal protein with great potential. Moreover, the growth of the shrimp industry has led to a large production of processing waste and shrimp heads alone represent 35-45% of the total production [(3)]. The aim of this study was to evaluate the impact on growth and nitrogen metabolism of using meal from cold-water shrimp (*Pandalus borealis*) waste (heads and peels) as a protein source included at different levels in diets formulated for European lobster juveniles.

Materials and Methods

Homogeneous groups of 15 *H. gammarus* juveniles (164 ± 55 mg) were individually reared on five semi-moist diets for 8 weeks in a raceway recirculation seawater system (18±0.5˚C temperature, 34±1 PSU salinity, >7mg/L dissolved oxygen, <0.1mg/L NH_4). The experimental diets were formulated to contain 50% crude protein [(1)] combining different proportions of shrimp waste meal (SWM) and fish meal (FM), thus FM was substituted by 0%, 10%, 20%, 30%, or 40% of SWM . Individual lobsters were hand-fed one pellet (approx. 55 mg) of the assigned diet each morning, and allowed to feed for 4h. Moulting frequency and mortality were recorded daily. Wet body weight (BW) and carapace length (CL) of individual lobsters were measured every second week. Nitrogen excretion rate was determined between the second and fourth week of the growth trial. Briefly, each lobster (previously starved for 24h) was transferred to a 130 mL seawater container. Water samples were collected manually from individual containers at time 0h, 2h, 6h, 12h, and 24h for baseline screening of total ammonia nitrogen (TAN) excretion rates. Following this period, lobsters were offered a pre-weighed pellet for 4h. The uneaten fraction was collected, filtered, and dried to estimate feed intake (FI). After the meal, lobsters were transferred to a similar container with clean seawater. Water samples were collected at the same sampling times for the determination of postprandial TAN excretion rates. N intake was calculated as 16% of protein intake.

Results

The experimental diets had a significant effect on survival highest for the SWM40 group (87 %) and the lowest (47%) for the SWM10 group (Fig. 1). Experimental diets with higher inclusion of SWM had a significant positive effect on FI (Table 1). Specific growth rate (SGR), carapace length increment (iCL), and intermoult period were not affected by the dietary treatments. Despite the generally higher N intake and N excretion at the highest replacement levels, no significant effect of dietary treatment was observed on nitrogen budgets (Table 1).

Discussion

Results showed that FM can be replaced by SWM in practical diets for juvenile *H. gammarus* up to 40% without negatively influencing growth or nitrogen retention. The replacement of FM by SWM had a positive effect on the feed intake and survival rate of the lobster juveniles. The increased survival rates of lobsters reared on the SWM40 could be the result of a higher chitin content in this diet. Shrimp meal is a natural source of chitin. After enzymatic degradation, chitin splits into N-acetyl glucosamine which can potentially be used to synthesize new chitin during the moulting process [(4)]. The SGR observed here is similar to what was reported for *H. gammarus* juveniles of similar size, reared under the same conditions, and fed a standard diet composed of fresh Antarctic krill (*Euphausia superba*) [(1)]. Nitrogen budget results showed that nitrogen retention is high for all dietary treatments, within the same range observed in a previous trial using extruded feeds and above the levels of the fresh standard dietary treatment [(1)]. Results suggest that European lobster juveniles were able to utilize efficiently the dietary protein in new tissue deposition when reared on all the experimental diets.

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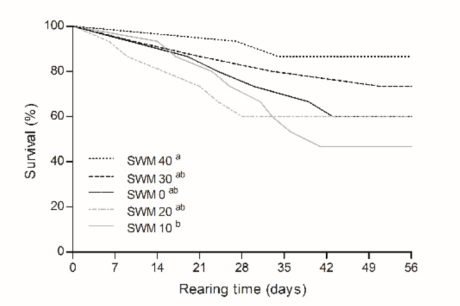

Fig. 1. Survival of *H. gammarus* (% of initial numbers) fed on experimental diets. Different letters denote statistically significant difference ($p<0.05$) determined by Log-rank test.

Table 1. Growth performance and nitrogen balance of *H. gammarus* fed the various experimental diets over an eight-week period.

	Levels of SWM as % of FM replaced				
	0	10	20	30	40
Growth performance					
SGR (%.d^{-1})	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
iCL(%.CL$_i$)	34.9 ± 4.7	33.6 ± 5.7	26.9 ± 3.7	24.1 ± 4.3	26.0 ± 2.9
Intermoult (days)	29.3 ± 1.6	26.0 ± 2.4	28.3 ± 2.0	30.7 ± 2.1	30.9 ± 1.8
FI (% BW d^{-1})	1.8 ± 0.3^{b}	1.9 ± 0.3^{b}	1.6 ± 0.5^{b}	2.6 ± 0.3^{ab}	2.9 ± 0.4^{a}
Nitrogen balance					
N_{int} (g g BW^{-1})	1464 ± 221	1323 ± 177	1241 ± 344	1880 ± 197	2046 ± 264
N_{exc} (g g BW^{-1})	140 ± 40	187 ± 45	144 ± 34	218 ± 33	226 ± 33
N_{ret} (% intake)	91 ± 2	88 ± 3	86 ± 4	89 ± 2	89 ± 2

SGR – specific growth rate; iCL – carapace length increment, FI – feed intake; N_{int} – nitrogen intake; N_{exc} – nitrogen excretion; N_{ret} – nitrogen retention. Values are means ± standard error. Different superscript letters indicate statistically significant differences between diets at $p < 0.05$ measured by one-way ANOVA followed by LSD test.

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References

1. Goncalves R, Lund I, Gesto M, et al. (2020) The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (Homarus gammarus, L.). *Aquaculture* **525**, 735334.
2. Hinchcliffe J, Powell A, Langeland M, et al. (2020) Comparative survival and growth performance of European lobster Homarus gammarus post-larva reared on novel feeds. *Aquac. Res.* **51**, 102–113.
3. Malaweera BO & Wijesundara WMNM (2014) Use of seafood processing by-products in the animal feed industry. In *Seaf. Process. By-Products Trends Appl.*, vol. 9781461495, pp. 315–339 [Kim SK, editor]. New York: Springer Science + Business Media.
4. Fox CJ (1993) The effect of dietary chitin on the growth, survival and chitinase levels in the digestive gland of juvenile Penaeus monodon (Fab.). *Aquaculture* **109**, 39–49.

CAN ATLANTIC SALMON PRODUCE LONG CHAIN POLYUNSATURATED FATTY ACIDS?

Xu Gong[1]*, Matthew Sprague[1], Monica B. Betancor[1], Rolf Erik Olsen[2], Ole Torrissen[3], Brett D. Glencross[1], Douglas R. Tocher[1]

[1] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA Scotland, UK
[2] Norwegian University of Science and Technology, 7491 Trondheim, Norway
[3] Institute of Marine Research, Matre Research Station, 5984 Matredal, Norway
E-mail: xu.gong@stir.ac.uk

Atlantic salmon (*Salmo salar*) have a limited capacity to endogenously biosynthesize n-3 long-chain polyunsaturated fatty acids (LC-PUFA),eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, from α-linolenic acid (ALA). However, linoleic acid (LA) in commercial feeds competes with ALA for LC-PUFA biosynthesis enzymes resulting in the production of n-6 LC-PUFA such as arachidonic acid (ARA).The aim of the project was to quantify the endogenous production of EPA and DHA from ALA in salmon fed from first feeding on diets that contain no EPA and DHA, and to determine the influence of dietary LA and ALA:LA ratio on LC-PUFA production. Atlantic salmon were fed for 22-weekswith three experimental diets, devoid of any EPA and DHA, formulated to provide ALA:LA ratios of approximately 3:1, 1:1 and 1:3, using sunflower and linseed oils. Endogenous production of n-3 LC-PUFA was 5.9, 4.4 and 2.8 mg.g $fish^{-1}$, whereas n-6 LC-PUFA was 0.2, 0.5 and 1.4 mg.g $fish^{-1}$ for salmon fed 3:1, 1:1 and 1:3 diets, respectively. The ratio of n-3:n-6 LC-PUFA production decreased from 27.4 to 2.0,and DHA:EPA ratio increased and EPA:ARA and DHA:ARA ratios decreased, as dietary ALA:LA ratio decreased. In conclusion, with a dietary ALA:LA ratio of 1, salmon fry/parr produced around28 μg n-3 LC-PUFA per g of fish per day, with a DHA:EPA ratio of 3.4. Production of n-3 LC-PUFA exceeded that of n-6 LC-PUFA by almost 9-fold. Reducing the dietary ALA:LA ratio reduced n-3LC-PUFA production, and EPA:ARA and DHA:ARA ratios, and increased n-6 LC-PUFA production, and DHA:EPA ratio.

ARCH-UK: MAXIMISING THE POTENTIAL OF COORDINATED NATIONAL RESEARCH

Joanna Gosling*[1], Andrew Rowley[2], Sam Martin[3], Charles Tyler[4], Herve Migaud[1]

[1.] Institute of Aquaculture, Natural Sciences, University of Stirling, FK9 4LA, UK
[2.] Centre for Sustainable Aquatic Research, Biosciences, Swansea University, SA2 8PP, UK
[3.] Scottish Fish Immunology Research Centre, Ecophysiology, University of Aberdeen, AB24 3FX, UK
[4.] Geoffrey Pope Building, University of Exeter, SA2 8PP, UK

Email: joanna.gosling1@stir.ac.uk

ARCH-UK is the only academia-led national aquaculture network in the UK and galvanises the knowledge exchange, capacity building and coordination of UK research. The network focusses on developing skills within aquaculture for Early Career Researchers, encouraging a culture of collaboration with industry. ARCH-UK also supports UK Government by providing expertise, strategic guidelines and priorities to inform research investment decisions.

Through eight working groups, ARCH-UK champions science which addresses the fundamental knowledge gaps preventing the sustainable development of UK aquaculture. The working groups invest in science communication, facilitate the uptake of research innovation by industry and create opportunities for multi-stakeholder collaboration.

ARCH-UK is funded by two UK Research and Innovation (UKRI) councils, the Natural Environment Research Council (NERC) and the Biotechnology and Biological Sciences Research Council (BBSRC) as part of their £5.1 million Aquaculture Initiative, which funded four large consortium and eight innovation research projects alongside the network in 2017.

To date, ARCH-UK has built a member-base of nearly 600 aquaculture stakeholders, working with industry leaders and academic experts to create over 25 workshops and training events on priority subjects. The impact of these workshops, specifically designed to bring together academics and industry members from different disciplines, has brought further investment and skills to the UK aquaculture sector, aiding members in gaining new perspectives and insight to solve long-term issues.

Moving forward, ARCH-UK will continue to align research objectives with priority industry issues in addition to translating research outputs for the development of national policy and strategy within the farmed aquatic food sector.

A MAJOR QTL FOR *Flavobacterium psychrophilum* RESISTANCE IN ATLANTIC SALMON (*Salmo salar*)

Grammes, F.[1*], Macchia, V.[2], Reeve, A.[3], Adams, S.[2], Moen, T[1] and Hoare, R[2]

[1]AquaGen AS, P.Box 1240 Torgard, N-7462 Trondheim, Norway
[2]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
[3]AquaGen Scotland Ltd, Holywood Salmon Farm, Dumfries, DG2 0RJ, Scotland, UK
E-mail:fabian.grammes@aquagen.no

Introduction

F. psychrophilum has been known for decades to affect fry and larger fish in freshwater hatcheries as well as in on-growing sites in Rainbow trout (*Oncorhynchus mykiss*) aquaculture worldwide. The disease, known as Rainbow trout fry syndrome (RFTS), can lead to high mortalities at affected sites, causing considerable economic losses and necessitating the use of antibiotics. However, recently *F. psychrophilum* has also been isolated from Atlantic salmon (*Salmo salar*) fry following disease outbreaks in freshwater sites in the UK and Norway, causing concern for the aquaculture industry.

Material and Methods

Using a newly developed *F. psychrophilum* immersion challenge model we conducted two challenge trials for two different year-classes (2018/19) of Atlantic salmon from the AquaGen nucleus . The challenges were conducted using different bacterial isolates of *F. psychrophilum* for the respective year-classes.

In the first trial 3064 Atlantic salmon with an average weight of 1.2 g were distributed into two tanks before starting the challenge. The trial lasted for 25 days and cumulative mortality reached 28%. In the second trial 1161 Atlantic salmon (1.4 g) were challenged in a single tank. Cumulative mortality was somewhat lower at 18%. All fish were genotyped on a high-density SNP chip containing 70,000 DNA-markers.

Results

Our genome wide association study (GWAS), testing for marker association with the trait (survival) led to the discovery of a major QTL for *F. psychrophilum* resistance in Atlantic salmon in the first challenge trial. The QTL was then confirmed in the second challenge trial. Since the same QTL was identified in two different year-classes of Atlantic salmon, each challenged with a different isolate we are confident that the QTL can be used to select Atlantic salmon (more) resistant to infection by *F. psychrophilum*.

GENOME WIDE ASSOCIATION STUDIES REVEALED ONE STRONG EFFECT QTL FOR VIRAL NERVOUS NECROSIS RESISTANCE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

R. Griot[a,b,c,*], F. Allal[c], F. Phocas[b], S. Brard-Fudulea[d], R. Morvezen[a], A. Bestin[a], P. Haffray[a], Y. François[a], T. Morin[e], C. Poncet[f], A. Vergnet[c], S. Cariou[g], J. Brunier[h], J-S. Bruant[g], B. Peyrou[h] and M. Vandeputte[b,c]

[a] SYSAAF, Station LPGP/INRAE, Campus de Beaulieu, 35042 Rennes, France
[b] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[c] MARBEC, Univ. Montpellier, Ifremer, CNRS, IRD, 34250 Palavas-les-Flots, France
[d] SYSAAF, UMR BOA, Centre INRA Val de Loire, 37380 Nouzilly, France
[e] INRAE-UCA, UMR GDEC, Clermont-Ferrand, France
[f] ANSES, Ploufragan-Plouzané-Niort Laboratory, Viral Fish Diseases Unit, National Reference Laboratory for Regulated Fish Diseases, Technopôle Brest- Iroise, 29280 Plouzané, France
[g] Ferme Marine Du Douhet, 17840 La Brée Les Bains, France
[h] Ecloserie Marine de Gravelines-Ichtus, 59273 Gravelines, France

MARBEC, Univ. Montpellier, Ifremer, CNRS, IRD, 34250 Palavas-les-Flots, France
E-mail : ronan.griot@ifremer.fr

Introduction
Viral Nervous Necrosis is considered as one of the most impacting disease for the European sea bass (*Dicentrarchus labrax*) industry, leading to mortality up to 90% (Le Breton et al., 1997). With the lack of efficiency of vaccines, selective breeding is a promising strategy to reduce the frequency and severity of the outbreaks. With the development of genomic tools, such as the ThermoFisher 57K DlabCHIP SNP array, the identification of genomic regions involved in the variation of the trait, named Quantitative Trait Locus (QTL), is now easier. This work presents genome-wide association studies (GWAS) preformed in three different populations in order to study the genetic architecture of VNN resistance in European seabass.

Material and methods
Four full-sib families were produced by mating four sires from three different geographic origins (western, north-eastern and south-eastern Mediterranean Sea) to four susceptible females from the western Mediterranean Sea. As the sires used came from a cross of resistant sires with susceptible females, the four backcross families are expected to segregate for disease resistance loci. In addition, two commercial cohorts (pop A and pop B) were produced from factorial design were added to the study. All individuals were challenged to nervous necrosis virus and genotyped on the ThermoFisher 57K DlabCHIP SNP array. After quality controls, 378, 454, 291 and 211 individuals for each backcross family were genotyped for 30,592, 23,592, 30,656 and 31,490 markers, respectively. In commercial cohorts, 1,089 and 476 individuals were genotyped on 40,623 and 41,166 markers, respectively. In backcross families, composite interval mapping was performed to detect QTLs in each family. In commercial cohorts, GWAS were performed using a GBLUP approach and a BayesCπ approach. For each QTL detected in the commercial cohorts, confidence interval for the location as well as part of the genetic variance explained were estimated.

Results and discussion
In composite interval mapping analysis, one QTL was detected on LG12 in three over four backcross families. In one of them, an additional QTL was located on LG8. In commercial cohorts, one QTL on LG12 was detected and shared by both cohorts. In pop A, four additional QTLs located on LG4, LG8, LG14 and LG19 were detected using a BayesCπ approach (Figure 1a). Similarly, two additional QTLs were revealed in pop B on LG15 and 20 (Figure 1b).

The QTL located on the LG12 explained 9.21% of the total genetic variance and the other ones explained 1% or less; The LG12 QTL and was located between 33.26 cM and 33.91 cM. This interval, equivalent to 3.7 Mb, remains too wide to find a candidate gene.

Our results show that VNN resistance is an oligogenic trait, that involves one strong effect QTL shared by different populations. This finding is innovative as VNN resistance was previously described as a polygenic trait (Palaiokostas et al., 2018).

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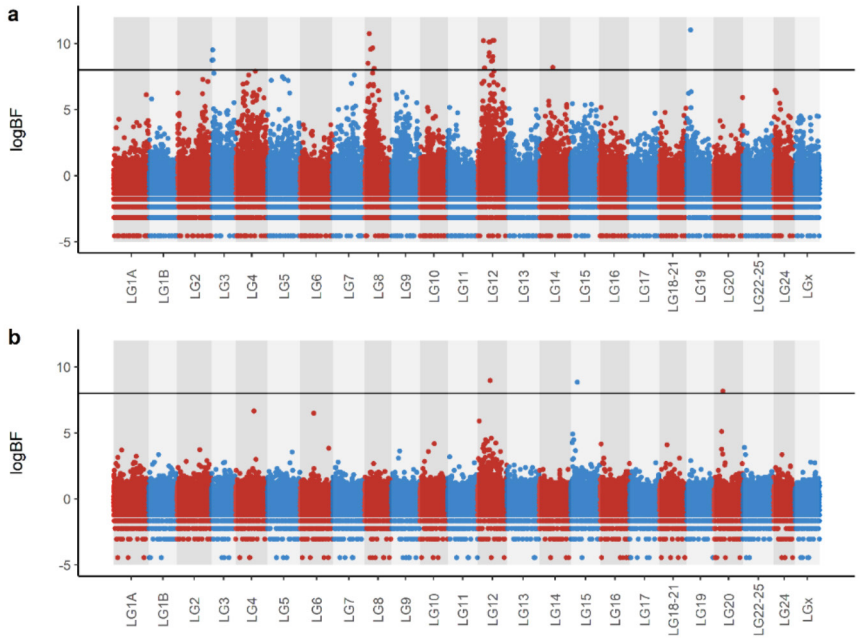

Figure 1 : Genome-wide logBF plot for VNN resistance across the genome in the European sea bass populations pop A (a) and pop B (b) using a BayesCπ model. Horizontal black lines represent the logBF threshold of 8, corresponding to strong evidence for the presence of a QTL.

Acknowledgements

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References

Le Breton, A., Grisez, L., Sweetman, J., Ollevier, F., 1997. Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass, Dicentrarchus labrax (L.). J. Fish Diseases 20, 145–151. https://doi.org/10.1046/j.1365-2761.1997.00284.x

Palaiokostas, C., Cariou, S., Bestin, A., Bruant, J.-S., Haffray, P., Morin, T., Cabon, J., Allal, F., Vandeputte, M., Houston, R.D., 2018. Genome-wide association and genomic prediction of resistance to viral nervous necrosis in European sea bass (Dicentrarchus labrax) using RAD sequencing. Genet. Sel. Evol. 50, 30. https://doi.org/10.1186/s12711-018-0401-2

PIKEPERCH – COMPARISON OF THE MEAT QUALITY FROM WILDLIFE AND AQUACULTURE FISH

K. Tönissen[1], R. Bochert[2], R. Pfuhl[1], B. Grunow[1*]

[1]Institute for Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[2]Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries (LFA MV) Institute of Fisheries; Research Station Aquaculture, Südufer 8, 18375 Born, Germany
E-Mail: grunow@fbn-dummerstorf.de

Introduction

Pikeperch (*Sander lucioperca*), a freshwater fish, gained popularity in the recent years due to its tender and low fat meat. Because of this attractiveness, pikeperch aquaculture is expanding (Policar et al., 2019). But does the filet of pikeperch from captivity have the same quality as the ones from wildlife animals? The aim of the aquaculture production is the supply of a permanently high quality fish to the consumer. The consumer's evaluation are depending on the obvious characteristics, such as the colour of the meat and its physical composition (e.g. tenderness, water content) measured by objective methods. For this reason, we evaluated animals from an aquaculture and a lake (pre-spawn and post-spawn) regarding the physical meat quality, in order to adapt the husbandry conditions in aquaculture, if necessary in this study.

Materials and methods

Adult *Sander lucioperca* were obtained from the Hohen Sprenz-lake in Mecklenburg-Vorpommern, Germany before (mid March) and after (mid May) spawning season as well as from the Institute of Fisheries in Born, Germany. Pikeperches from aquaculture were reared in a freshwater RAS and fed with commercial diet (Coppens Supreme-10 4.5 mm pellet size) at a feeding rate of 1.0 % per fish weight by automated feeders (Komolka et al., 2020).

The meat quality analysis of all fishes regarding pH, electrical conductivity and impulse-impedance was performed five minutes *post mortem* and a second time one hour later. Furthermore, shear force analysis was measured via Warner-Bratzler-Method (Sigurgisladottir et al., 1999, Komolka et al., 2020), water holding capacity via Hypress method (Große et al., 1975) and the colour via a CR-300 Chroma Meter.

Results

The studies showed considerable differences in some aspects. In particular, spawning time has a significant effect on the quality of the meat, in terms of water holding capacity, which was significantly higher in the filets sampled in May. Furthermore, we could validate that the filets of the wildlife animals are very different to the aquaculture animals. Thus, the specimen from aquaculture have a filet shear force that corresponds to about one sixth of that of the animals from the lake. Contrary, the water holding capacity is much higher. The colour examination also revealed differences between the three experimental groups. After spawning season, the yellowness of the filets was substantially lower than in the animals sampled two month earlier and also of the filets gained from the aquaculture animals. In addition, the filets of the aquaculture animals had a higher redness value compared to both groups from the lake.

Discussion

The study showed that spawning time has an influence on meat quality, but seems to be negligible compared to the differences of the wildlife and aquaculture groups in general. The meat quality therefore appears to be primarily dependent on the origin of the animals.

It is known that especially the feed has a strong impact on meat quality. Thus, on one hand it is not surprising that the aquaculture animals revealed a filet with a very high red value, as the consumer prefers this colour. On the other hand, the analysis on shear force and water holding capacity proved that further optimisations in husbandry are inevitable for reaching comparable qualities of the wildlife animals. Higher shear force and lower water holding capacity values are needed for a good cooking result. Both factors can be influenced by the feed, but also by swimming behaviour. Therefore, next to a food change, the influence of flow conditions could be investigated on the meat quality.

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Sources

Große, F., Bretschneider, U. & Saß, G. (1975) Eine Methode zur direkten Bestimmung des Preßsaftes von Fleisch, *Fleisch*, 29 p 104

Komolka, K.; Bochert, R.; Franz, G. P.; Kaya, Y.; Pfuhl, R.; Grunow, B. (2020). Comparison of physical meat quality parameters of Percidae and Salmonidae in aquaculture. *Foods* 9(4): 388, 1-13

Policar, T., Schaefer, F. J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., & Zarski, D. (2019). Recent progress in European percid fish culture production technology-tackling bottlenecks. *Aquaculture International,* 27 (5), 1151-1174.

Sigurgisladottir, S., Hafsteinsson, H., Jonsson, A., Lie, O., Nortvedt, R., Thomassen, M., & Torrissen, O. (1999). Textural Properties of Raw Salmon Fillets as Related to Sampling Method. *Journal of Food Science*, 64(1), 99–104.

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 – 4, rue Toussaint Flechaire – 84510 Caumont-sur-Durance, 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 is done automatically 6 times a day. The groups of shrimps received the respective diets at the predetermined percentages of their initial mean body weight and expected daily growth. This was adjusted daily according to the expected growth, and observed mortality and feed consumption per group.

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.

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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.

GENOTYPE BY ENVIRONMENT INTERACTION IN TWO COMMERCIAL PRODUCTION SITES OF GILTHEAD SEABREAM *Sparus aurata* IN THE MEDITERRANEAN

Benan Gulzari[1]*, Hans Komen[1], Varun Raj Nammula[1,2], John W.M. Bastiaansen[1]

[1]Department of Animal Breeding and Genomics, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen (The Netherlands)
[2]Department of Integrated Plant and Animal Breeding, University of Göttingen, Wilhelmsplatz 1, 37073 Göttingen (Germany)
E-mail: Benan.Gulzari@wur.nl

Introduction
Significant GxE between commercial fish production sites may reduce the effectiveness of genetic improvement programs. If the GxE between commercial fish production sites is quantified, breeding programs may adjust their decisions to reach a balanced performance in many locations rather than a high performance in only a single location. The issue of GxE attracts more attention as the breeding companies started to distribute genetically improved stock internationally.

Gilthead seabream is a key species in Mediterranean fish farming, and it is farmed over large distances and in different conditions. Genetically improved fingerlings of seabream are distributed internationally, which poses a potential for GxE. However, studies that quantify GxE for production traits of gilthead seabream between commercial fish farms are scarce.

The objective of the present study was to quantify GxE for production traits of gilthead seabream in two different production sites with distinct temperature profiles.

Materials and Methods
Data were obtained from two commercial gilthead seabream farms within the EU-project MedAID. The commercial farms were selected to represent the east (Galaxidi, Greece) and west Mediterranean (El Campello, Spain) conditions. Fish for this experiment originated from the same breeding program and are related. Data were collected from the commercially produced fish that were harvested after a grow-out period of 465 days in Greece (n = 999) and 500 days in Spain (n = 945).

After the fish was harvested, body weight (BW) was measured. Fillet fat measurements (FF) were taken using Distell fat meter, from eight points (four on each side) of the whole fish and the average of these eight measurements were used in the analyses. Then, the fish were slaughtered, viscera weight (VW) and fillet weight (FW) were recorded. Measurements were standardized between the two sites, except fillet was skin-off, trimmed in Greece and skin-on, not-trimmed in Spain. Fillet percentage (Fil%) was calculated as (FW / BW) * 100. The relatedness of the harvested fish in both locations was calculated (VanRaden, 2008)967 bulls and 50,000 markers distributed randomly across 30 chromosomes. Estimation of genomic inbreeding coefficients required accurate estimates of allele frequencies in the base population. Linear model predictions of breeding values were computed by 3 equivalent methods: 1 using the 30K MedFish SNP chip.

To quantify GxE, measurements in the two locations were treated as different traits and genetic correlations between them were estimated using genomic relationships. Deviation of the genetic correlation from unity was regarded as GxE.

Results
Descriptive statistics of performance in the two grow-out farms are in Table I. Coefficient of variation (as percentage) is presented as the measure of variation. On average, the harvested fish in Spain had higher average BW, FW and VW. However, the variation of these traits was similar in the two locations. Average Fil% was substantially higher in Spain; however, the CV was similar. Average FF was similar in the two locations and the CV in Spain was higher.

Measured differences in environmental conditions between the two locations are in Table II.

The GxE estimates for the traits are in Table III. Genetic correlations between Greece and Spain ranged from 0.41 to 0.86. BW, FW, Fil% and VW are moderately correlated, and FF is strongly correlated between the two environments.

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Table I. Descriptive statistics of grow-out performance of gilthead seabream

TRAIT	GREECE		SPAIN	
	Mean	(CV)	Mean	(CV)
BW	370.8	(17.6)	412.0	(17.1)
FW	117.6	(18.0)	179.7	(18.8)
Fil%	31.5	(7.7)	43.6	(7.1)
VW	27.1	(26.2)	29.2	(22.8)
FF	12.7	(18.0)	12.8	(21.7)

Table II. Measured average daily values in environmental conditions

VARIABLE	GREECE	SPAIN
Water temperature (°C)	20.5	21.4
Salinity (‰)	39.0	37.3

Table III. GxE estimates for the traits studied (as genetic correlation between the two sites)

TRAIT	GENETIC CORRELATION (SE)
BW	0.41 (0.12)
FW	0.47 (0.13)
Fil%	0.54 (0.26)
VW	0.64 (0.10)
FF	0.86 (0.06)

Discussion and Conclusion

This study quantifies GxE for production traits of gilthead seabream between east and west Mediterranean. Grow out conditions in terms of feed, cage size and density were standardized between the sites. BW and FW had the strongest and FF had the weakest GxE between the production sites. Moderate GxE for BW and FW indicates that the grow-out performance of genetically improved fish is expected to be different for these traits in east and west Mediterranean. Weak GxE for FF, however, indicates that the grow-out performance of genetically improved fish is not expected to be different for this trait. Navarro et al., 2009 estimated a weaker GxE for BW (0.70) and GxE was practically absent for FW (0.94) in seabream; however, production sites tested in their study were geographically close. Based on the current results, breeding programs will need to use data from east and west Mediterranean to produce fish that will perform well in both sites in terms of BW and FW.

Acknowledgment

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References

VanRaden, P.M., 2008. Efficient Methods to Compute Genomic Predictions. J. Dairy Sci. 91, 4414–4423.

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, 225–230.

WATER QUALITY DYNAMICS IN EARTHEN PONDS WITH AND WITHOUT FISH

S. Gyamfi*, R. E. Edziyie, K. A. Obirikorang, D. Adjei- Boateng and P.V. Skov

Kwame Nkrumah University of Science and Technology
Email:stephengyamfi40@gmail.com

Introduction

Tilapia is currently cultured in over 100 countries and increasingly becoming one of the most cherished protein sources globally. The global development of tilapia culture has passed through several phases of improvement over the last two decades. That notwithstanding, water quality management in the main culture system (ponds) for this species is yet to be fully optimised. This study was conducted to assess the water quality dynamics in tropical earthen ponds with and without fish.

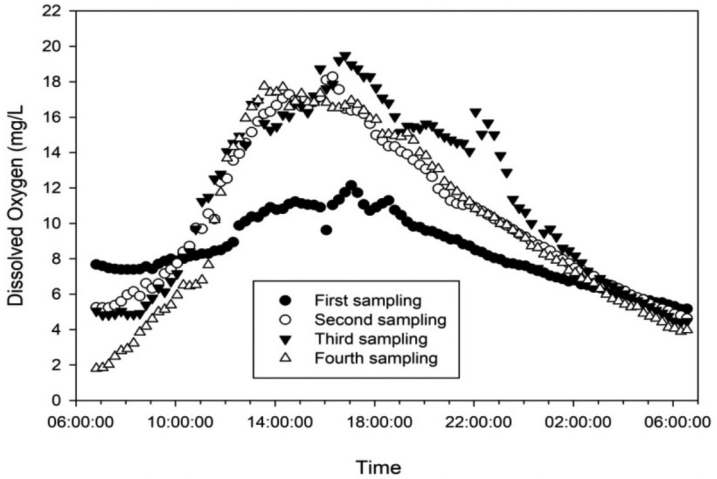
Materials and Methods

The study was conducted for three (3) months in four ponds (10×15×1m each) at an experimental fish farm in Kumasi, Ghana. Two treatments i.e. fed pond which comprised two ponds stocked with all male tilapia (38g) and fed twice daily with a commercial feed (CP-30%) and a control- two other ponds without fish/feed. Growth and feed utilization of the fish were assessed at the end of the study. Physicochemical and biological water quality parameters were monitored every four days and every three weeks, respectively. Twice a week, the dissolved oxygen (DO), pH, temperature and conductivity levels were measured *in-situ* with a multiparameter probe (Hach, Hd40Q) between 7 and 8 am while water samples were taken to the laboratory for turbidity, total suspended solids (TSS), total dissolved solids (TDS), alkalinity, NH_3, NH_4,ortho-phosphate, NO_2, NO_3 biological and chemical oxygen demand (BOD & COD), organic matter (OM) and organic carbon (OC) analysis. Furthermore, every three (3) weeks, a 24h O_2 monitoring was done in the experimental ponds and sludge accumulation and sludge characteristics were also determined. Data on water quality, growth and feed utilization were expressed as means and standard deviation using Microsoft Excel and graphs generated with GraphPad Prism 5 software. Data on nutrients were subjected to the Mann Whitney test while all physico-chemical and biological parameters were subjected to Students T-test (α =0.05).

Results

Physico-chemical parameters were relatively better in the control treatment ponds than the fed treatment ponds with highly significant differences observed in DO, temperature, TDS, TSS, turbidity, alkalinity and nutrients (NH_3, NH_4, NO_2, NO_3). Organic and biological parameters were significantly higher in the fed treatment ponds with BOD, COD, OM, OC and chlorophyll-*a* recording 22%,20.1%,14%,10.2%,35% respectively than the control treatment pond. Sludge measured was relatively higher in the fed ponds with better sludge characteristics in the control treatment. Water quality parameters recorded in the control ponds were within the recommended ranges for tilapia growth. Diel O_2 variation in both treatments decreased along sampling times.

Fig



1. Diel oxygen cycles in the control pond at depths of 20 cm beneath the pond water surface. Sampling was done every three weeks with 15 min interval oxygen readings during the study

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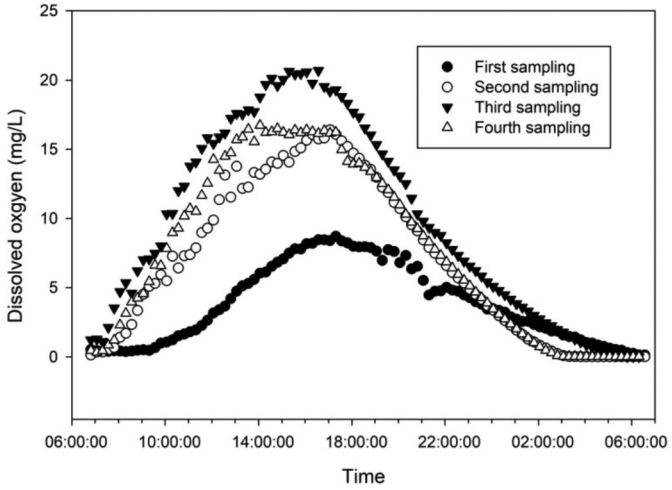
Fig



2. Diel oxygen cycles in the fed pond at depths of 20 cm beneath the pond water surface. Sampling was done every three weeks with 15 min interval oxygen readings during the study

A METHODOLOGY FOR CLASSIFYING PHYSICAL CONDITIONS AT OFFSHORE AQUACULTURE SITES IN WESTERN SCOTALND USING OUTPUT FROM A SPECTRAL WAVE MODEL

J. Hardwick[1]*, H. C. M. Smith[1], M. Holloway[2], D. Aleynik[2], K. Davidson[2]

[1]University of Exeter, Penryn Campus, TR10 9FE, UK
[2]Scottish Association for Marine Science, Oban, PA37 1QA, Scotland
*Email: j.p.hardwick@exeter.ac.uk

Introduction

The aquaculture industry in Scotland is worth £1.8 billion (2016) to the economy and has been identified as a key economic area for growth [1]. Expanding fin fish aquaculture into new locations will require investigation of more energetic environments. The Off Aqua research project is a multidisciplinary consortium investigating the potential opportunities and challenges encountered by moving fin fish aquaculture into more exposed offshore areas in the West of Scotland. This paper discusses the physical conditions at active and potential future aquaculture locations with a particular focus on the wave climate, from which a site classification methodology is being developed.

For a complete understanding of the conditions offshore sites will encounter, it is necessary to for a characterisation of the wave climate to be undertaken. A bespoke wave model is run to provide spectral wave output across the entire region with hindcast output computed for 30 years. When combined with current and wind data a picture of the physical conditions offshore is compiled.

This dataset and methodology will enable aquaculture operators to quantify the physical conditions at potential locations which when combined with other outputs from the project will provide an assessment that can assist in de-risking potential installations.

Wave Modelling

A SWAN spectral wave model was constructed covering the waters around the West coast of Scotland. The model calculates the wave climate across a detailed unstructured mesh using wind and flow inputs from [2]. A 30-year hindcast run of the model was undertaken providing hourly wave parameters at 439 locations. These locations comprise 419 existing aquaculture installations [1] along with 20 other locations of interest. The model has also provided mapped data and full spectral output at three case study sites.

In-situ data has been collected at sites around the region for both aquaculture and non-aquaculture reasons which has been used to calibrate and validate the model. In addition to informing the classification methodology the model data is used for reliability analysis and provides input to the other work across the project.

Site Classification

To provide the aquaculture community information about site conditions, a methodology is being developed to classify the physical conditions at sites across the domain. Aquaculture site classification has been attempted in earlier studies, for example in [3], sites in Norway are classified by estimating wave height using fetch calculated distance. Development of a bespoke wave model and an extreme value analysis of the results build on these earlier studies for a comprehensive assessment of the physical conditions.

This first stage of the site classification is a univariate analysis of a single parameter; the significant wave height output from the model. An extreme value model, comprised of Generalised Pareto Distribution is fit to the output at each of the 439 locations and extrapolated to find the 50 year expected waves, the sites can then be grouped by the results. An example of the extreme value model and a histogram showing the 50 year wave at the 419 active aquaculture sites are shown in figure 2.

A further analysis is being undertaken to develop a multi-parameter approach combining output from the wave model with predicted wind and current extremes, from these data multivariate environmental contours can be constructed [4] and used to predict the maximum expected combined conditions at a potential sites. As well as classifying the sites this output can feed into other models used to assess the viability, overall risk, and cost effectiveness of a project. This work combined with the other outputs from the Off Aqua consortium aims to improve the chance of successful deployments being undertaken in the more energetic environments further offshore.

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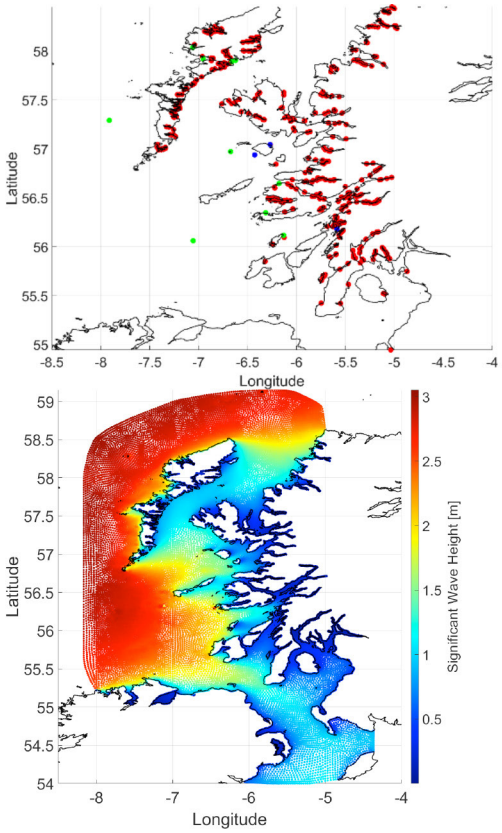

Figure 1 – *Left,* output locations from 30-year hindcast model (including active sites [red], case study sites [blue] and other sites of interest [green]). *Right*, average wave height across entire grid.

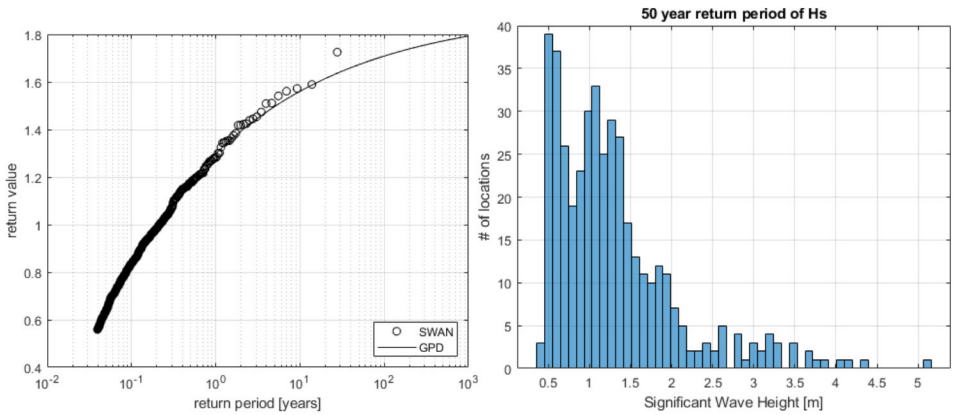

Figure 2 – *Left,* GPD extreme value model fit to Hs data from BSNC fish farm site. *Right,* histogram showing 50 year Hs at 419 active aquaculture sites.

References

[1] Aquaculture Scotland, [website] (2020). *Scotland's Aquaculture*. Retrieved from: aquaculture.scotland.gov.uk

[2] Aleynik, D., Dale, A. C., Porter, M., & Davidson, K. (2016). *A high resolution hydrodynamic model system suitable for novel harmful algal bloom modelling in areas of complex coastline and topography*. Harmful algae, 53, 102-117.

[2] Lader, P., Kristiansen, D., Alver, M., Bjelland, H. V., & Myrhaug, D. (2017). *Classification of aquaculture locations in Norway with respect to wind wave exposure.* 36th International Conference on Ocean, Offshore and Arctic Engineering.

[3] Mackay, E. & Jonathan, P. (2020). *Estimation of Environmental Contours Using a Block Resampling Method*. 10.1115/ OMAE2020-18308.

REARING WATER MICROBIOMES IN *Litopenaeus vannamei* LARVICULTURE ASSEMBLE STOCHASTICALLY AND ARE INFLUENCED BY THE MICROBIOMES OF LIVE FEED PRODUCTS

Jasmine Heyse[1*], Ruben Props[1], Pantipa Kongnuan[2], Peter De Schryver[2], Geert Rombaut[2], Tom Defoirdt[1], Nico Boon[1]

[1] Center for Microbial Ecology and Technology (CMET), Department of Biochemical and Microbial Technology, Ghent University, Coupure Links 653, 9000 Gent, Belgium
[2] INVE Technologies NV, Hoogveld 93, 9200 Dendermonde, Belgium
[*]Nico.Boon@UGent.be

Outbreaks of microbial diseases have posed one of the main impediments to the sustainable growth of the aquaculture industry. The development of effective management strategies to reduce the occurrence of these diseases is hampered by the limited knowledge on the microbial ecology of these systems. To advance our understanding of these communities, we studied the sources, dominant community assembly processes and microbiome dynamics in the rearing water of five replicate *Litopenaeus vannamei* larviculture tanks.

We found that the bacterial community undergoes two shifts that match with the dynamics of the algal abundances in the rearing water. Additionally, we found that the community assembly over time was dominated by stochasticity, which explains the observed heterogeneity between replicate cultivations. This stochasticity implies that the dynamics of these larviculture system are largely unpredictable and hence they necessitate continuous monitoring. Finally, we quantified the contribution of peripheral microbiomes, such as those of live and dry feeds, to the rearing water microbiome. We found that 37% of all bacteria in the rearing water were introduced through these peripheral microbiomes. The contribution of the algae was the largest, followed by the *Artemia*, the exchange water and the dry feeds. Our results illustrate that these peripheral microbiomes have an important contribution to the rearing water microbiome. Given this contribution, careful preparation and storage of these inputs will be paramount to maintain stable, healthy systems.

FOOD AND LOCAL, AGRICULTURAL, AND NUTRITIONAL DIVERSITY

K. Hoevenaars*, T. Bardócz and M. Setti

*AquaBioTech Ltd, Central complex, Naggar street,Targa Gap, Mosta, Malta
Email: kyh@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; to detect behaviour and preferences of consumers and producers, in order to customize innovations to local sensitiveness; to develop and implement organizational innovations, aimed at boosting coordination among food operators; to develop, test, and validate (open) technological innovations in laboratory and in the field; and 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 of 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 the cold chain in the 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 to reduce production costs and the use of imported fish feed. The specific RAS developed in the project will provide a technology with low operational costs to ensure the supply of high-quality fingerling with affordable prices for 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, and at the same time, 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.

HOW SLIPPER LIMPETS *Crepidula fornicata* ALTER THE HYDRODYNAMIC ENVIRONMENT AT THE SEABED, AND THE IMPLICATIONS FOR OYSTER *Ostrea edulis* RESTORATION

Zoë Holbrook*, Chris Hauton

University of Southampton, National Oceanography Centre, European Way, Southampton, SO14 3ZH, UK.
Email: zh5g12@soton.ac.uk

Appropriate site selection is essential for restoration of a sessile species such as European flat oyster *Ostrea edulis*, as abiotic factors including water temperature, salinity and hydrodynamics can influence the physiological performance of a population. Hydrodynamics can influence morphology and growth of individuals, can physically displace individual oysters and cultch, alter oxygen concentrations, nutrient and food availability, change larval swimming patterns, and impact the level of sedimentation, which could influence the success of restoration projects. Turbulence and the associated shear stress at the benthic boundary layer (BBL) play a role in bringing post pelagic-stage larvae to the seabed for settling and metamorphosis, but the irregularity of this turbulence and shear stress can hinder the attachment of these larvae to an appropriate substrate. The loss of native oyster beds around the UK has resulted in a shift in the benthic community, with invasive slipper limpet, *Crepidula fornicata*, dominating the epibenthos of southern, south western and south eastern UK coastal regions. The aim of this study was to understand if this change in biological community had altered the hydrodynamics such that a potentially permanent change in ecological system had occurred (*i.e.* the new 'state' (*C. fornicata*) preventing a return to the old state (*O. edulis*)). Herein, water velocity along the channel of an annular flume was measured at multiple elevations above (a) an *O. edulis* bed and (b) a *C. fornicata* bed for comparison. At the higher experimental water velocities (0.31, 0.42 and 0.52 m s^{-1}), *O. edulis* reduced water velocity immediately above the bed to almost half of the depth averaged speed, while the same velocities were not altered by the *C. fornicata* bed. The shear stress profiles showed a marked contrast between the two species; the oyster bed created higher shear stress (three times in magnitude) by comparison to that associated with the limpet bed. These data present implications for oyster feeding through re-suspension of particles available for consumption, and for larval settlement and consequently population recruitment. A lower water velocity immediately above an oyster bed might allow larvae more control over settlement on adult oyster shell without the hindrance of stronger currents. Restoration efforts must prioritise the development/rebuilding of substrate material appropriate for *O. edulis* settlement while taking into account the natural hydrodynamic environment and water velocity at the benthic boundary layer.

TESTING EXISTING MOLECULAR MARKERS ASSOCIATED WITH *Bonamia*-INFECTION

Zoë Holbrook*, Chris Hauton

University of Southampton, National Oceanography Centre, European Way, Southampton, SO14 3ZH, UK.
Email: zh5g12@soton.ac.uk

One key challenge for *Ostrea* spp. restoration lies in the potential impact of parasites such as the paramyxean *Marteilia refringens* (marteiliosis) and the haplosporidian *Bonamia* spp. (*B. ostreae*, *B. exitiosa*, *B. perspora*, and *B. roughleyi*) (bonamiosis) that have caused mass mortality of oysters worldwide. *Ostrea edulis* previously exposed to *B. ostreae* have demonstrated a tolerance to the infection, yet no *O. edulis* population has yet demonstrated full resistance. Programmes to develop *Bonamia*-'resistant' strains of *O. edulis* began in the mid-late 1980s in France and Ireland. 'Selected' oysters demonstrated inhibited phagocytic activity that served to reduce the spread of parasites to wider tissues, whilst the expression of apoptosis-related genes was upregulated. Indeed, molecular responses are likely to be shaped by previous exposure to parasites. Many contemporary studies have started to explore the expression of proteins, genes, and micro-RNAs associated with phagocytosis, respiratory burst, and apoptosis and have compared *Bonamia*-naïve and *Bonamia*-exposed oysters to identify underlying mechanisms that might support a differential phenotype (e.g. Morga *et al*., 2012). Morga *et al*. (2012) identified seven expressed sequence tags (ESTs) as potential markers of *Bonamia*-resistance (extracellular superoxide dismutase (*OeEcSOD*), inhibitor of apoptosis (*OeIAP*), fas-ligand (*OeFAS*), cathepsin B (*Cathep*), ferritin (*Oefer*), C1q (*OeC1q*), and Cyclophylin B (*Oepepti*)). Herein we test the potential of six genetic markers of resistance in different populations of oysters studied over a seasonal cycle using MIQE-compliant qPCR. We identified a significant difference in the expression of C1q (*OeC1q*), fas-ligand (*OeFAS*) and -actin (*ACT*) between *Bonamia*-infected and non-infected oysters. However, seasonal effects on this variation in expression of *OeC1q* and *OeFAS* confounded the response to infection and suggests the use of genetic markers is more nuanced than previously considered. We advocate longer term studies using multiple populations to validate markers of *Bonamia*-resistance for use with oyster restoration projects, and we recommend the parallel evaluation of other markers of disease and health in commercial populations of oysters and other bivalves.

Morga B. Renault T. Faury N. Arzul I. 2012. 'New insights in flat oyster *Ostrea edulis* resistance against the parasite *Bonamia ostreae*'. *Fish and Shellfish Immunology*. 32. 958-968.

TACKLING PROLIFERATIVE KIDNEY DISEASE (PKD): UTILITY OF FUNCTIONAL GENOMICS TO INFORM ON PARASITE VIRULENCE AND THERAPEUTIC APPROACHES

Jason W. Holland*, Marc N. Faber, Carolina Tafalla, Christopher J. Secombes

Scottish Fish Immunology Research Centre, University of Aberdeen, Scotland. UK
Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain
Email: j.holland@abdn.ac.uk

Introduction

PKD is one of the most serious parasitic diseases affecting farmed and wild salmonid populations in the UK, Europe, and North America. Caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae*, PKD is a temperature driven disease with both spore sac development in colonial bryozoans and onset of clinical disease in fish linked to increasing water temperatures. Recovering fish are known to have protective immunity to reinfection providing the impetus for vaccine studies. Developing therapeutics and diagnostic tools to manage parasite diseases is one of the biggest challenges facing fish aquaculture. The application of functional genomics offers a powerful means to uncover mechanisms of host exploitation, including parasite virulence. The additional use of functional approaches can help to pinpoint disease control strategies, including antigen selection for vaccine studies.

Methods and Results

In our PKD work, we have implemented newly developed parasite transcriptome assemblies in the selection of putative virulence factors and extracellular proteins (ECPs). Importantly, given that fish specific ECPs are likely to be important therapeutic targets, we have determined the host specificity of selected ECPs to aid further refinement of therapeutic targets. As in other parasite models, several secretory ECPs are currently of unknown function. We have used novel functional tools to uncover virulence and lipid scavenging mechanisms that may account for the unusual nature of PKD pathology. These tools have also been used to develop a non-invasive ELISA-based diagnostic that can be used as a valuable disease management tool. Our vaccine studies have yielded two partially protective unknown antigens, reinforcing the need to continue searching for fish specific ECPs that could be used collectively as a subunit vaccine for PKD.

Conclusions

Overall, our work has made major in-roads towards the development of PKD therapeutic and diagnostic approaches, which has wider relevance to the control of other fish parasites.

MODELLING OF AQUACULTURE IMPACTS AND THE MOVE TO MORE EXPOSED LOCATIONS: WHAT SCALES DO WE NEED TO RESOLVE?

Thomas Adams*, Dmitry Aleynik, Andrew Dale, Max Holloway, Keith Davidson

Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, PA37 1QA
Email: tom.adams@sams.ac.uk

Introduction
Industry targets for global aquaculture demand an increase in output over the next decade. Salmon farm operators seek to meet this need either by establishment of new sites, or by the expansion of existing sites. However, finding space for new sites can be challenging. The OFF-AQUA project (https://www.sams.ac.uk/science/projects/off-aqua/) is investigating a range of issues associated with moving salmon production sites to more exposed locations in Scottish waters. In order to tackle this challenge, nested physical models covering a range of spatial scales were developed to investigate processes operating at inshore and more exposed locations. Results of the physical models were compared against in-situ observations of vertical structure at sites. Outputs from the models were used to drive particle tracking simulations focussed on assessing the likely impacts of parasitic sea lice and harmful algal blooms in differing environments.

Methods
A hydrodynamic modelling system covering the west coast of Scotland was developed (Aleynik et al. 2016, 2018)"give n":"K."},{"family":"Burrows","given":"M. T."}],"issued":{"date-parts":[["2018"]]}},{"id":1007,"uris":["http://zotero.org/users/294646/items/X3CPSZ99"],"uri":["http://zotero.org/users/294646/items/X3CPSZ99"],"itemData":{"id":1007,"type":"article-journal","title":"A high resolution hydrodynamic model system suitable for novel harmful algal bloom modelling in areas of complex coastline and topography","container-title":"Harmful Algae","collection-title":"Applied Simulations and Integrated Modelling for the Understanding of Toxic and Harmful Algal Blooms (ASIMUTH, based on directly coupled unstructured mesh ocean (FVCOM; Chen et al. 2013) and atmospheric (WRF; Skamarock et al. 2008) models. This has been run operationally since 2013 (minimum horizontal element size c 130 m, 10 vertical layers). In 2019, the domain was expanded to incorporate more exposed environments. Fine-scale models of a study site in a relatively isolated and exposed location were developed, with a range of horizontal and vertical (10-60 layers) spatial resolutions (Figure 1). In-situ observations were collected using a mooring system which was deployed close to the study site in winter and summer conditions.

A biological particle tracking model (Adams et al. 2016) was used to simulate the spread of "sea lice" larvae and harmful algal bloom species at three focal fish farm sites, covering sheltered fjordic through to exposed open environments. Simulations covered a full year, giving a representative range of tide and weather conditions, which dominate local flow patterns (Edwards 2016), and simulations incorporated a range of biophysical interactions.

Results
Higher resolution physical models appear to provide an improved description of in-situ observations. However, they also suggest the existence of features which are difficult to identify empirically. Analysis of dispersal maps and between-site connectivity allowed characterisation of the proposed sites in the context of their surroundings (Figure 2); sites with intermediate exposure generally demonstrating highest connectivity for sea lice. We show some examples of the influence of refined small-scale modelling on dispersal patterns, and how this relates to biophysical interactions.

Discussion and conclusion
A range of factors must be taken into account when selecting the most sustainable approach to industry expansion, and choosing sites upon which to focus. More exposed sites offer an opportunity to reduce environmental impacts in terms of sea lice connectivity, with an associated reduction in outbreak frequency and risk to wild fish. The broader implications of model scale and resolution for predicting dispersal patterns depend on a range of factors and will be discussed.

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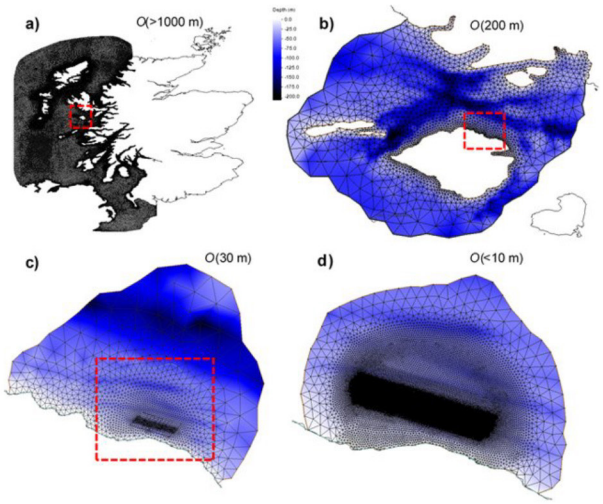

Figure 1: Hydrodynamic model domains for the west coast of Scotland, covering a range of spatial scales, with average horizontal element size of order a) 1000 m, b) 200 m, c) 30 m, and d) <10 m.

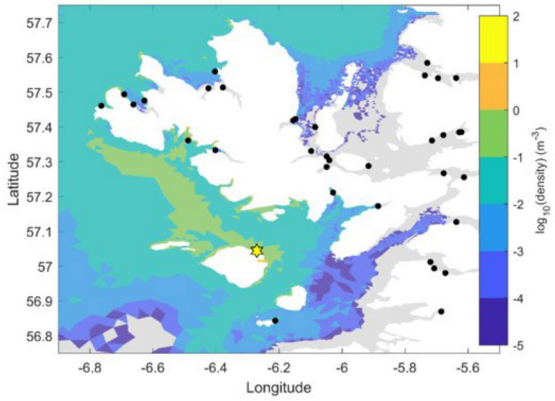

Figure 2: Mean dispersal pattern of model lice particles in the central region of the model domain, close to one of the study sites.

References

Adams TP, Aleynik D, Black KD (2016) Temporal variability in sea lice population connectivity and implications for regional management protocol. Aquac Environ Interact 8:585–596.

Aleynik D, Adams TP, Davidson K, Dale A, Porter M, Black K, Burrows MT (2018) Biophysical modelling of marine organisms: fundamentals and applications to management of coastal waters. In: *Handbook of Environmental Management of Marine Ecosystems, Ed. Md Nazrul Islam and Sven Erik Jørgensen*. Taylor and Francis, p 65–98

Aleynik D, Dale AC, Porter M, Davidson K (2016) A high resolution hydrodynamic model system suitable for novel harmful algal bloom modelling in areas of complex coastline and topography. Harmful Algae 53:102–117.

Chen C, Beardsley RC, Cowles G (2013) An Unstructured Grid, Finite-Volume Coastal Ocean Model: FVCOM User Manual, 4th Edition.

Edwards A (2016) The balance of tidal and wind-driven currents in west Scottish coastal waters.

Skamarock C, Klemp B, Dudhia J, Gill O, Barker D, Duda G, Huang X, Wang W, Powers G (2008) A Description of the Advanced Research WRF Version 3.

GENETIC PARAMETERS OF LIPID-RELATED PRODUCTION TRAITS AND MUSCLE FATTY ACIDS IN GILTHEAD SEABREAM (*Sparus aurata*)

S.S. Horn*[1], B. Ruyter[1], M.L. Aslam[1], K. Tsakoniti[2], B. Gulzari[3], J. Bastiaansen[3], C. Peñaloza[4], R. Houston[4] and A.K. Sonesson[1]

[1]Nofima, Norwegian Institute for Food, Fisheries and Aquaculture Research, NO-1433 Ås, Norway
[2]Galaxidi Marine Farm S.A., Galaxidi 330 52, Greece
[3]Wageningen University & Research, Animal Breeding and Genomics, PO Box 338, 6700 AH Wageningen, The Netherlands
[4]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

Lipids are important in all animal production because they are linked to production efficiency, health, and product quality. Excessive lipid deposition in and around internal organs is considered production loss and increases the risk of metabolic disorders and inflammation. Seabream is an important nutritional source of the health-promoting omega-3 fatty acids EPA and DHA in the Mediterranean diet. Studies in other fish species, particularly salmonids, have revealed a genetic component to the omega-3 levels of fillets (Horn et al. 2018; Leaver et al. 2011). The genetic parameters of fatty acid composition traits have not yet been explored in Seabream, and few studies have explored lipid-related production traits. Here we report on phenotypes and genetic parameters of lipid-related production traits and individual muscle fatty acids in gilthead seabream.

Materials and methods

Gilthead seabream originating from the Galaxidi Marine Farms were used in this experiment. Fish were fed commercial feed and kept in commercial sea cages in Galaxidi, Greece. Body weight and visceral weight were recorded at slaughter on 967 fish. Muscle samples from 232 of the 967 fish were analysed for muscle fat percentage and fatty acid composition, using Folch extraction, and methyl ester gas chromatography, respectively.

All fish were genotyped with the 60K MedFish SNP chip. Genotypic data was filtered using the Plink software (Purcell et al. 2007), approximately 27k SNPs passed filters and quality control. A genomic relationship matrix between the animals was firstly generated with the "-grm" function implemented in GCTA software, and afterwards considered for the estimation of the genetic parameters (Yang et al. 2011). 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 second allele and is the total number of SNP markers.

We used GCTA to perform univariate and bivariate restricted maximum likelihood (GREML) analyses to estimate heritability and genetic correlations between two traits, with "--reml" and "--reml-bivar" functions, respectively (Yang et al. 2011). No covariates were included in the model.

Results and discussion

On average, the fish weighed 371 grams and had a muscle fat content of 9.4%. The fatty acid (FA) composition of muscle was recorded as both proportional content (% of total FAs) and quantitative content (mg/g tissue). There was a low phenotypic variation in proportional content of the FAs, while the variation in quantitative content of FAs was large, reflecting the variation in muscle fat content. The major FAs in the muscle, constituting more than 60% of muscle FAs, were oleic acid (18:1n-9), palmitic acid (16:0), and linoleic acid (18:2n-6). The mean proportional content of EPA (20:5n-3) and DHA (22:6n-3) in the seabream muscle was 2.43 % and 6.95 %, respectively. The mean quantitative content of EPA and DHA was 2.14 and 6.08 mg/g, respectively, i.e. ca 800 mg per 100 g fish. This result should be seen in relation to the ISSFAL and GOED recommendations on daily intake of omega-3 FAs for the general human public of 250-500 mg EPA + DHA.

The heritability of muscle fat, viscera weight and viscera % were all above 0.3, indicating that substantial genetic gain can be expected if these traits are implemented in breeding programmes. Viscera % had an especially high heritability estimate of 0.44, while muscle fat had a heritability of 0.33. The genetic correlation of body weight with muscle fat and viscera weight was 0.77 for both traits, indicating that there is some genetic variation in these two traits that is independent of the weight of the fish.

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The estimated heritability of proportional content of both EPA and DHA was relatively high, at 0.39 and 0.34, respectively. These results diverge from what has been found in Atlantic salmon (Horn et al. 2018), where heritability estimates for EPA and DHA differed from each other, and the heritability of EPA was low (0.09). The fatty acid 16:1n-7, a marker of *de novo* lipogenesis, had a high heritability of 0.47, indicating that *de novo* lipogenesis in seabream is a heritable trait. The heritability of quantitative content of FAs reflected the heritability of muscle fat, thus all estimates were close to 0.33.

The proportional content of each FA was differently correlated to muscle fat. 18:1n-9 and 16:1n-7, both products of *de novo* lipogenesis, had the strongest genetic correlations with muscle fat. This indicates that a genetic predisposition for higher muscle fat is due to a higher inherent *de novo* lipogenesis activity. DHA had a negative genetic correlation with muscle fat (-0.50), while EPA had a weak positive correlation. Both EPA and DHA had very weak genetic correlations with body weight. These results need to be considered if selection for altered muscle fat level is applied, as this would likely influence the DHA level in fillets.

Conclusions

Genetic analysis shows the possibility of increasing EPA and DHA content in seabream fillets by selective breeding. Lipid deposition in viscera and muscle have moderate to high heritability. The fatty acid 16:1n-7, a marker of de novo lipogenesis, has a high heritability, indicating that there is a strong genetic component to this metabolic pathway in seabream. The studied lipid-related traits are important candidate traits for a breeding goal of gilthead seabream, because they affect both fish and human health, as well as production efficiency.

Acknowledgements

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References

Horn SS, Ruyter B, Meuwissen THE, Hillestad B, Sonesson AK. Genetic effects of fatty acid composition in muscle of Atlantic salmon. Genetics Selection Evolution. 2018;50.

Leaver MJ, Taggart JB, Villeneuve L, Bron JE, Guy DR, Bishop SC, et al. Heritability and mechanisms of n-3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comparative Biochemistry and Physiology D-Genomics & Proteomics. 2011;6(1):62-9.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American journal of human genetics. 2007;81(3):559-75.

VanRaden PM. Efficient methods to compute genomic predictions. Journal of dairy science. 2008;91(11):4414-23.

Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet. 2011;88(1):76-82.

ACCELERATING GENETIC IMPROVEMENT OF AQUACULTURE SPECIES USING GENOMIC TOOLS

R. D. Houston[1]

1. The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK
*E-mail: ross.houston@roslin.ed.ac.uk

The potential to grow aquaculture production via informed use of selective breeding and genomic technologies is huge, due to the relatively recent domestication and high fecundity of most species. In the more advanced, high value aquaculture sectors such as Atlantic salmon, genomic selection is routinely applied to increase selection accuracy and therefore cumulative genetic gain. This has been enabled by the development of high density SNP arrays and genotyping by sequencing technologies. To translate these benefits to many other aquaculture sectors, lower cost solutions are required, with effective technology translation from the more to less advanced sectors.

In parallel, high quality annotated reference genomes and functional genomic assays to profile transcriptional regulation can be utilised to prioritise putative causative variants in genomic regions associated with traits of economic interest. Genome editing (e.g. CRISPR/Cas9) can be used to demonstrate the causality of these variants, with potential for tackling major production barriers to aquaculture in the future.

This presentation will give an overview of applied genomic and selective breeding research aiming to take steps towards improvements in aquaculture breeding and production, with focus on an example UK-funded consortium project called ‘AquaLeap’.

EFFECT OF THE CELL DISRUPTION TECHNIQUE ON THE *IN VITRO* MICROALGAE ANTIMICROBIAL ACTIVITY

Iacumin L.[1*], Messina M. [1], Valente L.M.P. [2], Tulli F[1].

[1] Dept. of Agriculture, Food, Environment and Animal Science – University of Udine via Sondrio, 2 33100 Udine Italy
[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
* email: lucilla.iacumin@uniud.it

Introduction
The ability of microalgae to be natural sources of nutrients and bioactive compounds in diets for several finfish species has been evaluated in recent years. According to species, results have underlined poor nutrient utilization and reduced digestibility mainly hampered by the high complexity of the microalgal cell wall that allows only moderate levels of dietary inclusion of the microalgae biomass (Tulli et al. 2017). Different technological cell wall disruption methods have been successfully assessed as a first step to improve algal nutrient bioavailability (Batista et al., 2020). Anyway, as natural sources of several biomolecules recognized to offer health-promoting benefits, microalgae biomass in spite of its still high price, may contribute to the development of functional feeds able to support a sustainable aquaculture industry. This study was carried out to evaluate the effect of the disruption technique on the antimicrobial activity of dry microalgae biomass of two common species used for fish feeding.

Material and methods
Microalgal biomasses of *Tetraselmis suecica* and *Nannochloropsis oceanica* were produced by Allmicroalgae (Pataias, Portugal) and dried by convection and by spray-drying before used. Each biomass was subjected to two technological processes: a physical-mechanical rupture method and an enzymatic lysis applied to the physically disrupted microalgae according to Valente et al. (2019).

The antimicrobial activities were investigated *in vitro* against four pathogenic microorganisms (*Escherichia coli* DIAL1, *Bacillus cereus* DSMZ 2301, *Staphylococcus aureus* DSMZ 4910 and *Listeria innocua* DSMZ 20649) on the microalgal alcoholic extracts dehydrated and resuspended in 4% dimethyl sulfoxide (DMSO). The ability of extracts from microalgae (both treated and non-treated) to inhibit microbial growth was evaluated *in vitro* by analyzing the optical density at 600 nM (OD600) of the suspension inoculated to 0.1 ($\approx 10^7$ CFU/mL) of the target microorganism. The analyses were carried out in triplicate.

Results and discussion
All the microalgae tested, regardless of the cell disruption process , had a positive effect on the inhibition of the microbial growth of *B. cereus*. This effect was improved in the enzymatically processed *N. oceanica* and in the enzymatically processed *T. suecica*. An enhanced antimicrobial activity against *B. cereus* was also detected in the case of the unprocessed *T. suecica* biomass similarly to the results observed by Duraiarasan et al. (2014). On the contrary, the physical treatment resulted not effective in improving the antimicrobial ability for both the microalgae.

Physically and enzymatically processed *N. oceanica* biomass did not exhibit any antimicrobial effect against *S. aureus*. On the opposite, the unprocessed biomass inhibited the growth of the inoculated microorganism. An opposite effect was observed in the case of the unprocessed *T. suecica* biomass that enhanced the growth of *S. aureus* while the physically processed *T. suecica* inhibited its growth; the enzymatically processed microalgae stopped the growth of *S. aureus*.

Physically and enzymatically processed *T. suecica* extracts slowed down the growth of *Listeria innocua* over time while the other tested extracts did not inhibit the growth of the bacteria.

With the exception of unprocessed *N. oceanica*, all microalgae considered resulted effective in reducing the microbial growth of *E. coli* as previously observed by Dooslin et al. (2013) for *Dunaliella salina*.

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Conclusion

It can be stated that microalgae extracts are effective in inhibiting the growth of pathogens. In some cases, enzyme treated microalgae can even stop the growth of bacteria. With the exception of unprocessed *N. oceanica*, all microalgae extracts reduced the growth of *E. coli*. *T. suecica* proved its antimicrobial activity against *Listeria* spp., but also against *B. cereus* and *E. coli*.

References

Batista S., Pintado M., Marques A., et al., 2020. J. Applied Phycology, 2020, 32(5): 3429–3446 DOI: 10.1007/s10811-020-02185-2.

Dooslin V., Bai M., Krishnakumar S. 2013. International Journal of Pharmacy and pharmaceutical Sciences, 5, 17-23.

Duraiarasan S., Mani V., Abdul Razack S. et al., 2014. J. Coastal Life Medicine 2(11) DOI: 10.12980/JCLM.2.2014APJTB-2014-0138

Tulli F, Pascon G, Niccolai A. et al.,. 2017. Effect of sonication on the nutrient digestibility of *Chlorella sorokiniana* in rainbow trout (*Oncorhynchus mykiss*). In: Aquaculture Europe 2017, Dubrovnik, Croatia

Valente L.M.P., Pintado M.M.E., Batista S.M.G., et al., 2019. Method for obtaining proteins or rich-protein extract from 1038 algae, extracts and uses therefore. Patent WO/2019/171293, PCT/ 1039 IB2019/051811

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EFFECTS OF FIRST FEEDING REGIME ON DEVELOPMENT OF DIGESTIVE SYSTEM IN PIKEPERCH (*Sander lucioperca)* LARVAE

Aiman Imentai[1]*, Božidar Rašković[2], Christoph Steinbach[1], Carlos Yanes-Roca[1], Tomas Policar[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, Zátiší 728/II, 389 25, Vodňany, Czech Republic.
[2]University of Belgrade, Faculty of Agriculture, Institute of Animal Science, Nemanjina 6, Zemun, 11080 Belgrade, Serbia
E-mail: aimentai@frov.jcu.cz

Introduction
Higher survival and growth in pikeperch larviculture were obtained by using rotifers and *Artemia* as first diet (Yanes-Roca et al., 2018). However, within the larval growth rotifers become too small prey to sustain a proper development. Therefore, the aim of the present study was optimization of the first feeding regime for pikeperch larvae using rotifers and *Artemia* and to investigate the development of digestive system in pikeperch larvae under different feeding regimes (Imentai et al., 2020).

Materials and methods
Experimental culture of pikeperch larvae with rotifers *B. plicatilis* was performed in the Experimental Fish Facility of the Faculty of Fisheries and Protection of Waters, University of South Bohemia (Czech Republic). Hatched pikeperch larvae originating from nest spawning of pond-cultured broodstock were acclimated to experimental recirculating aquaculture system with water temperature of 15 ± 0.5°C at 3 days post-hatch (DPH). Then, the larvae at 4 DPH (total length= 5.62 ± 0.03 mm, body weight = 0.66 ± 0.16 mg) were divided into five experimental groups with four replicates at initial density of 100 larvae per liter. All larvae at 5 DPH were initially fed with rotifers for 3 days and thereafter from 8 to 17 DPH were divided to 5 (A-E) different feeding regimes and fed with rotifers and *Artemia* as follows: (A) larvae fed only with rotifers till 17 DPH; (B) larvae fed with rotifers till 14 DPH followed by feeding with *Artemia* till 17 DPH; (C) larvae fed with rotifers till 11 DPH followed by feeding with *Artemia* till 17 DPH; (D) larvae fed only with *Artemia* till 17 DPH; (E) larvae fed a combination of rotifers and *Artemia* till 17 DPH. Rotifers and *Artemia* were provided as live feed to larvae three times per day with residual counts prior to each feeding. Feeding densities were steadily increased based on residual counts, performed prior to each feeding.

Twelve larvae (3 per replicate) were sampled at 11, 14 and 17 DPH for histological analyses. Whole larvae was sacrificed humanely by immersion in overdose of MS-222 anaesthetic, immediately transferred to Davidson's fixative (preserved overnight) and subsequently transferred into ethanol (70%). The samples were dehydrated in ascending ethanol concentrations (70%, 95% and 100%), cleared in xylene, embedded in paraffin, and cut into a series of 5 μm longitudinal sections using a rotary microtome (Galileo, Italy). Sections were stained with hematoxylin and eosin. The slides were assessed for general histopathological alterations and tissue structure, and later assessment of selected cells in each tissue was conducted. Anterior portion of intestine was analyzed for: (a) surface areas of enterocyte nuclei and (b) height of enterocytes; liver was analyzed for: (c) surface areas of hepatocyte nuclei (d), vacuolation of hepatocytes and (e) frequency of small (possibly pyknotic) nuclei. All measurements were done at 1-3 serial sections of the same fish. Histological slides were analyzed and photographed using an Olympus EX51 light microscope fitted with Canon E600 digital camera.

Growth performance and survival rate were assessed at 11, 14 and 17 DPH. All data were statistically analyzed using RStudio while differences were considered significant at $p<0.05$ using one-way ANOVA followed by Tukey post hoc test.

Results
The groups fed rotifers for initial 3 days followed by feeding on *Artemia* (group D) (53 ± 5.43 %) and combination of rotifers and *Artemia* (group E) (68 ± 5.51 %), respectively, for the following 9 days showed significantly ($P<0.05$) higher survival rates than the other groups (36-50%). The group fed merely on rotifers (group A) exhibited significantly lower specific growth rate (SGR) than the other groups, and the highest SGR was found in the group fed with combination of rotifers and *Artemia* after 3 day rotifer feeding.

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The histological examination of the anterior intestine, liver and pancreas revealed no specific alterations in the histological organization. The highest values of enterocyte height in the anterior intestine were observed in groups C, D and E at 14 DPH which were significantly different from those of A and B groups. However, there were no significant differences among groups at 11 and 17 DPH. The highest profile area of enterocytes nuclei was found in group D at 11 DPH. The highest profile area of hepatocyte nuclei was detected in groups E and D at 11 and 14 DPH, respectively. The appearance of hepatocytes was typical for the liver of fish. The variable size/number of vacuoles were detected in the cytoplasm of hepatocytes in most of fishes. Frequency of cytoplasm vacuolation at 11 DPH was statistically different among the groups, where group A had higher scores compared to D and E groups ($P < 0.05$). A vast majority of cells in group A had cytoplasm almost entirely occupied with vacuoles at 11 DPH, but that statistical trend did not continue at other sampling points where the degree of cellular vacuolation decreased in group A at 14 DPH while this trend continued in all groups at 17 DPH. Mean profile area of nuclei showed a significant difference among groups at first two sampling points. At 11 DPH mean profile area of nuclei was significantly higher in E group compared to the other groups ($P < 0.05$), while at 14 DPH profile area of nuclei was the highest in D group ($P < 0.05$). Detailed breakdown of profile area of hepatocyte nuclei to classes showed domination of larger nuclei during 11 DPH and 14 DPH sampling points, while small, possibly pyknotic nuclei (profile area less than 8 μm^2) averaged only 1.2% and 0.9% of total nuclei, respectively. At 17 DPH, smallest class of nuclei accounted for 6.8% of total nuclei, while largest classes reduced frequency in total number of nuclei.

This study concluded that feeding pikeperch with rotifers during first three days (from 5 DPH till 8 DPH) and afterwards replacing with *Artemia* is proper feeding regime that supports larval development with minimal investments in live feed and labor.

References

Imentai, A., Rašković, B., Steinbach, Ch., **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 https://doi.org/10.1016/j.aquaculture.2020.735636

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, *497*, 260-268.

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CLIMATE CHANGE INDUCED EXTREME AMBIENT WINTER COLD TEMPERATURE EFFECTS IN EUROPEAN SEABASS (*Dicentrarchus labrax*): GROWTH PERFORMANCE, METABOLIC AND PHYSIOLOGICAL RESPONSES

M. Jakiul Islam[1,2, 3*], 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
Faculty of Biology and Chemistry (FB 02), University of Bremen, Germany
Email: jakiul.islam@awi.de

Introduction

Extreme temperature and precipitation indices showed trends consistent with global warming (Knapp et al., 2015; Sun et al., 2016)extreme climatic events have been a major issue worldwide. Regional assessments on various climates and geographic regions are needed for understanding uncertainties in extreme events' responses to global warming. The objective of this study was to assess the annual and decadal trends in 12 extreme temperature and 10 extreme precipitation indices in terms of intensity, frequency, and duration over the Loess Plateau during 1960-2013. The results indicated that the regionally averaged trends in temperature extremes were consistent with global warming. The occurrence of warm extremes, including summer days (SU. Due to global climate changes, winter temperatures in some parts of the Mediterranean sometimes plunge to 9.5 to 10.6 °C (Besson et al., 2016; Llorente and Luna, 2013). Shifts in precipitation patterns can alter water temperature and salinity (Troia and Giam, 2019)N. rubricroceus, Etheostoma rufilineatum, E. chlorobranchium. Temperature and salinity shifts outside of normal tolerable ranges are stressful for fish and cause suboptimal growth, metabolic, osmotic, and physiological performance (L'Honoré et al., 2020)with an average 30% mortality rate. In this study, we bring new evidence of mechanisms underlying freshwater tolerance in sea bass at gill and kidney levels. In fresh water (FW. The present study aimed to elucidate the effects of an environmentally realistic extreme winter cold temperature in European seabass before acclimatization at different salinities under controlled laboratory conditions.

Materials and methods

European seabass acclimatized at 3, 6, 12, and 30 PSU salinities were subjected to an ambient extreme winter cold event (8° C), monitored 20 days for growth and physiological performance. For sample collection, fish were euthanized on days 1, 10, and 20. Serum metabolites, biochemical parameters, and selective gene expression were analyzed.

Results

During cold stress exposure, osmoregulatory activities, metabolic and physiological performances were markedly affected in 3 and 30 PSU fish. Blood Na+, Cl- and K+ concentrations significantly ($p<0.05$) increased in 30 PSU fish. Cortisol, glucose, blood urea, hepatic enzymes increased significantly ($p<0.05$) in 3 and 30 PSU fish, whereas opposite trends were observed for serum protein, lactate, and triglycerides content during cold stress progression. The abundance of HSP70, TNF-α, and CFTR genes were significantly ($p<0.05$) increased in 3 and 30 PSU fish. In contrast, Igf1 gene expression was significantly lower in 3 and 30 PSU groups. Besides, Na+/K+ ATPase α1 and Na+/K+/Cl-cotransporter-1 were significantly upregulated in 30 PSU followed by 12, 6, and 3 PSU groups on day 20.

Discussion and conclusion

Results demonstrate that ambient extreme winter cold event induces metabolic and physiological stress responses and provide a conceivable mechanism by which growth and physiological fitness are limited at cold thermal events. However, European seabass exhibit better physiological fitness in 12 and 6 PSU water during ambient extreme cold (8 ° C) events, providing possible insight into future aquaculture management options.

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References

Besson, M., Vandeputte, M., van Arendonk, J.A.M.A.M., Aubin, J., de Boer, I.J.M.J.M., Quillet, E., Komen, H., 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.

Knapp, A.K., Hoover, D.L., Wilcox, K.R., Avolio, M.L., Koerner, S.E., La Pierre, K.J., Loik, M.E., Luo, Y., Sala, O.E., Smith, M.D., 2015. Characterizing differences in precipitation regimes of extreme wet and dry years: implications for climate change experiments. Glob. Chang. Biol. 21, 2624–2633. https://doi.org/10.1111/gcb.12888

L'Honoré, T., Farcy, E., Blondeau-Bidet, E., Lorin-Nebel, C., 2020. Inter-individual variability in freshwater tolerance is related to transcript level differences in gill and posterior kidney of European sea bass. Gene 741, 144547. https://doi.org/10.1016/j.gene.2020.144547

Llorente, I., Luna, L., 2013. The Competitive Advantages Arising from Different Environmental Conditions in Seabream, *Sparus aurata* , Production in the Mediterranean Sea. J. World Aquac. Soc. 44, 611–627. https://doi.org/10.1111/jwas.12069

Sun, W., Mu, X., Song, X., Wu, D., Cheng, A., Qiu, B., 2016. Changes in extreme temperature and precipitation events in the Loess Plateau (China) during 1960-2013 under global warming. Atmos. Res. 168, 33–48. https://doi.org/10.1016/j.atmosres.2015.09.001

Troia, M.J., Giam, X., 2019. Extreme heat events and the vulnerability of endemic montane fishes to climate change. Ecography (Cop.). 42, 1913–1925. https://doi.org/10.1111/ecog.04576

EFFECT OF STOCKING DENSITY ON THE ANTIOXIDANT ENZYME ACTIVITIES OF GRASS CARP (*Ctenopharyngodon idella*) REARED IN A FRESHWATER INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM IN CAGES

Jane Jacob*, Kiran Dube, Chadha, N.K., Reddy, A.K., Chandrakant M.H., Abisha, R., Raju, K .D.

ICAR-Central Institute of Fisheries Education
(Deemed University)
Panch Marg, Off Yari Road, Versova, Andheri (W),
Mumbai-400061, India
* Corresponding author. E-mail: jane.aqcpa904@cife.edu.in

Introduction

Cage culture is done in a subsistence level in India and other developing nations whereas the commercial cage culture of salmon and other high value fishes is an entirely different industry in the concerned places (Tacon and Halwart, 2007). However, these cages threaten the biodiversity and quality of water in the bodies where they are installed in terms of nutrient loading, fish escape, antibiotic flushing, etc. A sustainable alternative to this monoculture is IMTA (Integrated multi-trophic aquaculture), it employs extractive species such as mussels and sea cucumbers to remove the nutrients while providing income (Troell et al., 2009). IMTA is predominantly employed in marine waters but other variants such as the Brackishwater IMTA has surfaced over the years (Balasubramaniam et al., 2018). This experiment is a trial of a freshwater variant of IMTA in floating net cages. It helps to decide the basic stocking density of fish and extractive animals by analysing the anti-oxidant enzymes produced by the fishes. If successful, FIMTA can replace traditional monoculture and increase the income for the people while reducing the impact it can potentially cause.

Material and methods

The experiment was conducted in cages in a freshwater reservoir (Dimbhe, Maharashtra, India). It was designed as per completely randomised design (CRD) with five treatments (Different stocking density of fish with prawn and mussels) in triplicates in a total of 15 cages. The fishes were grass carp (*Ctenopharyngodon idella*) as the fed species and freshwater prawn (*Macrobrachium rosenbergii*) along with freshwater mussel (*Lamellidens marginalis*) as the extractive species. It may be deemed as a partial FIMTA as the plant component for extractive species was not used. The control for the experiment was monoculture of freshwater prawn with mussels and grass carp alone in another treatment. The fishes were sampled at the beginning and end of the experiment of 120 days. The water quality was assessed every fortnightly. At the end of the experiment, the samples of liver were taken and analysed for antioxidant enzymes activity. The superoxide dismutase (SOD) activity was estimated by the method of Misra and Fridovich (1972) based on oxidation of epinephrineadrenochrome transition by the enzyme. SOD was expressed as units per mg protein-1min-1 at 37° C (amount of protein required to give 50 % inhibition of epinephrine autooxidation). Protein was estimated using Lowry's method (Lowry, 1951). Catalase (CAT) activity was determined by the method of Takahara et al. (1960). Catalase activity was expressed as nanomole of H2O2 decomposed/min/mg protein. Statistical analysis of different growth and physiological parameters were analyzed by using one-way analysis of variance (ANOVA) via SPSS 22.0 for windows. For post hoc comparison of mean, Duncan's test was applied.

Result

The physiological response of the fish towards any stress induced through the integration was analysed in terms of the quantity of SOD and Catalase present in the liver expressed in units/mg protein. It was observed that there was no significant variation ($p>0.05$) in the values of SOD and CAT within the treatments. There was also no difference in the values of SOD and CAT between the treatment with only fish and the integrated systems of various fish stocking densities. The values obtained are graphically represented in fig 1.

Discussion

Stress in fish is induced when exposed to unsuitable or unfavourable conditions. Stressor can enhance or suppress of immune system thus leading to vulnerable conditions (Tort, 2011). In fish, exposure to pollution, pesticides and or other contaminants such as heavy metals can induce oxidative stress and it is mitigated by production of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The enzyme SOD detoxifies the toxic superoxide anion radical and catalase is the primary antioxidant defence component. In the study there is no significant ($p>0.05$) trend observed in the quantity of SOD/CAT secretion. However, several authors have studied the effect of different stressors on carps and the corresponding values are higher than that obtained in the highest treatment in this study (Wen et al., 2015; Ciji et al., 2012; Shahbaz et al., 2010). This indicates that the fish in all densities was in its normal physiological condition when integrated in the FIMTA systems i.e. the presence of prawns in the cages did not affect the homeostasis of the fishes.

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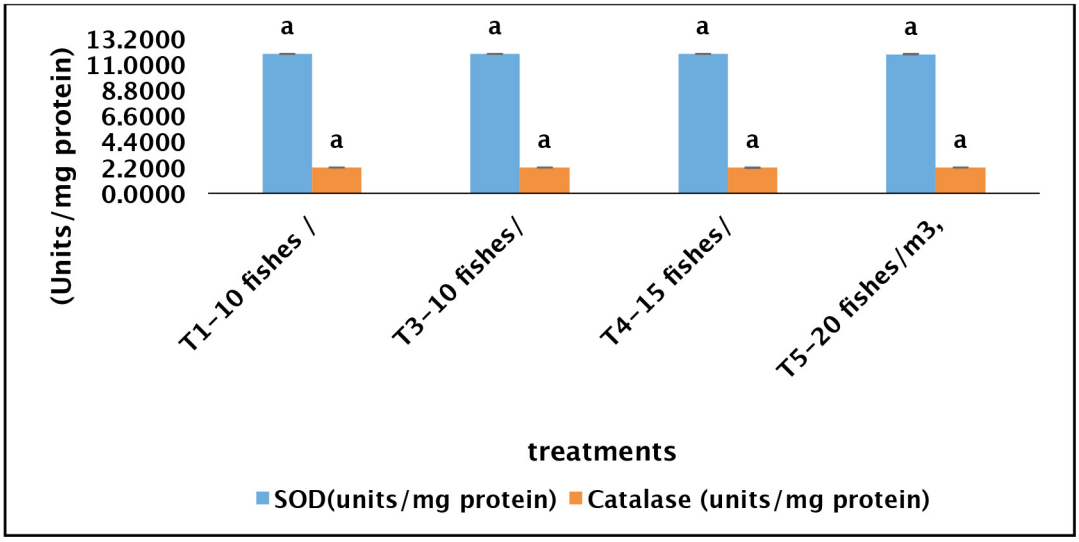

Fig 1: Graphical representation of SOD and CAT enzymes observed in various treatments

Conclusion

This study was a preliminary study on the integrated multi-trophic aquaculture in freshwater floating net cages and hence it is essential to analyse the stress response of the fed species on integration with the other species. The antioxidant enzyme levels were within limits and this shows that future studies can be taken up with higher stocking densities of the fishes and extractive animals for integration.

Reference

Balasubramanian, C.P., Mhaskar, S.S., Sukumaran, K., Panigrahi, A., Vasagam, K., Kumararaja, P., Thigale, D., Sawant, R., Vijayan, K.K. and Vasudevan, N., 2018. Development of integrated multi-trophic aquaculture (IMTA) for tropical brackishwater species in Sindhudurg District, Maharashtra, west coast of India. *Indian Journal of Fisheries*, *65*(1), pp.59-64.

Ciji, A., Sahu, N. P., Pal, A. K., Dasgupta, S. and Akhtar, M. S., 2012. Alterations in serum electrolytes, antioxidative enzymes and haematological parameters of Labeo rohita on short-term exposure to sub lethal dose of nitrite. Fish physiology and biochemistry, 38(5), pp.1355-1365.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the phenol folin reagent. J Biol Chem, *193*, 265-275.

Misra, H. P. and Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247(10), pp.3170-3175.

Shahbaz Akhtar, M., Pal, A. K., Sahu, N. P., Alexander, C., Gupta, S. K., Choudhary, A. K., Jha, A. K. and Rajan, M. G., 2010. Stress mitigating and immunomodulatory effect of dietary pyridoxine in Labeo rohita (Hamilton) fingerlings. Aquaculture Research, 41(7), pp.991-1002.

Tacon, A.G. and Halwart, M., 2007. Cage aquaculture: a global overview. *FAO Fisheries Technical Paper*, *498*, p.3.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T., 1960. Hypocatalasemia: a new genetic carrier state. The Journal of Clinical Investigation, 39(4), pp.610-619

Tort, L., 2011. Stress and immune modulation in fish. Developmental and Comparative Immunology, 35: 1366–1375.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H. and Fang, J.G., 2009. Ecological engineering in aquaculture—potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture*, *297*(1-4), pp.1-9.

Wen, J., Jiang, W., Feng, L., Kuang, S., Jiang, J., Tang, L., Zhou, X. and Liu, Y., 2015. The influence of graded levels of available phosphorus on growth performance, muscle antioxidant and flesh quality of young grass carp (Ctenopharyngodon idella). Animal Nutrition, 1(2), pp.77-84.

STRENGTHS AND WEAKNESSES OF MACROALGAE CULTIVATION IN THE BALTIC PROPER AND ADJACENT BASINS

Magdalena Jakubowska*, Tomasz Kulikowski

National Marine Fisheries Research Institute, Kołłątaja 1, 81-332 Gdynia, Poland
mjakubowska@mir.gdynia.pl

Macroalgae have been used in human diets since very early times. Apart from direct consumption, seaweeds being rich in protein, dietary fibers and various bioactive compounds are also used as additives to enhance the nutritional quality of the food products as well as in cosmetic, pharmaceutical and agriculture industries. In the last decade, the global cultivation of macroalgae has doubled. Production of seaweed in the world currently amounts to almost 30 million tons per year. Although, the main producing countries are China, Indonesia and the Philippines, the demand for seaweed products in Europe is growing every year.

In the Baltic Sea Region the cultivation of macroalgae is still at an early stage. Conditions in the Western Baltic are suitable for farming commercially valuable species - *Laminaria digitata* and *Saccharina latissima*, for which many pilot projects have been implemented, technology is available and recently commercial cultivation is ongoing. In the rest of the Baltic Sea - Baltic Proper and bays, the low salinity prevents from cultivation of these commercially important species. However, the interest of growing local, valuable or potentially valuable species such as *Fucus vesiculosus*, *Ulva intestinalis* or *Furcellaria lumbricalis* is increasing in recent years.

During the implementation of GRASS project (Growing algae sustainably in the Baltic Sea), which aims to raise awareness and build capacity on macroalgae cultivation, harvesting and use among public authorities and other relevant stakeholders, we have identified strengths and weaknesses of macroalgae cultivation in the Baltic Sea, focusing on the Baltic Proper and adjacent basins. Strengths (e.g.: presence of valuable species in the Baltic Sea, possibility to provide various ecosystem services by seaweed cultivation, possibility to provide product unique in the region - fresh macroalgae), weaknesses (e.g.: various legislative challenges, limited technology dedicated to Baltic species and conditions, higher production costs than in Asian counties), opportunities (e.g.: excellent seaweed consumer's perception, potential synergies with other seawater uses, possibility to subsidize macroalgae cultivation as an environmental service) and threads (e.g.: limited space for macroalgae farming, no confirmation of production costs in practice, necessity to position products high on the market) related to farming macroalgae in this region have been identified, listed, and the most important of them described in details.

THE CONTRIBUTION OF LOW TROPHIC AQUACULTURE (SHELLFISH) TO CIRCULAR FOOD SYSTEMS

Henrice Jansen*

Wageningen University and Research (WUR), Wageningen, The Netherlands
Email: henrice.jansen@wur.nl

Introduction

There are growing doubts as to the long term sustainability of many existing food production systems, including aquaculture, to meet the future increasing global demands (Tacon et al 2009). Whereas marine capture fisheries and aquaculture within Europe focus on high trophic level carnivorous fish species, the production of lower trophic level species, including shellfish, offer vast opportunities to contribute to circular food systems.

Framework

Through an integrated framework of Product, Effect and Circularity indicators we will explore the contribution of bivalve aquaculture to sustainability in our food systems. The framework reviews examples of shellfish aquaculture from different regions and production types.

Product indicators relate to the (potential for future) production levels to deliver food, feed and bio-based products. Production of food is the principle aim for shellfish aquaculture. However, the carbon-rich shells, traditionally considered a waste of aquaculture activities, have recently acquired an interest under the framework of zero waste circular economy (Alonso et al 2021). To make smart circular connections between sectors (e.g. shellfish and concrete industries) a success, the issue of volume and logistics becomes relevant.

Traditionally sustainability is measured based on *effect indicators* such as Life Cycle and Environmental Impact Assessments (LCA, EIA). Hilborn et al (2018) demonstrate that shellfish aquaculture has the lowest the ecological footprint in comparison to other animal protein products, both marine and terrestrial. Effect indicators also include the numerous positive contribution of shellfish to deliver ecosystem services (Smaal et al 2019).

Currently *circularity indicators* gain interest in the realm of food system analysis, and highlight for example resource (re) use in input and output parameters. In this context we will discuss the nutrient re-use and carbon sequestration potential. The use of bivalves in circular IMTA farming systems will be discussed from local and regional perspective.

References

Alonso and X. A. Alvarez-Salgado and L. T. Antelo (2021) Assessing the impact of bivalve aquaculture on the carbon circular economy. Journal of Cleaner Production 279: 123873. DOI:10.1016/j.jclepro.2020.123873

Hilborn R, J Banobi, SJ Hall, T Pucylowski, TE Walsworth 2018. The environmental cost of animal source foods. Frontiers in Ecology and teh Environment 16(6):329-335. doi.org/10.1002/fee.1822

Smaal, A., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø. (Eds.) 2019. Goods and Services of Marine Bivalves. eBook ISBN 978-3-319-96776-9. DOI 10.1007/978-3-319-96776-9

Tacon AGJ, MM Giovanni M. Turchini & SS De Silva (2009) Responsible Aquaculture and Trophic Level Implications to Global Fish Supply, Reviews in Fisheries Science, 18:1, 94-105, DOI: 10.1080/10641260903325680

WHAT GOES WRONG DURING EARLY DEVELOPMENT OF ARTIFICIALLY REPRODUCED EUROPEAN EEL (*Anguilla anguilla*)? CLUES FROM THE LARVAL TRANSCRIPTOME AND GENE EXPRESSION PATTERNS

Pauline Jéhannet [a]*, Arjan P. Palstra [a], Leon T.N. Heinsbroek [b], Leo Kruijt [a], Ron P. Dirks [c], William Swinkels [d], Hans Komen [a]

[a] Animal Breeding and Genomics, Wageningen University & Research, Wageningen 6708 PB, The Netherlands
Email: pauline.jehannet@wur.nl.
[b] Wageningen Eel Reproduction Experts, Wageningen 3708 AB, The Netherlands
[c] Future Genomics Technologies B.V., Leiden, 2333 BE The Netherlands
[d] DUPAN foundation, Wageningen 6708 WH, The Netherlands

Introduction

Closing the life cycle of the European eel in captivity is urgently needed to gain perspective for the commercial production of juvenile glass eels. Larvae are produced weekly at our facilities but large variations in larvae mortality are observed during the first week after hatching. Although much effort has been devoted on investigating ways to prevent early larval mortality, it remains unclear what the causes are. The aim of this study was to perform a transcriptomic study on European eel larvae in order to identify genes and physiological pathways that are differentially regulated in the comparison non-viable *vs*. viable larvae.

Material and methods

Larvae collected at 1 day post-hatch (dph) from batches that survived for at least a week were classified as viable larvae, while those from batches that survived less than 3dph were classified as non-viable larvae. RNA was isolated from these samples, RNA-seq was performed and differentially expressed genes were analysed between non-viable *vs*. viable larvae. The major histocompatibility complex class-I (*mhc1*) gene, M-protein (*myom2*), the dopamine 2B receptor (*d2br*), the melatonin receptor (*mtr1*) and heat-shock protein beta-1 (*hspb1)* showed strong differential expression in the RNA-seq data. Consequently, expression patterns of these genes were investigated in 1, 8 and 15dph larvae (Fig. 1) by RT-PCR to further comprehend their role during early ontogeny.

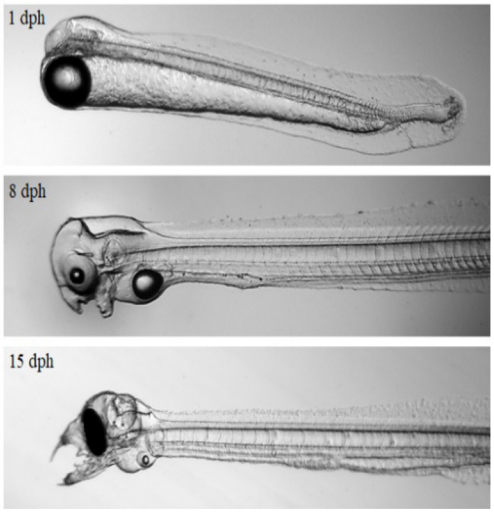

Figure 1. Larvae at 1, 8 and 15dph. At 1dph, larvae hang vertically in the water column and have yolk-sac reserves with large oil droplet. At 8dph, larvae start swimming and develop lower and upper jaws. At 15 dph, the yolk-reserves are almost depleted and the protruding teeth are formed which marks the start of exogeneous feeding.

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Results and discussion

Expression of genes involved in inflammation and host protection were higher in non-viable *vs*. viable larvae suggesting that non-viable larvae suffered from microbial infection. Expression of genes involved in osmoregulation were higher in non-viable *vs*. viable larvae implying that non-viable larvae were possibly damaged and tried to maintain homeostasis by strong osmoregulatory adaptation. Myogenesis, neural and sensory development were reduced in non-viable *vs*. viable larvae, probably because non-viable larvae invested energy in the immune response and homeostasis at the cost of developmental processes. Expression of *d2br*, *hspb1* and *mtr1* increased during ontogeny which may reflect the increase in movement at the start of active swimming (8 dph) and feed searching behaviour (15 dph). Expression of *mhc1* was highly expressed at all time points reflecting an active immune system immediately after hatching. Expression of *myom2* decreased during ontogeny reflecting the investment in growth that decreased in line with the consumption of yolk-sac reserves.

In conclusion, larvae exhibit immune competency. Non-viable larvae initiated an immune response but suffered from microbial infection. Non-viable larvae tried to maintain ionic and water homeostasis by strong osmoregulatory adaptations. Microbial control and salinity reduction might benefit eel larvae in terms of lower mortality and improved development by lowering the costs of immune functioning and osmoregulation.

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DIETARY ANTIOXIDANT SUPPLEMENTATION BOOSTS INTERMEDIARY METABOLISM IN SENEGALESE SOLE POSTLARVAE

I. Jerez-Cepa[1+], M.J. Xavier[2,3,4,5+], L.E.C. Conceição[3], L.M.P. Valente[4,5], S. Engrola[2*]

[1] Department of Biology, Faculty of Marine and Environmental Sciences, International Campus of Excellence in Marine Science (CEI·MAR), University of Cádiz, 11510 Puerto Real, Cádiz, Spain
[2] Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[3] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[4] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, 4450-208 Matosinhos, Portugal
[5] Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313 Porto, Portugal
*E-mail address: sengrola@ualg.pt
[+]Both authors contributed equally for this work

Introduction

Aquaculture industry is seeking new ways to improve production, and nutrition is one of the major driven factors to increase robustness of larvae and juveniles. Recently, the inclusion of functional additives in microdiets has been gained attention, as a strategy to promote juvenile quality. Plant-derived extracts have been shown to have a variety of biological functions, such as appetite enhancers, growth promoters and immunostimulants in aquaculture fish species. As an example, Xavier et al. (under review, 2020) investigated how the dietary supplementation of natural extracts from curcuma, green tea and grape seed could affect the metabolism of Senegalese sole (*Solea senegalensis*) postlarvae.

As growth is a highly demanding metabolic process, this study aimed at determining the effect of dietary natural antioxidant extracts (curcuma, green tea and grape seed) on the energy metabolism of *S. senegalensis* postlarvae. Changes in enzymatic activities from the intermediary metabolism of carbohydrates, lipids and amino acids were assessed to support the results described in Xavier et al. (under review, 2020).

Materials and Methods

Senegalese sole (*S. senegalensis*) postlarvae (70 DAH) were sampled from the experimental design described in Xavier et al. (under review, 2020). Briefly, postlarvae (45 DAH), were fed for 25 days with three experimental diets and a commercial diet used as control. The experimental diets were supplemented with curcumin (CC), green tea extract (GT); and grape seed extract (GS). The doses of each antioxidant extract are under a patent pending application (PCT/IB2020/056001), and test feeds were prepared by SPAROS Lda. (Olhão, Portugal).

The enzymatic activities were analyzed in lyophilized postlarvae (n = 12). The complete postlarvae were weighed and then homogenized by mechanical disruption in ice-cold buffer. The homogenate was then centrifuged and the enzymatic activities assayed in the supernatant. The homogenization buffer composition and the enzymatic assays were according to previous studies (Jerez-Cepa et al., 2020).

Results and Discussion

The supplementation with the antioxidant extracts has stimulated the intermediary metabolism in Senegalese sole postlarvae. In general, the inclusion in the diet of curcumin (CC), green tea extract (GT) and grape seed extract (GS), increased the enzymatic activity associated to carbohydrates, lipids and amino acids metabolic pathways in comparison to control (CTRL) postlarvae (Figure 1). However, the magnitude of this effect depended on the nature of the extract supplemented in the diet. Thus, the inclusion of curcumin (CC) boosted the glycolysis (HK, PK) and the gluconeogenesis (LDH, FBP) in the postlarvae, as well as the lipid synthesis (G6PDH, GPDH), and the amino acid catabolism (ALT, AST, GLDH). This increase in energy metabolism due to curcumin addition is in line with the increase in growth and the modulation of myogenic factors described in Xavier et al., (2020), for the same postlarvae. GT diet induced a comparable effect in the postlarvae, also enhancing glycolysis and gluconeogenesis pathways, as well as amino acid catabolism, but not lipid synthesis. On the contrary, although the activity in most of the enzymes assessed tended to increase in GS sole, no significant differences were determined against any of the remaining treatments. Xavier et al., (2020) also described an enhanced growth in the postlarvae supplemented with grape seed, but the expression of the myogenic factors remained as in the control fish.

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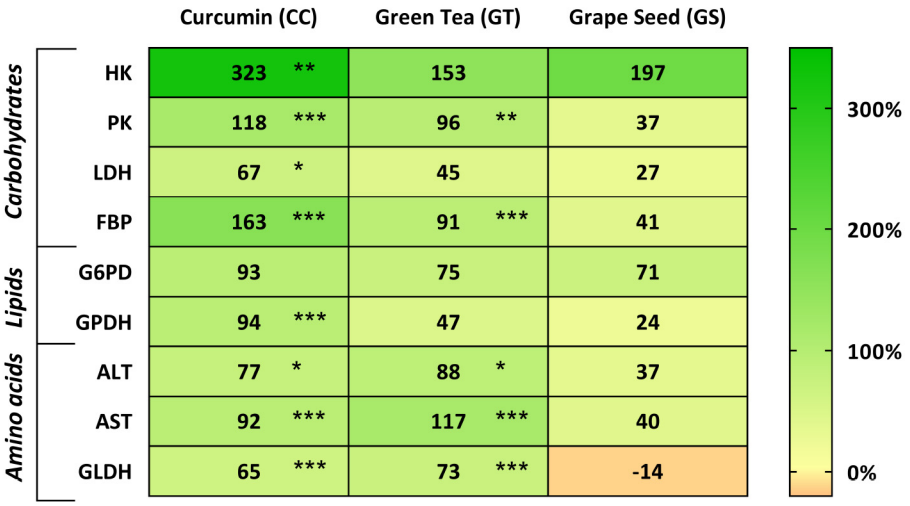

Figure 1. Heat map of the enzymatic activities (U larvae^{-1}) assayed in *Solea senegalensis* 70 DAH postlarvae after 25 days fed with experimental diets (control, curcumin, green tea and grape seed extracts). Data were normalized to percentage of variation in comparison to CTRL fish responses: ((test diet value/CTRL diet value)-1)*100. Statistically significant differences against CTRL fish are indicated with asterisks (*: p <0.05; **: p <0.01; ***: p <0.001).

Conclusions

The results show a stimulated metabolism of carbohydrates, lipids and amino acids in Senegalese sole due to the diets supplemented with the natural antioxidants, especially with curcumin. The increase in energy metabolism is most likely related to the increased growth described before for the same postlarvae (Xavier et al., 2020), and corroborate the potential benefit of using these natural antioxidants to improve the aquaculture of Senegalese sole.

References

Jerez-Cepa, I., Marín-Rincón, A., Martínez-Rodríguez, G., Ruiz-Jarabo, I., Mancera, J.M., 2020. A natural additive in the diet to improve growth and reduce energy expenditure of gilthead seabream (*Sparus aurata* L.): Attenuation of high stocking density stress responses. *Aquaculture* 524, 735263. 10.1016/j.aquaculture.2020.735263

Xavier, M.J., Colen, R., Carballo, C., Manchado, M., under review. Dietary Natural Plant Extracts Modulate Growth Performance and Antioxidant Status of Senegalese Sole Postlarvae. *Biology Open*

Xavier, M.J., Engrola, S., Conceição, L.E.C., Manchado, M., Carballo, C., Gonçalves, R., Colen, R., Figueiredo, V., Valente, L.M.P., 2020. Dietary Antioxidant Supplementation Promotes Growth in Senegalese Sole Postlarvae. *Front. Physiol.* 11, 1–11. 10.3389/fphys.2020.580600

Acknowledgements

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IN OVO AMINO ACIDS SUPPLEMENTATION MODULATES INTERMEDIARY METABOLISM IN ZEBRAFISH LARVAE CHALLENGED AT HIGH TEMPERATURE

I. Jerez-Cepa[1*], C. Navarro-Guillén[2a], A. Lopes[2], J.M. Mancera[1], S. Engrola[2]

[1] Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), International Campus of Excellence in Marine Science (CEI·MAR), University of Cádiz, 11510 Puerto Real, Cádiz, Spain
[2] Centre of Marine Sciences (CCMAR), Campus de Gambelas, Faro, Portugal
[a] Current affiliation: Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro, 11510 Puerto Real, Spain
*E-mail address: ismael.jerez@uca.es

Introduction
In the current context of global climate change, the aquaculture industry is facing new challenges to improve its sustainability, being fish nutrition a well-known key factor. The improvement of digestive efficiency and feeding protocols are necessary to increase the productivity and reduce the environmental impact. With this objective, nutritional programming during early development phases can play an interesting role to obtain robust larvae. This imprinting may result in long-term effects on growth and physiological status. Therefore, dietary amino acids have been probed to modulate fish digestive capacity and consequently growth performance.

This study aimed at determining the effect of *in ovo* supplementation of arginine and glutamine amino acids as long-term modulators of zebrafish (*Danio rerio*) larvae digestive capacity, and growth performance at optimal (28 ºC) and challenging temperature (32 ºC). As growth is a highly demanding metabolic process, changes in enzymatic activities from the intermediary metabolism of carbohydrates, lipids and amino acids were assessed. These results support the conclusions of Navarro-Guillén et al. (AE 2020 Online, EAS).

Materials and Methods
The amino acid (AA) supplementation was performed in 3.5 h post-fertilization zebrafish (*D. rerio*) eggs using the sonophoresis technique. Embryos were supplemented with either arginine (ARG, 50x) or glutamine (GLN, 50x). An additional pool of eggs was exposed to ultrasound without amino acids as control group. After the procedure, eggs were placed in rearing tanks and maintained at two different temperatures (28 ºC and 32 ºC) until larvae reached 898 growing degree-days (GDD).

The enzymatic activities were analyzed in lyophilized larvae (n = 16). Larvae were weighed and then homogenized by mechanical disruption in ice-cold buffer. The homogenization buffer composition and the enzymatic assays of glycogen phosphorylase (GP); hexokinase (HK); pyruvate kinase (PK); lactate dehydrogenase (LDH); fructose 1,6-bisphosphatase (FBP); glycerol-3-phosphate dehydrogenase (GPDH); 3-hydroxyacyl-CoA dehydrogenase (HADH); alanine aminotransferase (ALT); aspartate aminotransferase (AST); and glutamate dehydrogenase (GLDH) were according to previous studies (Faught and Vijayan, 2019; Jerez-Cepa et al., 2019)we generated a ubiquitous GR knockout (GRKO. Principal Component Analysis (PCA) was performed to unravel response patterns related to *in ovo* supplementation of AA and intermediary metabolism of *D. rerio* larvae.

Results and Discussion
In ovo supplementation with ARG and GLN modulated the metabolic response to the challenging temperature (32 ºC) in zebrafish larvae. However, the magnitude of this effect differed according to the amino acid and the temperature. At optimal temperature (28 ºC), the metabolic pattern of ARG-larvae was associated to the enhancement of glycolysis (HK and PK) and gluconeogenesis pathways (GPDH) through amino acids catabolism (ALT and GLDH); while GLN-larvae enhanced mainly lipid catabolism (HADH). At the challenging temperature (32 ºC), the metabolic pattern of ARG-larvae also increased GP and FBP activities; while in GLN-larvae it was similar to untreated animals. The boosted metabolism determined for ARG-larvae at 32 ºC is in accordance with Navarro-Guillén et al. (AE Online 2020, EAS); where ARG supplementation was associated with an improvement of fish performance and gut maturation at this temperature.

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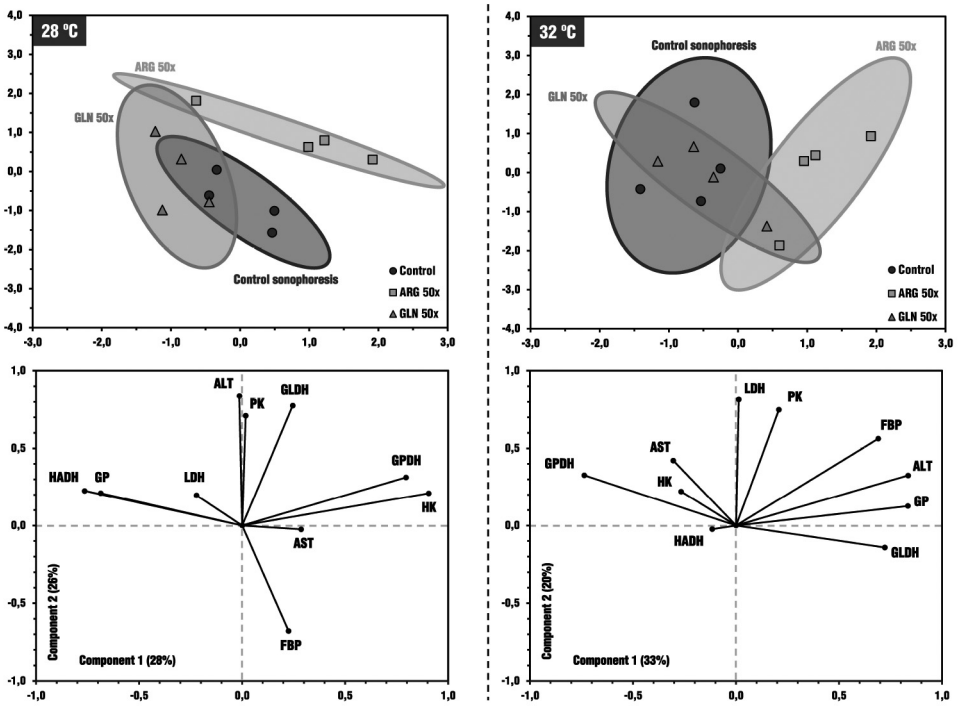

Figure 1. Principal component analysis (PCA) of enzymatic activities from intermediary metabolism in *Danio rerio* larvae at two different temperatures (28 and 32 ºC) after 898 growing degree-days (GDD). Upper graphs are the factor score plots for each individual included in the PCA analysis, and grouped by 95 % confidence ellipses, for each treatment. Bottom graphs represent the parameters correlation to principal components (PC1 and PC2).

Conclusions

This study validates *in ovo* sonophoresis as early programming method to modulate larvae metabolism through amino acids supplementation. Arginine supplementation induced major changes in comparison to glutamine at both temperatures (28 and 32 ºC), and promoted an enhanced metabolism that helped fish to cope with higher temperature (32ºC).

References

Faught, E., Vijayan, M.M., 2019. Loss of the glucocorticoid receptor in zebrafish improves muscle glucose availability and increases growth. *Am. J. Physiol. Endocrinol. Metab.* 316, E1093–E1104. 10.1152/ajpendo.00045.2019

Jerez-Cepa, I., Gorissen, M., Mancera, J.M., Ruiz-Jarabo, I., 2019. What can we learn from glucocorticoid administration in fish? Effects of cortisol and dexamethasone on intermediary metabolism of gilthead seabream (*Sparus aurata* L.). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 231, 1–10. 10.1016/j.cbpa.2019.01.010

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WATER SALINITY AS A MODULATING FACTOR OF GROWTH AND FILLET QUALITY IN THE GREATER AMBERJACK (*Seriola dumerili*) IN RAS SYSTEMS

F. Sánchez-García[1#], I. Jerez-Cepa[2#*], A. Roldán[1], I. Ruiz-Jarabo[3], M.T. Jiménez[4], J. Roo[5], J.L. Muñoz[4], J.M. Mancera[2]

[1] Department of Chemical Engineering and Food Technology, Faculty of Sciences, University of Cádiz, 11510 Puerto Real, Cádiz, Spain
[2] Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), International Campus of Excellence in Marine Science (CEI·MAR), University of Cádiz, 11510 Puerto Real, Cádiz, Spain
[3] Department of Animal Physiology, Faculty of Biological Sciences, University Complutense, Madrid, Spain
[4] IFAPA Centro El Toruño, Junta de Andalucía, 11500, El Puerto de Santa María, Cádiz, Spain
[5] Grupo de Investigación en Acuicultura (GIA), Instituto Universitario Ecoaqua, Universidad de Las Palmas de Gran Canaria, 35214, Telde, Las Palmas, Canary Islands, Spain
* E-mail address: juanmiguel.mancera@uca.es
[#] These authors contributed equally to this work

Introduction

The greater amberjack (*Seriola dumerili*) is an interesting species for aquaculture diversification in Europe, especially in Spain and other Mediterranean countries. This carnivorous finfish species is appreciated for the flavor and quality of its meat. In addition, it is a fast-growing species with voracious feeding habits and high growth rates compared to other species extensively farmed in Southern Europe, as seabass (*Dicentrarchus labrax*) or seabream (*Sparus aurata*). Some companies are developing its culture in recirculation systems (RAS), where the physicochemical and biological parameters are better controlled, and the consumption of water is reduced. In this way, RAS systems allow for the optimization of production processes and the reduction of environmental impact generated by traditional aquaculture farms.

Water salinity is of paramount importance in aquaculture productions, as it is directly associated to energy management and growth performance of fish (Ruiz-Jarabo et al., 2018). Furthermore, the modulation of energy metabolism, as well as the osmoregulatory performance of fish can affect also the quality of meat. Thus, the objective of this study aimed at defining the effects of water salinity in growth performance and fish-meat quality in the greater amberjack (*S. dumerili*) reared in controlled RAS systems.

Materials and Methods

Greater amberjack (*S. dumerili*) juveniles (N = 420; initial weight = 12.5 ± 2.8 g) were randomly distributed in four independent RAS systems at IFAPA "El Toruño" facilities (Cadiz, Spain). Each RAS system, consisting in four 1000-L fiber glass tanks, was maintained at a constant salinity (15, 22, 29 or 36 ppt) for 108 days. The experiment was performed according to the guidelines from the European Directive 2010/63/UE for animal experimentation.

Fish were periodically sampled to take biometric parameters (weight and length) and determine growth performance indexes (*K*, *SGR*, *FCR*). At the end of the trial, fish were euthanized to determine physiological parameters in plasma related to osmoregulatory performance (pH, CO_2, HCO_3^-, osmolality, sodium, potassium, calcium and magnesium), as well as physicochemical parameters in fillets related with the quality of their meat (pH, moisture, cohesiveness, consistency, crispiness, firmness, stringiness, viscosity and work of penetration).

Results and Discussion

The results confirmed the effect of water salinity in the growth performance of greater amberjack (*S. dumerili*). In general, animals maintained at the salinities above 15 ppt presented better growth performances, resulting in higher *SGR*. For the lowest salinity, fish body mass at the end of the trial was 20% lower compared to the other treatments, which was associated with lower plasma pH and HCO_3^-, and also higher concentrations of sodium and potassium. Although no changes in plasma osmolality were observed, the salinity of 29 ppt seemed to be optimal point for this species, and the good growth performance at this salinity could be associated to a lower osmoregulatory expenditure (Figure 1). The lowest *FCR* were determined at 29 and 36 ppt (1.29 and 1.55 respectively), confirming the potential of this fast-growing species to be cultured in controlled RAS systems. The better management of energy resources observed in those fish maintained at the

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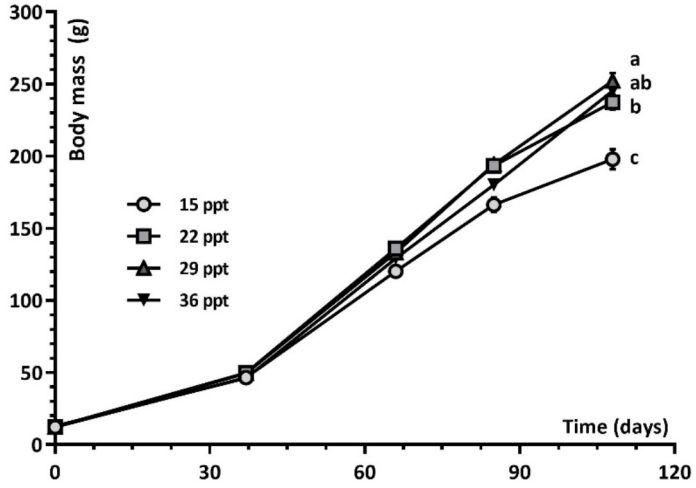

Figure 1. Body mass (g) of greater amberjack (*Seriola dumerili*) along 108 days at four water salinities (15, 22, 29 and 36 ppt). Letters represent statistical groups at the same sampling time (one-way ANOVA, Tukey test; $p < 0.05$).

highest salinities is reflected in a higher condition factor (*K*) and a higher body mass after 15 weeks. Regarding fish fillets properties, higher salinities reduced the percentage of moisture in the muscle, while muscle pH presented a similar pattern to plasma pH. The texture parameters assessed in fillets (cohesiveness, consistency, crispiness, firmness, stringiness, viscosity and work of penetration) did not vary statistically among treatments; however, fillets from fish reared at 29 ppt presented generally better texture properties.

Conclusions

The greater amberjack (*S. dumerili*) can be successfully reared in RAS systems with a water salinity above 15 ppt. The best growth performance for this species is obtained at 29 ppt, with an interesting low *FCR* (1.29) and a high *SGR* (2.8% d^{-1}). Fish-meat quality and fillets texture seemed to be better also in fish reared at 29 ppt.

References

Ruiz-Jarabo, I., Márquez, P., Vargas-Chacoff, L., Martos-Sitcha, J.A., Cárdenas, S., Mancera, J.M., 2018. Narrowing the range of environmental salinities where juvenile meagre (*Argyrosomus regius*) can be cultured based on an osmoregulatory pilot study. *Fishes* 3, 48. 10.3390/fishes3040048.

Acknowledgements

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ENERGY AND WATER EFFICIENCY IN THE AQUACULTURE SECTOR: AN E-LEARNING PLATFORM TO SUPPORT AQUACULTURE PROFESSIONALS' TRAINING NEEDS. HTTPS://EWEASPROJECT.EU

M. Johnson*, A. Cano Cabanero, A. Dominguez Mas, A. Fabris, M. Kadoič, P.A. Salvador, M. Štular, E. C. Tylko Ausias, A. Vitkovska, M. Rodriguez-Caro de la Rosa

AquaTT, Dublin, Ireland
Email martin@aquatt.ie

EWEAS aims to improve water and energy efficiency in aquaculture facilities through development of a new training course designed to reduce water and energy consumption in aquaculture which will offer improved management practices and environmentally safe and cost-effective solutions. This will help the aquaculture sector across Europe and beyond to reduce CO_2 emissions and water usage as well as increase profitability.

The EWEAS e-learning tool will be free-to-use and will promote interdisciplinary cooperation and facilitate exchange of good practices among the aquaculture professionals, who often work in remote areas, making face-to-face training more difficult.

The training programme being developed by EWEAS will provide an essential resource for aquaculture professionals to learn how to benchmark and improve the energy and water efficiency of their aquaculture systems, including estimation of the economic benefit of such improvements.

EWEAS' robust training platform will provide expert-led content and promote work-based learning in the aquaculture sector. The platform will benefit plant managers, farm technicians and other aquaculture professionals, providing them with the knowledge to carry out self-management of energy and water consumption. Not only will this improve company productivity, but this will also vastly improve the sustainability of the sector and protect the environment.

EWEAS is an EU funded ERASMUS+ project led by SGS TECNOS, Madrid with partners in Ireland (AquaTT), Latvia (Eurofortis), Italy (Associazione Piscicoltori Italiani) and Slovenia (Kmetijsko gozdarska zbornica Slovenije).

IMPROVED RAPESEED PROTEIN PRODUCTS AS FISH FEED IN AQUACULTURE

F. Kaiser*, H.-J. Harloff, R.-P. Tressel, D. Steffens, T. Kock, A. L. Grassl, F. Parsche, C. Schulz

Christian-Albrechts-Universität zu Kiel, Christian-Albrechts-Platz 4, 24118 Kiel, Germany
Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum, Germany
Email: kaiser@gma-buesum.de

Introduction
With growing demands and a limited availability of fishmeal, the search for suitable alternatives has never been more urgent (Tacon et al. 2010; Hermann et al. 2016). Proteins from rapeseed and canola have been extensively studied in the past; however, anti-nutritive substances in rapeseed together with non-optimal nutritional quality have limited its use as an ingredient in fish feeds (Francis et al. 2001; Thiessen et al. 2004). The present three studies focus on new methods for improving the effectiveness of rapeseed protein products as fishmeal alternatives.

Materials and methods
The first experiment used different levels of supplemented glucosinolates and phytic acid to determine their threshold concentrations for rainbow trout. In the second and third experiment, highly purified rapeseed protein isolates with different protein compositions were tested as substitutes for fishmeal. Additionally, nutrient digestibility of both isolates was determined and a coating technique of experimental diets was applied in the third experiment to reduce negative effects on feed intake of rainbow trout. Juvenile rainbow trout were fed twice a day to apparent satiation for 56 days in all three experiments.

Results
Results show a significantly impaired growth performance at dietary phytic acid inclusion levels of 3%. Glucosinolates did not affect growth performance parameters significantly at either level of inclusion (0.5-1.7 µmol/g).

The first rapeseed protein isolate tested had a crude protein digestibility of 95.2 ± 1.7% and was able to replace fishmeal up to 66% without significantly impairing growth. Total replacement led to significantly impaired feed intake.

The second rapeseed protein isolate had the highest crude protein digestibility recorded for rainbow trout using stripping method (99.8 ± 1.6%). However, feed intake and feed conversion were both significantly negatively affected at substitution levels of 66% and higher. Coating of experimental diets was not able to affect voluntary feed intake significantly.

Discussion
In accordance with other literature (von Danwitz & Schulz, 2020), inclusion of glucosinolates showed no effect on growth performance at applied levels of supplementation. Deactivation of myrosinase during processing of rapeseed protein concentrate used in this study might have prevented formation of brake down products, which were mainly made responsible for negative effects on growth performance from glucosinolates (Francis et al., 2001). Negative effects from phytic acid were most likely due to impaired diet acceptance, as feed conversion was not affected negatively. This was also reported by previous literature (Denstadli et al., 2006; Liu et al., 2018; Rasid et al., 2017). Inclusion of glucosinolates and phytic acid should be kept under 1.7 µmol/g and 3% respectively.

Determination of nutrient digestibility of both rapeseed protein isolates showed that complex carbohydrates are the main factor impairing digestibility at low inclusion of phytic acid. However, despite high digestibility, total replacement of fishmeal was not possible without significantly impairing growth. Negative effects from glucosinolates or phytic acid are negligible since concentrations of both ANFs are well below levels with significant impact (experiment 1; Burel, et al., 2000). Results of the second and third experiment suggest that nutrient composition, most of all dietary amino acid composition is the main factor influencing growth performance of highly purified rapeseed protein products. Coating of diets showed that post-absorptive factors could mainly determine feed intake regulation in rainbow trout. Optimization of dietary amino acid profiles could lead to successful replacement of fishmeal with rapeseed protein products.

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References

Burel, C., Boujard, T., Escaffre, A. M., Kaushik, S. J., Boeuf, G., Mol, K. A., van der Geyten, S., & Kühn, E. R. 2000. Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition, *83*(6), 653-664.

Denstadli, V., A. Skrede, Å. Krogdahl, S. Sahlstrøm, T. Storebakken. 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture 256 (1-4), S. 365–376.

Francis, G., H.P.S. Makkar, K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199 (3-4), S. 197–227.

Hermann, B. T., T.B.H. Reusch, R. Hanel. 2016. Effects of dietary purified rapeseed protein concentrate on hepatic gene expression in juvenile turbot (*Psetta maxima*). Aquacult Nutr 22 (1), S. 170–180.

Liu, L. W., Liang, X. F., Li, J., Yuan, X. C., & Fang, J. G. 2018. Effects of supplemental phytic acid on the apparent digestibility and utilization of dietary amino acids and minerals in juvenile grass carp (*Ctenopharyngodon idellus*). Aquaculture Nutrition, *24*(2), 850-857.

Rasid, R., Brown, J. H., Pratoomyot, J., Monroig, O., & Shinn, A. P. 2017. Growth performance, nutrient utilisation and body composition of *Macrobrachium rosenbergii* fed graded levels of phytic acid. Aquaculture, *479*, 850-856.

Tacon, A.G.J., M. Metian, G.M. Turchini, S.S. De Silva. 2010. Responsible Aquaculture and Trophic Level Implications to Global Fish Supply. Reviews in Fisheries Science 18 (1), S. 94–105.

Thiessen, D. L., G.L. Campbell, P.D. Adelizi. 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. Aquac Nutrition 9 (2), S. 67–75.

von Danwitz, A., & Schulz, C. 2020. Effects of dietary rapeseed glucosinolates, sinapic acid and phytic acid on feed intake, growth performance and fish health in turbot (*Psetta maxima* L.). Aquaculture, *516*, 734624.

IMPROVED RAPESEED PROTEIN PRODUCTS AS FISH FEED IN AQUACULTURE

F. Kaiser*, H.-J. Harloff, R.-P. Tressel, D. Steffens, T. Kock, A. L. Grassl, F. Parsche, C. Schulz

Christian-Albrechts-Universität zu Kiel, Christian-Albrechts-Platz 4, 24118 Kiel, Germany
Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum, Germany
Email: kaiser@gma-buesum.de

Introduction
With growing demands and a limited availability of fishmeal, the search for suitable alternatives has never been more urgent (Tacon et al. 2010; Hermann et al. 2016). Proteins from rapeseed and canola have been extensively studied in the past; however, anti-nutritive substances in rapeseed together with non-optimal nutritional quality have limited its use as an ingredient in fish feeds (Francis et al. 2001; Thiessen et al. 2004). The present three studies focus on new methods for improving the effectiveness of rapeseed protein products as fishmeal alternatives.

Materials and methods
The first experiment used different levels of supplemented glucosinolates and phytic acid to determine their threshold concentrations for rainbow trout. In the second and third experiment, highly purified rapeseed protein isolates with different protein compositions were tested as substitutes for fishmeal. Additionally, nutrient digestibility of both isolates was determined and a coating technique of experimental diets was applied in the third experiment to reduce negative effects on feed intake of rainbow trout. Juvenile rainbow trout were fed twice a day to apparent satiation for 56 days in all three experiments.

Results
Results show a significantly impaired growth performance at dietary phytic acid inclusion levels of 3%. Glucosinolates did not affect growth performance parameters significantly at either level of inclusion (0.5-1.7 µmol/g).

The first rapeseed protein isolate tested had a crude protein digestibility of 95.2 ± 1.7% and was able to replace fishmeal up to 66% without significantly impairing growth. Total replacement led to significantly impaired feed intake.

The second rapeseed protein isolate had the highest crude protein digestibility recorded for rainbow trout using stripping method (99.8 ± 1.6%). However, feed intake and feed conversion were both significantly negatively affected at substitution levels of 66% and higher. Coating of experimental diets was not able to affect voluntary feed intake significantly.

Discussion
In accordance with other literature (von Danwitz & Schulz, 2020), inclusion of glucosinolates showed no effect on growth performance at applied levels of supplementation. Deactivation of myrosinase during processing of rapeseed protein concentrate used in this study might have prevented formation of brake down products, which were mainly made responsible for negative effects on growth performance from glucosinolates (Francis et al., 2001). Negative effects from phytic acid were most likely due to impaired diet acceptance, as feed conversion was not affected negatively. This was also reported by previous literature (Denstadli et al., 2006; Liu et al., 2018; Rasid et al., 2017). Inclusion of glucosinolates and phytic acid should be kept under 1.7 µmol/g and 3% respectively.

Determination of nutrient digestibility of both rapeseed protein isolates showed that complex carbohydrates are the main factor impairing digestibility at low inclusion of phytic acid. However, despite high digestibility, total replacement of fishmeal was not possible without significantly impairing growth. Negative effects from glucosinolates or phytic acid are negligible since concentrations of both ANFs are well below levels with significant impact (experiment 1; Burel, et al., 2000). Results of the second and third experiment suggest that nutrient composition, most of all dietary amino acid composition is the main factor influencing growth performance of highly purified rapeseed protein products. Coating of diets showed that post-absorptive factors could mainly determine feed intake regulation in rainbow trout. Optimization of dietary amino acid profiles could lead to successful replacement of fishmeal with rapeseed protein products.

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References

Burel, C., Boujard, T., Escaffre, A. M., Kaushik, S. J., Boeuf, G., Mol, K. A., van der Geyten, S., & Kühn, E. R. 2000. Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition, *83*(6), 653-664.

Denstadli, V., A. Skrede, Å. Krogdahl, S. Sahlstrøm, T. Storebakken. 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture 256 (1-4), S. 365–376.

Francis, G., H.P.S. Makkar, K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199 (3-4), S. 197–227.

Hermann, B. T., T.B.H. Reusch, R. Hanel. 2016. Effects of dietary purified rapeseed protein concentrate on hepatic gene expression in juvenile turbot (*Psetta maxima*). Aquacult Nutr 22 (1), S. 170–180.

Liu, L. W., Liang, X. F., Li, J., Yuan, X. C., & Fang, J. G. 2018. Effects of supplemental phytic acid on the apparent digestibility and utilization of dietary amino acids and minerals in juvenile grass carp (*Ctenopharyngodon idellus*). Aquaculture Nutrition, *24*(2), 850-857.

Rasid, R., Brown, J. H., Pratoomyot, J., Monroig, O., & Shinn, A. P. 2017. Growth performance, nutrient utilisation and body composition of *Macrobrachium rosenbergii* fed graded levels of phytic acid. Aquaculture, *479*, 850-856.

Tacon, A.G.J., M. Metian, G.M. Turchini, S.S. De Silva. 2010. Responsible Aquaculture and Trophic Level Implications to Global Fish Supply. Reviews in Fisheries Science 18 (1), S. 94–105.

Thiessen, D. L., G.L. Campbell, P.D. Adelizi. 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. Aquac Nutrition 9 (2), S. 67–75.

von Danwitz, A., & Schulz, C. 2020. Effects of dietary rapeseed glucosinolates, sinapic acid and phytic acid on feed intake, growth performance and fish health in turbot (*Psetta maxima* L.). Aquaculture, *516*, 734624.

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

Integrated Multi Trophic Aquaculture (IMTA) is identified 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 (Chopin *et al*, 2001). Culture of extractive species with fed species in the same aquaculture locations is encouraged, and this practice is shown to remove waste materials from fed species and lower the nutrient load in the water (FAO,2018). As yet, IMTA is not widely adapted at a commercial level and understanding the validity of the 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/) is working to promote the eco-intensification of aquaculture by demonstrating the eco-efficiency and minimization of environmental impacts, the socio-economic benefits and ecosystem services, and promoting the transition towards a circular economy business model. IMPAQT has developed and deployed an autonomous data acquisition and communication system, an advanced IMTA model and an integrated management platform to achieve a holistic approach addressing this complete system view.

Results

The overview outputs from the IMPAQT project will be presented.

IMPAQT has developed and deployed an autonomous data acquisition and communication system comprising data from both novel and off the shelf sensors, remote and crowd sourced data to determine environmental quality, stock welfare and assist farm management. The support data acquisition, data aggregation and power management tools have been developed, along with an integrated management system (IMS), operating at the scale of an IMTA farm and comprising analysis and decision support functionalities, to monitor and manage IMTA production and enable enhanced operational decisions for animal welfare, production optimization, 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.

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. Based on field trials carried out at the Impaqt pilot sites the project outputs are providing data to demonstrate the eco-efficiency and reduced environmental from IMTA as well as the socioeconomic benefits, cost effectiveness, and the ecosystem services provided by IMTA to push towards a more circular aquaculture production.

References

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche□González, J.A., Yarish, C. and Neefus, C. (2001) Integrating Seaweeds into Marine Aquaculture Systems: A Key Toward Sustainability. *Journal of Phycology*, 37: 975-986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

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.

Acknowledgments

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ROLE OF SELECTIVE BREEDING IN THE IMPROVEMENT OF FEED EFFICIENCY AT RAINBOW TROUT FARMS

Antti Kause[1*], Antti Nousiainen[1], and Heikki Koskinen[1]

[1] Natural Resources Institute Finland (Luke), Finland
Email: antti.kause@luke.fi

Introduction

Feed conversion ratio (FCR), the ratio of feed input to fish biomass output, has a major impact on profitability and environmental impact of aquaculture (Knap and Kause, 2018). Nitrogen (N) and phosphorus (P) are main nutrients that micro- and macroalgae need for growth. Elevated levels of these nutrients cause eutrophication which is regarded as a negative impact in many instances. Specific nutrient loading from aquaculture can be quantified as the amount of nutrients ending up in water (kg) per produced 1000 kg of fish. Similar to FCR, environmental loading defined in this way is a measure of efficiency of aquaculture production. The objectives were: **1)** To quantify the long-term trend from year 1980 onwards in the farm-level FCR and in specific N and P loading in commercial rainbow trout industry in Finland; and **2)** To quantify the degree to which which genetic improvement made by the national breeding programme has improved FCR and the consequent reduction in nutrient loading.

Material and methods

The study was conducted using two data sets. To quantify the phenotypic trend at commerical fish farms, annual values of the amount of feed provided, fish biomass grown, and specific phosphorus and nitrogen loading of farms were extracted from the database of the ELY Centre, Finland. ELY monitors the licencing of fish farms, and the data consists of all the farms that have been approved a farming licence at the coast of the mainland Finland between years 1980 and 2016. For each farm in each year, farm-level feed conversion ratio was calculated as: FCR_{Farm} = Total feed use (kg) / Fish biomass growth (kg). Nutrient loading was calculated as:Specific P loading (kg P / 1000 kg fish) = (Fish biomass growth (kg) × P concentration in fish - Total feed use (kg) × P concentration in Feed) / 1000 kg of fish. Specific N loading was calculated in an equivalent way.

To quantify the genetic trend in FCR, the data of the national breeding programme maintained by Luke was used. The data included 23 year classes from 1992 to 2014 with 537 262 individuals with phenotypes plus the base population of year classes 1989 and 1990. Offspring were generated from 3260 sires, 3270 dams, and 5821 full sib-families. The parents for each generation were selected based on their estimated breeding values (EBV) for growth (since 1992), maturity age (since 2001), external appearance (since 2001), skeletal deformations (since 2002), fillet colour (2003-2012), cataract caused by *Diplostomum* parasite (since 2003), visceral percentage (2005) and survival (since 2010) (Kause et al., 2005; 2007, Vehviläinen et al., 2012).

Results and discussion

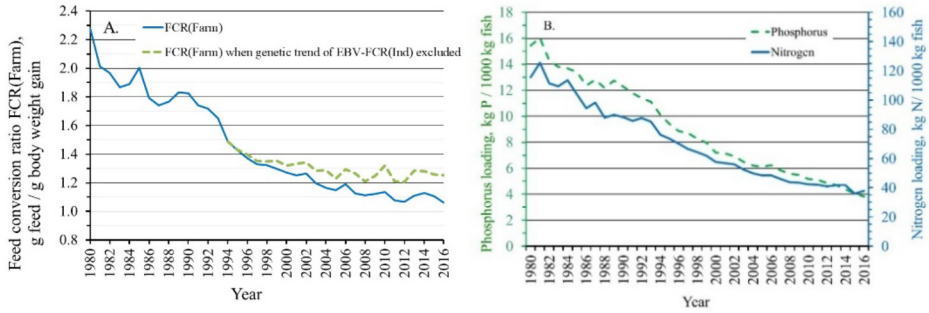

Figure 1. a) Trend of farm-level FCR_{Farm} (in blue), and re-calculated FCR_{Farm} when the genetic trend of FCR_{Ind} has been subtracted from FCR_{Farm} (in dotted green). The area between the two lines is genetic improvment in FCR_{Ind}. **b)** Nitrogen (in blue) and phosphorus (dotted green) loading from commercial farms located at the coast of the mainland Finland during 1980-2016.

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Genetic trend of FCR was estimated in five steps. First, genetic, common environmental and residual (co)variances of the selected traits were estimated (discussed in Kause et al., 2005, 2007; Vehviläinen et al., 2010). Second, FCR_{Ind} as well as fillet% and muscle lipid% (corrected for body weight) were recorded only in one or two generations, and their (co) variances (Kause et al., 2007, 2016) were merged into the (co)variance matrix obtained in step 1. To record FCR_{Ind}, feed intake was recorded using the x-ray technique 9 times on fish growing from an initial average weight of 143.5 g to the final average weight of 2113 g, and FCR_{Ind} was calculated as: Feed intake / Body weight gain. The key genetic parameters influencing the genetic trend of FCR were heritability of 0.07 for FCR_{Ind}, and its genetic correlations of -0.47 with body weight, -0.50 with fillet%, and +0.54 with muscle $lipid\%_{BW}$. Third, the full phenotypic data of the selected traits across year classes was updated with dummy observations for the traits, FCR_{Ind}, fillet% and muscle $lipid\%_{BW}$. Fourth, the updated full phenotypic data, the phenotypic and genetic parameters, and the full pedigree were used to estimate breeding values across the generations (MiX99 software; Lidauer et al., 2017). In this way, the genetic changes in FCR_{Ind}, fillet% and muscle $lipid\%_{BW}$ are correlated genetic changes because these traits are not directly selected. Finally, the genetic trend in FCR_{Ind} was integrated into the annual trend of farm-level FCR_{Farm}, by using the year 1994 as the first year when fish from the breeding programme were at the sea (i.e. year class 1992 fish at on-growing).

Results and discussion

The farm data showed that from the peak year of 1980 to 2016, FCR_{Farm} recorded at farms has improved by 53.4 % (Fig. 1a). Hence, to produce 1 kg of fish today, only half of the feed is needed now compared to 80's. Since 1994 when FCR_{Farm} was 1.49, FCR_{Farm} has improved by 28.7%, that is -0.427 FCR units (Fig. 1a). Since then, the genetically selected rainbow trout have been available for on-growing. The genetic trend analysis showed that FCR_{Ind} has genetically improved 1.96% per generation, and cumulatively by 12.9%, i.e. by -0.192 FCR units (Fig. 1a). This cumulative genetic improvement in FCR_{Ind} is equal to 45% of the total phenotypic improvement in FCR_{Farm} that occurred during that time. The remaining improvement in FCR is due to improvements in feeds, feeding practices, farm management, error in any estimation, and other unexplained causes.

From the peak year of 1981, nitrogen and phosphorus loading to have reduced by 76 % and 70 %, respectively (Fig. 1b). It can be assumed that the 12.9 % improved FCR due to breeding has lead to at least similar reduction in nitrogen and phosphorus loading. This is a feasible scenario when focusing only on the improvement in FCR. The amount of N and P loading may be also impacted by changes in body composition and retention efficiencies of N and P but further more detailed analysis would be required to quantify these impacts.

To conclude, these improvements in resource efficiency are a win-win for both industry and environment - the same amount of sea food can be produced with significantly reduced amounts of raw materials and reduced environmental impact.

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References

Kause, A., A. Kiessling, S. A. M., Martin, D. Houlihan, and K. Ruohonen. 2016. *Br. J. Nutr.* 116:1656-1665.
Kause, A., T. Paananen, O. Ritola, and H. Koskinen. 2007. *J. Anim. Sci.* 85:3218-3227.
Kause, A., O. Ritola, T. Paananen, H. Wahlroos, and E. A. Mäntysaari. 2005.*Aquaculture* 247:177-187.
Knap, P. W., and A. Kause. 2018. *Front. Genet.* 9:184.
Lidauer, M., K. Matilainen, E. Mäntysaari, T. Pitkänen, M. Taskinen, and I Strandén. 2017. MiX99 : Technical reference guide for MiX99 solver. Luke.
Vehviläinen, H., A. Kause, H. Kuukka-Anttila, H. Koskinen, and T. Paananen. 2012. *Evol. Appl.* 5:732-745.

ESTABLISHMENT OF A NEW CELL LINE FROM FIN TISSUE OF *Coregonus maraena* (MARAENA WHITEFISH)

Yagmur Kaya[1*], Katrin Tönißen [1], Bianka Grunow[1]

[1] Institute of Muscle Biology and Growth, Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany
E-mail: kaya@fbn-dummerstorf.de

Introduction

As an anadromous freshwater fish of the *Salmonidae, Coregonus maraena* (maraena whitefish) is an economically important fish species. However, its population is on the verge of extinction especially in the Baltic Sea region due to extensive fishing, anthropogenic eutrophication of spawning areas and habitat fragmentation caused by human interference (Brietzke et al., 2016; Olsson et al., 2012). In the light of threatened fish species and the requirement of supporting the tenets of the 3Rs of animal research (replacement, reduction, and refinement) (Russell and Burch, 1959), *in vitro* cell lines are growing into an essential tool in fish-research (Sneddon et al., 2017). In this study, we established a fish cell line from *C. maraena* (CMA-fin1), which can be used as an *in vitro* model and help to reduce the use of animals in research.

Materials and Methods

For cell isolation, the dorsal fin of adult *C. maraena* was digested with a 0.1% trypsin/EDTA solution. Cells were cultivated at 20°C. Cell number, viability and cell size were determined via the Countess® Automated Cell Counter. Mean ± S.E.M. was calculated for all cell passages of CMA-fin1 cells.

To reveal the optimal cell growth conditions for CMA-fin1 cells, Leibovitz 15 (L-15) medium with four different FBS (fetal bovine serum) concentrations (5%, 10%, 15%, 20%) and different cell seeding numbers (0.25 x 10^4 or 0.5 x10^4 cells per well) were examined via IncuCyte® S3 Live-Cell Analysis System at 20°C. Cell proliferation of 4 replicates for each group was measured every 15 min for 5 days at passage 19 (P19). Cell proliferation was quantified by analyzing the occupied area of cell images over time (% confluence) by using the Incucyte® Cell-by-Cell Analysis Software Module. Statistical significance was calculated using two-way ANOVA followed by Tukey's post-hoc modification. P value < 0.05 was considered significant.

Results

48 hours after cell isolation, single cells, 3D layers, and tissue fragments could be observed. Within higher passages, particularly at P5, the cells started to proliferate in a monolayer, and tissue fragments were not present anymore. Cells demonstrated different shapes particularly in the first 3 passages ranging from large, round cells, polygonal cells, and cells with a spindle-shaped appearance. As the passage number increased, the cells became more uniform and homogenous. Cell size increased from 10.9 ± 0.27 µm in P2 to 13.70 ± 0.12 µm in P9 and remained stable in further passages (13.2 ± 0.32 µm in P22).

IncuCyte® S3 Live-Cell Analysis, which is a label-free, non-invasive, cellular confluence assay, indicated that the best growth condition is L-15 medium with 15% FBS as well as 10% FBS. Growth curve under these two cultivation condition were not significantly different and therefore demonstrated almost the same increasing confluences with both cell seeding densities. With regard to the 3R guidelines, 10% FBS is observed to ensure optimal growth conditions for CMA-fin1 cells.

Discussion

As a remarkably growing sector, aquaculture is increasingly in the need of easy manageable *in vitro* models to enable efficient research on fish. Cell lines represent an essential biological tool to conduct examinations for fish virology, physiology, toxicology etc. (Lakra et al., 2011; Rakers et al., 2018). Furthermore, *in vitro* models provide a cost-effective tool under controlled conditions and supporting the principles of the 3Rs by not requiring the use of animals.

Until to date, our newly established CMA-fin1 model is a stable fast-growing cell-line, which could be cultivated and cryopreserved over 22 passages so far. Therefore, this *in vitro* model can lead to an important tool for research on *C. maraena*.

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References

Brietzke, A., Borchel, A., Altmann, S., Nipkow, M., Rebl, A., Brunner, R.M., and Goldammer, T. (2016). Transcriptome sequencing of maraena whitefish (Coregonus maraena). Mar Genomics 29, 27-29.

Lakra, W.S., Swaminathan, T.R., and Joy, K.P. (2011). Development, characterization, conservation and storage of fish cell lines: a review. Fish Physiol Biochem 37, 1-20.

Olsson, J., Florin, A.-B., Mo, K., Aho, T., and Ryman, N. (2012). Genetic structure of whitefish (Coregonus maraena) in the Baltic Sea. Estuarine, Coastal and Shelf Science 97, 104-113.

Rakers, S., Ondrusch, A.K., Gruening, M., Adamek, M., Moeckel, B., and Gebert, M. (2018). Monitoring changing cellular characteristics during the development of a fin cell line from Cyprinus carpio. Comp Biochem Physiol B Biochem Mol Biol 225, 1-12.

Russell, W.M.S., and Burch, R.L. (1959). The principles of humane experimental technique (Methuen).

Sneddon, L.U., Halsey, L.G., and Bury, N.R. (2017). Considering aspects of the 3Rs principles within experimental animal biology. The Journal of Experimental Biology 220, 3007-3016.

FREE AMINO-ACIDS MIX MADE OF POULTRY KERATIN IMPROVES SURVIVAL OF WHITELEG SHRIMP POST LARVAE (*Litopenaeus vannamei*) IN CASE OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE AND WHITE SPOT SYNDROME VIRUS CHALLENGES

Kersanté Pierrick*, Le Reste Guillaume, Diringer Benoit, Quimi Juan, Sergheraert Renaud, Duperray Joël

BCF Life Sciences, Boisel, 56140 PLEUCADEUC – France
pkersante@bcf-lifesciences.com

Introduction

With their low molecular weight and high level of assimilation, mixes of free amino acids (MFAA) obtained from poultry keratin extensive hydrolysis are potential interesting candidates for aquaculture feeds dedicated to first development stages. In addition to previous investigations, underlining MFAA positive effect on shrimp zootechnical performances (Le Reste et al., 2019), these two studies were conducted to evaluate potential of MFAA as new efficient solutions to improve immune response of white shrimp, *L.vannamei*, in case of bacteriological and viral challenges.

Protocol

Two trials were conducted to evaluate the effects of MFAA on whiteleg shrimp Post Larvae (PL) *Litopenaeus vannamei*. In these two trials I and II, PL were fed four diets (control; control+1% MFAA; control+5% MFAA; control+10% MFAA) respectively for 28 days and 21 days. Following this growth phase, animals were experimentally infected with *Vibrio parahaemolyticus,* with a toxin gene-bearing plasmid responsible for acute hepatopancreatic necrosis disease (AHPND group) or White Spot Syndrome Virus (WSSV group) or mock infected (non-infected control), considering four diets treatments (control; control+1% MFAA; control+5% MFAA; control+10% MFAA) during 28 days for trials I and II. In these two trials, survival and biomass reached higher rates in WSSV infection groups, for PL fed with MFAA. For AHPND infection group in trial II, survival and biomass were also higher for PL fed with MFAA. Those results show the potential of MFAA to enhance shrimp PL performance and their application as shrimp feeding ingredients with functional benefits on animal survival in case of immune challenge.

Results for the infectious challenge period

The AHPND and WSSV infected control groups showed a significant decrease of survival and biomass while these parameters remained steady in non-infected groups. In WSSV infected groups of trials I and II and in AHPND infected group of trial II, animals fed with MFAA treatments showed significantly higher survival rates from the second week to the end of the infection phase (Graphs 1 and 2), in comparison with the animals fed with the control diet without MFAA.

Discussion

During these two experimentations, infected groups faced a strong drop of the PL population in comparison with the non-infected control groups. In these very challenging conditions, it is noteworthy that animals fed with feed supplemented with different levels of MFAA showed significantly higher survival and biomass increase in case of AHPND and WSSV infection. In general, research studies mainly focus on single amino acids and their individual effect on feed performances. To our knowledge, there was no previously available scientific work in shrimp nutrition underlining the role of MFAA in case of immune challenge. The purpose of AA supplementation is today mainly oriented for nutritional balance and present results are opening new possibilities for AA utilization in aquafeed formulations. This new field of application confirms their interest as a sustainable protein source converted into an efficient functional ingredient for shrimp nutrition.

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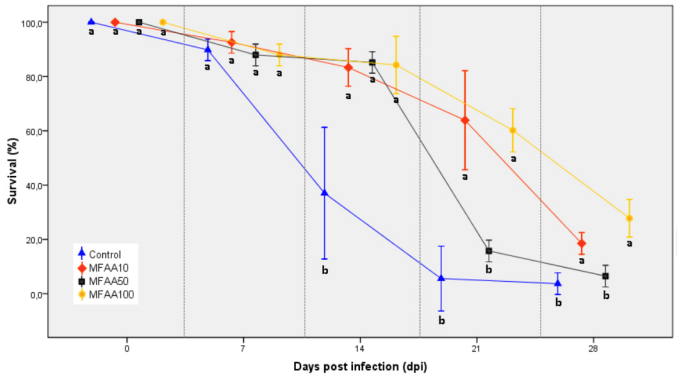
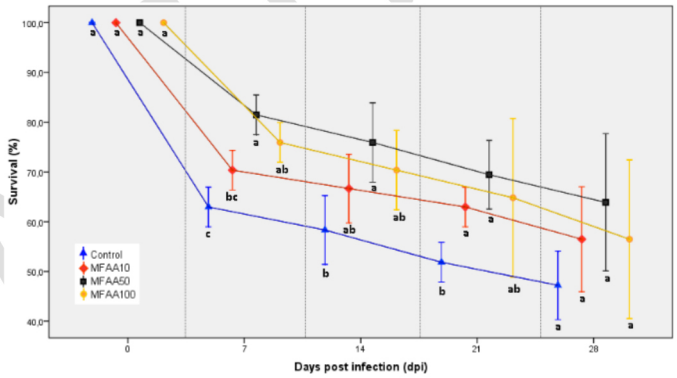
Graph 1. Evolution of Survival week by week after WSSV infection in Trial I (95% CI of averages and Duncan test by week)



Graph 2. Evolution of Survival week by week after AHPND infection in Trial II (95% CI of averages and Duncan test by week)



Bibliography

[1] Le Reste Guillaume, Kersanté Pierrick, Duperray Joël. 2019. Free Amino-Acids Mix made of Poultry Keratin as a new Functional Ingredient for White Shrimp (Litopeaneus vannamei) feed. Universal Journal of Agricultural Research 7(6): 203-209.

THE IMPACT OF UV RADIATION ON PACIFIC OYSTER *Crassostrea gigas* HEALTH AND DEVELOPMENT OF PATHOGENS *Vibrio aestuarianus* AND OSTREID HERPESVIRUS-1 (OsHV-1 µVar)

Gary F. Kett*[1], Sharon A. Lynch[1], Marcel A.K. Jansen[1], Sarah C. Culloty[1,2]

[1] School of Biological, Earth & Environmental Sciences, and Aquaculture & Fisheries Development Centre, Environmental Research Institute, University College Cork, Ireland
[2] MaREI Centre, Environmental Research Institute University College Cork, Ireland
E-mail: gary.kett@ucc.ie

Introduction
Solar ultraviolet radiation (UVR) is an important element of terrestrial and aquatic ecosystems. UVR can have both positive (e.g. immune system modulation) and negative impacts (e.g. mortality) on aquatic invertebrates. UV-B can damage DNA and other macromolecules leading to adverse effects on cells, individuals, and populations. In this study, lab and field experiments were used to assess the impact of UVR on the Pacific oyster *Crassostrea gigas* and the development of two of its common pathogens *Vibrio aestuarianus* and Osterid herpesvirus-1 (OsHV-1) and variants. *C. gigas* is a commercially important species to Irish and global aquaculture. In recent years, oyster cultivation sites around Europe have experienced increasing 'mass mortality events' occurring during summer months and often resulting in up to 80% oyster mortality. Both *V. aestuarianus* and OsHV-1 µVar have been associated with these mortality events.

Materials and methods
In initial lab trials, oyster seed (n = 880) were exposed to 0.7 kJ/m^2 of UV-B for three days and recovery was monitored for four days. Oyster mortality was quantified daily, and gill tissue samples were collected for pathogen detection using molecular methods. Field trials conducted in 2018 involved *C. gigas* seed (n = 3000) relayed at 'low' and 'high' shore points, with ~2 hrs of aerial exposure differential. Oysters were sampled for mortality and pathogen infection weekly during the summer months and environmental conditions were recorded from Met Éireann, the Irish meteorological service.

Results
Exposure to UVR was found to increase mortality in oyster seed but also to reduce both prevalence and intensity of *V. aestuarianus* infection. OsHV-1µVar was only detected in the 2018 field trial when the infection prevalence was higher in high shore oysters. These results show that pathogen partitioning exists between different tidal zones and that UV is an important factor to consider in animal health and disease transmission.

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REPURPOSING OF BRIDGE MATERIALS FOR OYSTER REEF RESTORATION

C. John Klein, Dorothy Leonard

CoastWatch Engineering LLC
c.johnklein@yahoo.com

The re-purposing of previously used construction materials is examined for the purposes of oyster reef creation in selected estuaries. The paper examines the use of materials resulting from the demolition of transportation infrastructure such as bridges and tunnels. These materials consist of riprap, gabions, gravel, steel and re-enforced concrete. The advantage of using repurposed concrete is the sequestration of CO2 within the materials eliminating the additional CO2 emissions from the manufacture of new concrete. The manufacture of cement produces about 0.9 pounds of CO2 for every pound of cement. Since cement is only a fraction of the constituents in concrete, manufacturing a cubic yard of concrete (about 3900 lbs.) is responsible for emitting about 400 lbs. of CO2. The overview also includes proprietary methods of demolition to size and process the materials to uniquely adapt them for use as reef substrate.

SUB-OPTIMAL WATER TEMPERATURE PROMOTES MUSCLE FIBRE RECRUITMENT IN NILE TILAPIA *Oreochromis niloticus*

G.D.P. Konnert*[1,2,3], F. Muhammad[1], Y. van Leur[1], S.W.S. Gussekloo[2], W.J.J. Gerrits[3], K. Masagounder[4], J. Mas-Muñoz[5], J. W. Schrama[1]

[1] Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[2] Experimental Zoology Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3] Animal Nutrition Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[4] Evonik Nutrition & Care 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

Fish post-embryonic muscle growth results not only from the enlargement of existing fibres (hypertrophy), but also from the recruitment of new muscle fibres (hyperplasia). In most farmed fish species, trunk white muscle fibres (i.e. fillets) account for a large proportion of total body mass (30 – 60%). Thus, recruitment and enlargement of trunk white muscle fibres are prime determinants of overall body growth (Rowlerson and Veggetti, 2001). Although post-embryonic muscle fibre hyperplasia declines and presumably stops after a certain body size is reached, its rate and duration may be modulated by environmental and dietary factors, among others (Johnston, 2006). Modulation of the rate of hyperplasia during early-life could offer means to enhance growth performances in later life stages, when muscle growth only results from hypertrophy. Growing European sea bass (*Dicentrarchus labrax* L.) embryos and larvae at low temperature (14°) was shown to prolong seasonal hyperplasia at later life stages, thereby improving growth (Alami-Durante et al., 2007). An experiment was conducted to evaluate whether similar effects occur when water temperature contrasts are applied to small Nile tilapia (*Oreochromis niloticus* L.), a tropical freshwater fish species, and whether temperature-induced contrasts in muscle growth affect responses to dietary digestible protein-to-energy ratio (DP:DE) at a later life stage.

Material and methods

The experiment consisted of two phases, in which all-male Nile tilapia (initial bodyweight = 7.7 g, SD = 0.20) were subsequently exposed to contrasts in water temperature and feeding level (Phase 1, 37 days) and in dietary DP:DE (Phase 2, 42 days). An incomplete factorial design was implemented in Phase 1, with two groups kept at an average water temperature of 32 °C, of which one was fed to apparent satiation (32-Sat) while the other (32-Res) was fed at a fixed feeding level (20 g/kg$^{0.8}$/d) equal to that fed to the third group (24-Res), which was kept in 24°C water. In Phase 2, fish were allocated to one of two diets differing in their DP:DE (17.2 and 26.8 g/MJ) and fed to apparent satiation in 28°C water. Fish were randomly allocated to 120 and 60 litres tanks in Phase 1 and 2 (n = 12 and 18, respectively) and connected to recirculation aquaculture systems. Nutrient apparent digestibility was determined using yttrium oxide as an inert marker in both phases. Final body composition was analysed from 10 fish per tank at the end of both phases. At the end of both phases, 10 mm^3 epaxial trunk muscle blocks were collected from 6 fish per treatment at the level of the 5th dorsal fin. These were immediately snap-frozen in liquid nitrogen and later cut into 10 μm-thick sections using a cryotome. Sections were mounted on glass slides and stained with Mayer's haematoxylin and 2% eosin. High-resolution scans were made with an upright microscope (Leica DM6b, Leica Microsystems, Wetzlar, DE) at a magnification of 200. The cross-sectional area and minimal Feret's diameter of a minimum of 650 fibres per fish were semi-automatically measured. Treatment effects on growth performance and fish morphometric data were analysed by analysis of variance (ANOVA), while muscle fibre diameter distribution frequencies were analysed via Kruskal-Wallis (KW) test and represented using nonparametric Kernel density estimates. Post-hoc pairwise comparisons were assessed on the basis of the Tukey and Dwass, Steel, Critchlow-Fligner tests following ANOVA and KW tests, respectively. All statistical analyses were made using the version 9.4 of the Statistical Analysis Software (SAS Institute Inc., Cary, NC, USA).

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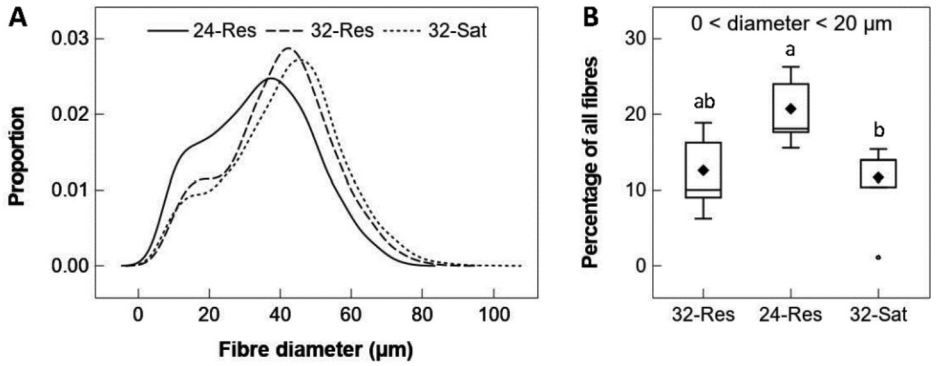

Figure 1. Distribution of epaxial white muscle fibre diameter in Nile tilapia subjected to 37 days of restricted feeding at 24 and 32°C (24-Res and 32-Res) and of feeding to apparent satiation at 32°C (32-Sat). A: curves represent kernel density estimates. B: black filled diamonds indicate treatment mean proportion of muscle fibres falling within the 0-20 μm diameter class. The means of treatment with differing letters differ at $P < 0.05$.

Preliminary results

At the end of the first phase, fish fed restrictively at 24 and 32 °C reached similar mean bodyweights (34.3 and 34.6 g, respectively), which both differed ($P < 0.0001$) from that of the fish fed to apparent satiation (46.7 g). Water temperature was found to affect the distribution of cross-sectional muscle fibre diameter (Fig. 1A). The mean proportion of muscle fibres falling in the 0-20 μm diameter class was higher in fish grown at 24 than at 32°C (Fig. 1B), thereby indicating prolonged white muscle fibre hyperplasia at 24°C. In Phase 2, a significant ($P < 0.05$) interaction was found between Phase 1 effects (Temperature-feeding level) and Phase 2 effects (dietary P:E) on the feed conversion ratio, thereby suggesting that temperature-induced changes in muscle fibre recruitment affect feed efficiency later on. Complete nutrient balances and analyses of the muscle samples collected at the end of phase 2 will provide additional read-outs to understand the mechanisms at stake.

References

ALAMI-DURANTE, H., OLIVE, N. & ROUEL, M. 2007. Early thermal history significantly affects the seasonal hyperplastic process occurring in the myotomal white muscle of Dicentrarchus labrax juveniles. *Cell and Tissue Research,* 327**,** 553-570.

JOHNSTON, I. A. 2006. Environment and plasticity of myogenesis in teleost fish. *Journal of Experimental Biology,* 209**,** 2249-2264.

ROWLERSON, A. & VEGGETTI, A. 2001. 5 - Cellular Mechanisms of Post-Embryonic Muscle Growth in Aquaculture Species. *In:* JOHNSTON, I. A. (ed.) *Fish Physiology.* Academic Press.

WEATHERLEY, A. H., GILL, H. S. & LOBO, A. F. 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *Journal of Fish Biology,* 33**,** 851-859.

FISH-AI: DEVELOPING AN ARTIFICIAL INTESTINE FOR THE SUSTAINABLE FARMING OF HEALTHY FISH

Trond M. Kortner[1*] Åshild Krogdahl[1] Kirsti Præsteng[1], Elin Valen[1], Marcelo Chacon[2], Liat Koch[2], Amir Bitan[2], Amos Tandler[2], Anna Szabó[3], Sandra Van Vlierberghe[3], Rebecca Heavyside[4], Grethe Rosenlund[4], David Peggs[4], Pedro F. Costa[5], Ricardo Ribeiro[5], Radmila Pavlovic[6], Sara Panseri[6], Nicole Verdile[7], Rolando Pasquariello[7], Tiziana A.L. Brevini[7], Fulvio Gandolfi[7]

[1]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
[2]National Center for Mariculture, Israel Oceanographic & Limnological Research, [3]Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Ghent, Belgium
[4]Skretting Animal Research Centre, Stavanger, Norway
[5] Biofabics Lda, Porto, Portugal
[6] Department of Health, Animal Science and Food Safety, University of Milan, Italy
[7] Department of Agricultural and Environmental Sciences, University of Milan, Italy.
*E-mail address: trond.kortner@nmbu.no

Introduction

The forecasted increase in fish production demands the search for new feed ingredients. Besides evaluation of nutritional properties of these, thorough investigation of possible specific positive or negative effects on gut health must be conducted to secure optimal production and fish welfare. Designing and conducting feeding trials to study nutritional and health characteristics of novel feed ingredients are challenging and demand high numbers of experimental animals. For screening purposes, in vitro tools modelling the functions of the digestive tract, may be used. In the FISH-AI project, funded by FET open H2020, our purpose is to model the complex digestive, immune and barrier functions of the rainbow trout gut by developing a novel in vitro model system; i.e. an in vitro assay for luminal digestion and a mucosal barrier platform based on new 3D-printed polymer-based scaffolds and differentiated intestinal epithelial cells. The model system will be used as an effective screening to predict the health and nutritional value of novel low-trophic fish feed resources, as well as increasing the knowledge of cellular and molecular mechanisms underlying the observed effects.

Results and discussion

1. Derivation, characterization and cultivation of rainbow trout intestinal epithelial cells.

From tissue samples and primary cell cultures derived from the rainbow trout intestine, we have studied the expression and localization of well-characterized mammalian intestinal stem cell (ISC) markers, and markers of enterocyte and goblet cell functionality. Our results so far demonstrate that mammalian ISC markers are expressed in the rainbow trout intestine but have a different function (Figure 1). Furthermore, different cell types are found in cultivated cell lines, including stem cells, differentiating and mature epithelial cells, expressing enterocyte and goblet cell markers, as well as connective cells.

2. Scaffold development

Our purpose is to develop a scaffold material which can mimic the extracellular matrix of the rainbow trout intestine to the greatest extent possible. Hydrogels are promising candidates to build biopolymer scaffolds of. Currently, functional features of the hydrogels such as strength, permeability to nutrients, and interaction with the intestinal cell lines are being explored. In parallel, processing of the most suitable hydrogels is investigated in order to develop a 3D intestinal structure, which can function as base for a life-like, in vitro intestine model. The most promising combinations of the scaffolds and the intestinal cell lines will be further explored in a closed cell cultured chamber produced by 3D printing (Figure 2).

3. In vitro digestion of complete fish feeds

Our aim is to develop an in vitro digestion procedure for fish feed, which produces a profile of digested nutrients that resembles the nutrient profile found in the intestine of fed rainbow trout. We have produced enzyme extracts from the stomach and pyloric caeca of rainbow trout, and used these enzyme extracts to in vitro digest fish feed under different test conditions. The in vitro produced digesta are currently being characterized by global and targeted metabolome analyses, and compared against gut content collected from the corresponding fish (Figure 3).

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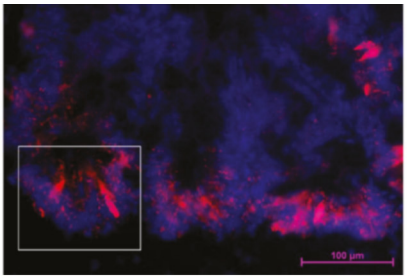

Figure 1. Rainbow trout columnar epithelial cells showing an intense *sox9* expression at the fold base in the second segment of the mid-intestine (Verdile N et al. 2020. https://doi.org/10.3390/ijms21239192.)

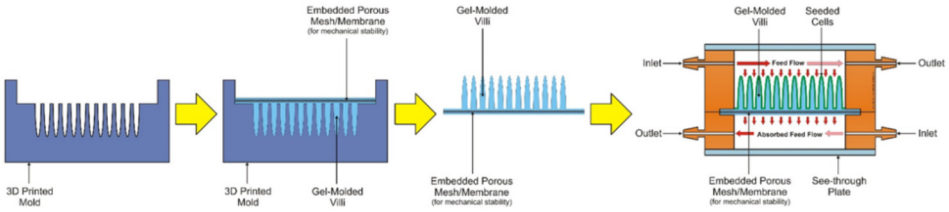

Figure 2. Fish-AI prototype design. The successful combination of biopolymer scaffolds and seeded intestinal cells will be employed in a closed perfusion chamber.

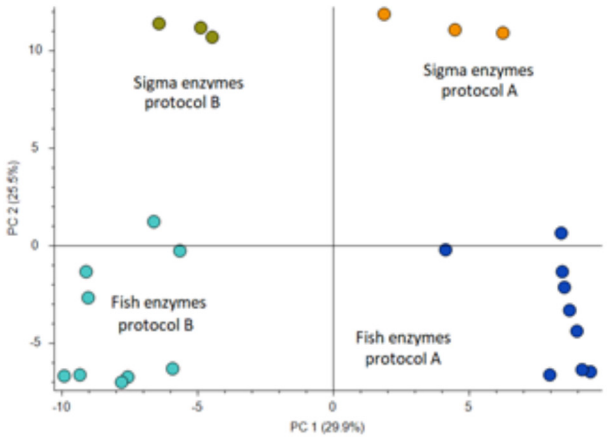

Figure 3. PCA analysis of amino acids, their metabolites and small peptides extracted from *in vitro* digested fish feed under different test conditions.

Acknowledgements
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TRACE ELEMENT SOURCE EFFECTS AND SUPPLEMENTATION LEVEL OPTIMISATION IN DIETS FOR ALTANTIC SALMON

K. Kousoulaki[1*], L. Sveen[2], M. Kokkali[1], A. Krasnov[2] and J. Sweetman[3]

[1]Nofima - Norwegian Institute of Food, Fisheries and Aquaculture Research, Department of Nutrition and Feed Technology, Kjerreidviken 16, N-5141 Fyllingsdalen, Norway
[2]Nofima - Norwegian Institute of Food, Fisheries and Aquaculture Research, Department of Fish Health, Osloveien 1, NO-1433 Ås, Norway
[3]Alltech Inc, Summerhill Rd, Sarney, Dunboyne, Co Meath, Ireland.
E-mail: katerina.kousoulaki@nofima.no

Introduction
There is significant variation in the dietary levels of essential nutrients in the Norwegian salmon feed market: Fe (108-340 ppm), Zn (126-232 ppm), Cu (7-14 ppm), Mn (21-70 ppm), and Se (0.3-1.8 ppm) (Sele et al., 2018). Different nutrient sources, being in inorganic or different organic forms have different bioavailability (Maage and Sveier, 1998; Standal et al., 1999) and physiological effects (Berntssen et al., 2018), whereas research has shown positive effects on fish performance supplementing essential trace elements beyond the known requirements (Taylor et al., 2019). The scope of this study was to investigate physiological effects of graded dietary supplementation levels of the essential trace metals Mn, Cu, Fe, Zn and Se in either organic (OM) or inorganic (IM) form in Atlantic salmon smolt.

Materials and Methods
A 2x4 design with 2 types of minerals at 4 dietary supplementation levels (IM/OM1-IM/OM4 ranging at equal intervals per metal between 10-24 ppm for Cu, 55-90 ppm for Mn, 80-180 ppm for Zn, 300-450 for Fe and 0.7-1.2 for Se) with 3 replications at each experimental point was applied. Raw materials, the master raw material mix used to produce the 8 experimental diets and the final extruded diets were analyzed for the 5 variable trace metals before the start of the feeding trial. Fish were fed without disturbance for 6 weeks, then subjected to 3 weekly handling stress treatments and finally fed 4 weeks again without additional treatments while water oxygen level was allowed to decline gradually as tank biomass increased. At trial start, end and during the stress treatments, blood samples were taken. At trial end, growth, feeding rates, FCR, biometrics, fillet and skin technical quality, tissue trace element levels, skin histology, macronutrient and trace element ADC, and skin transcriptomics were evaluated.

Results and Discussion
Briefly, selected results include significantly higher feed intake (p=0.012) and growth (p=0.041) after 6 weeks and the first stress treatment (p=0.020), and nearly significantly higher growth (p=0.054 for TGC) and feed intake (p=0.080) until the end of the trial in the OM as compared to the IM treatments. Overall, mineral levels did not influence growth significantly. No significant treatment differences were seen in FCR.

At trial end, OM fish had significantly higher gutted weight (p=0.043), length (p=0.033) and harvest yield (p=0.000), significantly lower intestinal fat (p=0.006), and tendency for higher fillet yield (p=0.084), as compared to the IM fish groups, with IM4 having significantly lowest fillet yield. Kousoulaki et al. (2016) saw that OM improved fatty acid ADC inducing 13% salmon fillet DHA increase. Our current results indicate that OM may be more efficient than IM in bioconverting ALA into EPA and DHA as there was significantly more EPA and a tendency for more DHA in of OM as compared to IM whole fish. Fe (Stangl and Kirchgeßner, 1998) and Zn (Eder and Kirchgessner, 1994) are among the metals that are important for this function in mammals. OM3 and OM4 tended to have higher gutted weight and bone strength with no such correlation in IM. No effect was seen on fin and liver score, cataract, CF, skin strength or pigmentation, or fillet pH.

Tendency for higher blood glucose in OM after the first handling stress event ($p = 0.102$), significantly higher blood protein ($p = 0.015$) and cholesterol ($p = 0.024$) after the second handling stress event, significantly higher blood cortisol ($p = 0.026$) after the third handling stress event, and a tendency for lower blood K ($p = 0.086$) and higher CK ($p = 0.056$) after the third handling stress event was seen as compared to IM. At trial end only blood glucose was significantly higher ($p = 0.006$) in OM as compared to the IM groups.

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As in Kousoulaki et al. (2016), we observed only small effects on the test metal tissue levels. Whole fish Se tended to be higher in OM ($p = 0.065$). Within IM and OM significant increase in skin Zn up to IM3 and OM2 was seen. Whole body Zn increased up to IM2 with no correlation within OM. Only level affected dietary nutrient ADC but not type of mineral. Cu, Zn Mn, Fe and P ADC was highest in IM1 and decreasing at higher IM levels, whereas the opposite effect was seen for Se. In OM ADC of Cu, Zn and Se decreased beyond OM3 but no negative effect of increasing dietary mineral level was seen in ADC of P, Mn, or Fe. Liver Se and Fe increased beyond IM1, but no such effect was seen in OM as also seen in Prabhu et al. 2020.

Few weak differences were observed in skin microanatomy between the groups. Lower number of mucous cells in the OM ($p = 0.036$), with cells positioned closer to the apical surface in the OM ($p = 0.014$). Mucous cell area ($e = 0.364$) and number ($e = 0.342$) was positively correlated to the mineral level but only within the OM group.

Conclusion

Overall, growth, biometric and blood parameters show improved Atlantic salmon performance at both undisturbed and stressful conditions when fed OM against IM mineral mix in the diet with only small effects on FCR, tissue mineral levels and nutrient ADC.

AQUAVIP EXPERIMENTS FOR THE FUTURE OF THE AQUACULTURE IN THE POMERANIA REGION, POLAND

H. Łądkowska*, B. Dmochowska, H. Kendzierska, M. Klin, U. Janas, K. Ocalewicz, F. Pniewski, A. Zgrundo

University of Gdańsk, Bażyńskiego 8, Gdańsk, 80-309, (Poland)
Email: hanna.ladkowska@ug.edu.pl

Introduction

Since aquaculture represents a growing contributor to the production of aquatic food worldwide, there is an increasing need for qualified personnel and knowledge in modern sustainable aquaculture. At the University of Gdańsk, a dynamically developing institution of higher education, on the basis of AquaVIP (Aquaculture Virtual Career Development Platform for the South Baltic Region, Interreg South Baltic Programme) experiments on innovative technology and promising species have been developed to serve further research, education and training. The experiments are focused on *Litopenaeus vannamei* (whiteleg shrimp), its cultivation in the small-scale laboratory recirculating aquaculture system (RAS 500), macro- and microalgae selection and cultivation in conjunction with shrimps, and cultivation of native and non-native invertebrates from the Baltic Sea as an alternative food source for humans, or as feed in the fish farms.

Materials and methods

Research carried out on *L. vannamei* in RAS 500 system at the University of Gdansk, as the first whiteleg shrimp RAS in Poland, serves as a baseline for the experiments including farming of *L. vannamei* using algae scrubber on a trickling filter and green algae as biofilters. The experiment design foresees: system preparation, transport and acclimation, cultivation, harvesting, and analysis. In case of *L. vannamei* growth and nutritional value will be examined. Algae scrubber on a trickling filter and macroalgae will be tested for their effectiveness in water treatment in the RAS system. Their biomass growth and the potential application will be analysed with the purpose to determine the possibilities of its commercial use for production of valuable biomolecules, as a fertilizer or feed additive.

L. vannamei culturing water will also be used for the aquaponic experiment including microalgae, which focuses on the selection of local strains that will grow efficiently on this medium. Special focus will be put on salinity and a nitrogen source influence. Biochemical characterization of biomass will be performed with the purpose to determine the possibilities of commercial use for wastewater treatment, production of pigments, lipids and their use as an additive to plant biomass for biogas production, or protein-rich biomass to be used as a feed additive. An assessment of algae growth in bioreactors and preparation of inoculum for cultivation on a semi-technical scale is planned in the further stage of the experiment, as well as the reassessment of growth rate and biochemical composition to determine the stability of biomass characteristics when changing the way algae are grown.

Another studies focus on native and non-native invertebrates living in the Baltic Sea as a food source for humans or in the future fish farms, serving as a good source of long-chain poly-unsaturated fatty acids (LC-PUFAs). The experiment focuses on the estimation of nutritional value of benthic invertebrates living in the Baltic Sea e.g. prawns: *Palaemon elegans, Palaemon adspersus,* beachflea *Platorchestia platensis* and bivalve *Rangia cuneata*. Organic matter, carbon, nitrogen, lipids and PUFAs analysis will be performed.

Results

The experiments and their results will serve AquaVIP service AquaYouth – Aquaculture Youth career development: training and education, which includes AquaVIP Gdynia summer school. The event will serve as an arena for experiments demonstration among participating students. The summer school is aimed to introduce participants to background theoretical skills in modern aquaculture biotechnology: main types, biological and technological processes and development trends. Moreover educational video materials on the experimental work will serve both students and professionals willing to improve their skills. Results received from the demonstrations will serve stakeholders as part of the e-learning materials included in the virtual platform. We hope that these actions will in turn spill over into creating conditions for changes in the labor market.

THE IMPORTANCE OF THERMAL CYCLING FOR PROPER GAMETOGENESIS AND SUCCESSFUL EGG AND SPERM PRODUCTION IN MEAGRE (*Argyrosomus regius*)

S. Lancerotto, P.H. Mello, P. Tsoukali, L. Calloni, S. Köpper, R. Sarracino, I. Fakriadis 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

Meagre (*Argyrosomus regius*) is a species with great potential for the diversification of Mediterranean aquaculture, but reproduction in captivity still remains a problem (Duncan *et al*., 2013). Due to biosecurity reasons (lack of pathogens), marine hatcheries use borehole seawater for their broodstock of relatively constant temperature during the year (18-20°C). Therefore, it would be cost-effective if fish could undergo reproductive maturation without the need to heat or cool the water during the year. It has been shown in gilthead seabream (*Sparus aurata*) that constant annual temperatures (18-20°C) had no negative effects on spawning kinetics and egg quality (Karamanlidis et al., 2017). Here, we manipulated the temperature when gametogenesis and maturation take place, and using established hormonal therapies to induce spawning, evaluated the effect on the reproductive performance of the broodstock.

Materials and Methods

Two broodstocks were maintained, under an attenuated seasonal temperature regime (SeasT, 16 to 20°C) or a constant temperature (CoT, 19.4±0.6°C), using borehole water. During the spawning season (May 2019), eight couples were placed in separate 5-m^3 tanks, and using gonadotropin-releasing hormone agonist (GnRHa), induced to spawn. Females were treated once a week (n=4) with a GnRHa injection (50 µg kg^{-1}) and males with GnRHa implants every 15 days (50 µg kg^{-1}). Throughout the study, oocyte development and sperm quality, as well as spawning success, were monitored. Spermiation was evaluated using a subjective index as follows: 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 amount of sperm release with very little pressure. Statistical analyses were done using *t-test* or Friedman's test (both at $P<0.05$).

Results

Females exposed to a constant temperature showed a smaller mean oocyte diameter ($P=0.02$) (Fig. 1A), and males did not release any sperm before GnRHa treatment (Fig. 1B). Subsequent GnRHa treatments slightly improved the reproductive outputs of the CoT group. Males partially started expressing sperm, though with a spermiation index lower than their SeasT counterparts ($P=0.002$) (Fig. 1B). Females spawned fewer times, with a mean total relative fecundity three times lower than SeasT females ($P=0.02$) (Fig. 1C), and embryo survival 24h after spawning dropped to 35 % ($P=0.02$) (Fig. 1D).

Discussion

Constant temperature significantly compromised, but did not prevent gametogenesis in both sexes. Females had initially a lower mean oocyte diameter, being less than what is

considered optimal for spawning induction in this species (Duncan et al., 2013, Mylonas et al., 2016). Males did not express any sperm upon gentle abdominal pressure at the beginning of the study. Fish from the SeasT group exhibited better reproductive parameters than the CoT group before any GnRHa administration. In response to GnRHa implants, CoT males produced progressively more sperm, even though the spermiation index remained low. Besides, SeasT females spawned well after hormonal treatments and exhibited a higher total relative fecundity than the CoT group. Although, the repeated hormonal therapy during the spawning season partially reduced the negative effects of constant temperature regime during gametogenesis and enabled CoT breeders to spawn, and to produce fertilized eggs both relative fecundity and embryo survival were reduced. Therefore, a seasonal thermal regime -even an attenuated one- was necessary for the proper development of the gametes, allowing for the successful spawning induction using the established GnRHa induction protocol.

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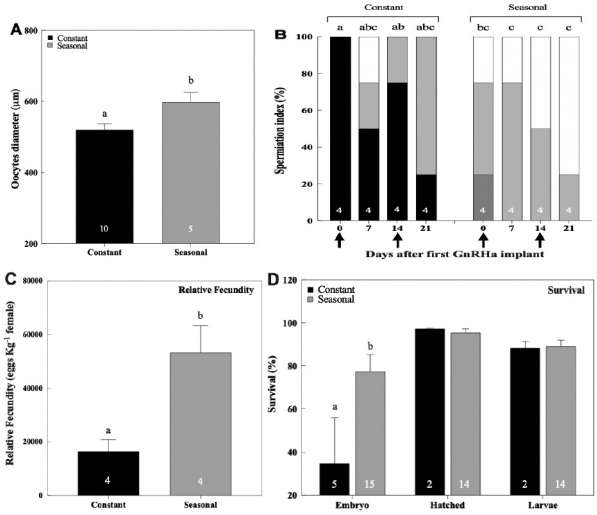


Fig 1. Reproductive parameters in meagre (*Argyrosomus regius*) in constant T and seasonal T groups. A) Mean (±SEM) oocyte diameter before hormonal treatment. The numbers inside the bars indicate the number of females evaluated making the mean. B) Spermiation index (% of fish at each category). C) Mean (±SEM) weekly relative fecundity. The numbers inside the bars indicate the number of weeks considered per mean. D) Mean (±SEM) percentage of survival of embryos and larvae. The numbers inside the bars indicate the number of spawns per mean.

References

Duncan, N.J, Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, F.J., Schuchardt, D., Vallés, R. 2013. Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: Allan G, Burnell G (eds) Advances in Aquaculture Hatchery Technology. Woodhead Publishing Limited, Cambridge, UK, p. 519-541.

Mylonas, C.C., Salone, S., Biglino, T., Mello, P.H., Fakriadis, I., Sigelaki, I., Duncan, N. 2016. Enhancement of oogenesis/spermatogenesis in meagre *Argyrosomus regius* using a combination of temperature control and GnRHa treatments. Aquaculture 464: 323-330.

Karamanlidis, D., Sigelaki, I., Papadaki, M., Fakriadis, I., Mylonas, C.C., 2017. Evolution of sex ratio and egg production in a population of gilthead seabream (*Sparus aurata*) over the course of five reproductive seasons, Aquaculture Europe 2017. European Aquaculture Society, Aquaculture Europe 2017, Dubrovnik, Croatia.

GUIDELINES AND RECOMMENDATIONS TO ENHANCE WATER BIOSECURITYAND PROMOTE FISH PRODUCTION USING ULTRAVIOLET DISINFECTION

Aran Lavi*, Ytzhak Rozenberg, Yariv Abramovich

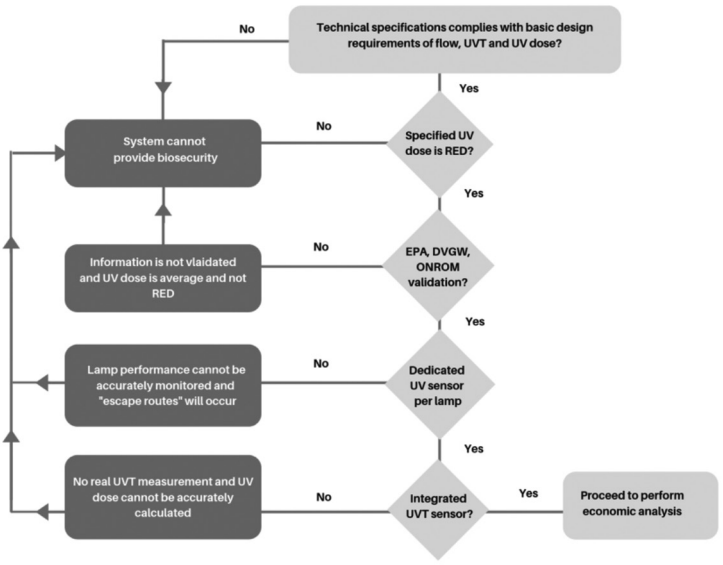
Atlantium Technologies
11 Hamelacha Street
Bet Shemesh, Israel 9910001
aranl@atlantium.com

Aquaculture has been identified and welcomed as an important "remedy" to successfully address the challenges to global food security arising from climate change and intensified by the projected 2050 world population of 9.5 billion (National Research Council, 2015). However, marine and land-based aquaculture facilities are being subjected to an increase of biosecurity threats resulting from intensified production and cross pollution on the quality of their influent water. Ultraviolet (UV) disinfection is probably the safest, most effective for treatment of pathogens such as bacteria, microorganisms, and viruses.

At present, there are more than 20 different kinds of commercially available UV technologies offered into the aquaculture market, varying in performance and price. UV systems are very sensitive to water conditions, depend on proper operation of all lamps, and require routine but dedicated maintenance as control and monitoring of UV systems is difficult at best and often times, there can be no real-time indication as to the effectiveness of the UV treatment. Despite its popularity, little information is available to producers about how to qualify UV systems according to site-specific needs. Navigating the selection can be overwhelming and a systematic approach is needed. A UV system performance and cost evaluation process diagram (TABLE 1) has been developed to assist aquaculture facilities in the selection of the most appropriate UV technology to meet their application specific needs. In addition, the convoluted UV terminology needs to be simplified and clearly designated. With these tools facilities can more appropriately select a UV technology based on a defined set of parameters and requirements which will increase the chances of the UV system to provide the site with the required water biosecurity.

Following this systematic approach will aid in prevention, control, eradication of risks to life and health, and a reduction in the economic impact of diseases.

TABLE 1: UV System Performance and Cost Evaluation Process Diagram



MOLECULAR EFFECTS ON SPERMATOZOA OF *Mytilus galloprovincialis* EXPOSED TO MERCURY CHLORIDE

Gennaro Lettieri*; Rosaria Notariale; Alessia Ambrosino; Alfredo Di Bonito, Caterina Manna, Marina Piscopo

Affiliation: University of Naples Federico II, Department of Biology
Email: gennarole@outlook.com

Introduction
Significant amounts of pollutants (toxic metals, plastics, persistent organics, etc.) are released into the marine ecosystem every year [1]. Bivalves, for their benthic and sedentary lifestyles, are easily exposed to environmental pollution and bioaccumulate these toxic substances [2]. For this reason, in the field of environmental toxicology, these species are usually used as bioindicators [3]. Mercury (Hg) is one of the most toxic non-essential metals [4]. Mercury contamination in seawater is a concern for the environment and human health. In seawater, the concentration of mercury is very low, about 1 pM [5], but it is still sufficient for bio-accumulation in marine organisms and may pose ecological health risks and as part of the human diet [6]. At the best of our knowledge, literature lacks informations regarding the effects of mercury concentrations, similar to those present in the waters of the Mediterranean basin and the North Atlantic oceans, on *Mytilus galloprovincialis* protamine-like proteins (PL proteins) and DNA. To this aim, we exposed *M. galloprovincialis* for 24 h to three picomolar doses (1, 10 and 100 pM) of $HgCl_2$. After exposures we measured the accumulation of mercury in the gonads of exposed mussel. Moreover, we analysed the electrophoretic pattern of PL proteins and the expression of the stress genes *mt10* and *hsp70* in spermatozoa. Further, we evaluated the DNA binding ability of PL proteins and their capacity to protect DNA from the action of free radicals.

Materials and methods
Mussels were immersed in artificial seawater in laboratories tanks, contaminated with $HgCl_2$. A tank, without $HgCl_2$, was also prepared and used as control.

Thirteen mussels in six litres of artificial seawater (ASW), were maintained at 18°C for 24 h. In mussels unexposed and exposed to $HgCl_2$, the accumulation of mercury, in the gonads, was evaluated by ICP-MS mass spectrometry with the digestion procedure UNI EN 13805:2014. Spermatozoa were collected using a Pasteur pipette and after sex identification by light microscopic evaluation, PL proteins, from unexposed and exposed mussels, were extracted under acidic conditions. The electrophoretic protein profile of PL proteins was then assessed on urea acetic acid polyacrylamide gels and by SDS-PAGE. DNA binding affinity of PL proteins from unexposed and exposed mussels, was then assessed by electrophoretic mobility shift assays (EMSA). The DNA protection capacity of the PL proteins from unexposed and exposed mussels was evaluated adding these proteins to a mixture of appropriate concentrations of a plasmid DNA (150 ng), $CuCl_2$ (5μM) and H_2O_2 (10μM) such as to produce, after 30' at 37°C the formation of 50% plasmid DNA in the relaxed form. Finally, the expression levels of two stress genes, *mt10* and *hsp70*, in spermatozoa of unexposed and exposed mussels were measured.

Results
Accumulation analysis carried out by ICP-MS on the gonads of exposed mussels showed an increase for all three conditions of exposure compared unexposed ones, particularly at 10 pM $HgCl_2$ in which the value was 2.5 times higher than that of unexposed mussels.

The electrophoretic analysis of PL proteins by acetic acid urea, did not show significant differences after the three conditions of $HgCl_2$ exposure. Differently, the SDS-PAGE analysis showed the presence of protein bands with low electrophoretic mobility, in particular, for 1 pM and 100 pM $HgCl_2$ conditions, indicative of probable protein aggregates. Given the differences found in SDS-PAGE analysis, the DNA-binding capacity of these proteins extracted from exposed mussels was evaluated. For these PL proteins, in fact, the DNA saturation, i.e the condition in which all plasmid DNA is close to the well, was not observed even at PL/DNA ratio 2 whereas with PLs from unexposed mussels, DNA saturation is generally obtained at PL/DNA ratio 1. The DNA protection assay, also showed a difference results for PL proteins extracted from unexposed and exposed mussels. The addition of the PL proteins from exposed mussels to the mixture of DNA, $CuCl_2$ and H_2O_2 did not prevent oxidative DNA damage. In fact, the intensity of the relaxed DNA band remained the same compared to that of the control condition in which PL had not been added. In contrast, the adding to this mixture of PL of unexposed mussels produced a decrease of plasmid DNA damage already at protein/DNA ratio of 0.4 while at 0.6 and 0.8 protein/DNA ratios, DNA damage was not observed, indicative that these latter PL proteins, differently from those extracted from exposed mussels were able to generate complexes capable of protecting DNA.

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Finally, the analysis of gene expression, on two stress genes, shown that *mt10* was about three fold over the control after 10 and 100 pM $HgCl_2$ exposure. After 100 pM $HgCl_2$ exposure, *hsp70* was hyper-expressed at the same time, whereas after 10 and 1 pM $HgCl_2$ exposure, this gene was hypo-expressed, especially at 1 pM $HgCl_2$. In this latter condition, the hypo-expression of this gene was roughly 1.5 times relative to the control condition.

Discussion

In this work we provide additional information that offers new insights into the mechanisms of mercury toxicity on the reproductive system of *M. galloprovincialis*. In particular we demonstrate that *M. galloprovincialis* exposure for 24 h to picomolar doses of $HgCl_2$ produces transcriptional responses in spermatozoa and alterations in PL proteins properties. 100 pM $HgCl_2$ exposure, differently from 1-10 pM, up-regulated *hsp70* transcription, probably to enhance the resistance of mercury, as high levels of HSP protect against the harmful effect of metals on protein integrity [7,8]in particular heavy metals, this system may be expected to play a major role in the course of local, microevolutionary events leading to the acquisition of toxicant resistance. Seven clones of Daphnia magna from different geographical regions were characterized regarding their sensitivity to Cd, their hsp70 expression, and Cd accumulation. In an acute immobilisation assay, the tested clones showed remarkable differences in their sensitivity to Cd. The highest EC(50. Moreover, the alterations observed in PL proteins properties, leading to a change in their ability to bind and condense DNA, could negatively affect the reproductive health of this organism.

References

1. Zebral, et al., doi:10.3390/ijms20123092.
2. Piscopo, M. et al., doi:10.1007/s12210-016-0564-0.
3. Piscopo, M. et al., doi:10.1002/mrd.23240.
4. Piscopo, M.; et al., doi:10.3390/molecules25143278.
5. Cinnirella, S.; et al., doi:10.1038/s41597-019-0219-y.
6. Mergler, D.; et al., doi:10.1579/0044-7447(2007)36[3:meahei]2.0.co;2.
7. Haap, T.; et al., doi:10.1016/j.aquatox.2009.06.006.
8. Singer, C.; et al., doi:10.1016/j.aquatox.2005.07.004.

THE POTENTIAL OF COMBINING MARINE RENEWABLE ENERGY AND OFFSHORE AQUACULTURE

Matt Lewis [1] , Rory O'Hara Murray [2], Simon Neill [1] Peter Robins [1] Jonathan Demmer [1] and Alice Goward-Brown[1]

[1] School of Ocean Sciences, Bangor University, UK
m.j.lewis@bangor.ac.uk
[2] Marine Scotland Science, The Scottish Government, UK
R.Murray@MARLAB.AC.UK

The potential for offshore aquaculture is immense, yet uncertainty of resilience to physical conditions makes identifying potential locations difficult (e.g. sites of waves < 4 m and currents < 1 m/s). Offshore aquaculture co-location with Marine Renewable Energy (MRE) devices has often been suggested as a way to reduce cost to both industries, and, we hypothesise, could also improve resilience for offshore aquaculture to greatly increase the number of suitable sites. Using data from two ocean models (1km resolution ROMS tidal model and 2018 ERA5 wave data), we mapped the oceanographic conditions of offshore aquaculture and Marine Renewable Energy (MRE) to understand (1) if the two industries could be co-located and (2) to resolve interactions between the co-located industries to better quantify industry potential. Uncertainty to co-location potential was also explored, for example aquaculture broadly needs regions of <1m/s currents and <4m wave height do not appear possible if annual maximum (as oppose to annual mean) values are used. Furthermore, lower rated power wave and hydrokinetic energy designs appear needed for the low energy ocean conditions and modest power requirements of aquaculture (~1-10 kiloWatts); for example, MegaWatt-scale tidal-stream energy-convertor technologies typically require current speeds above 2 m/s to produce economically viable power. We hypothesise the configuration of infrastructure could enhance the local-scale hydrokinetic energy resource whilst also reducing the local wave climate; making co-location possible (e.g. the bio-optimisation of a site with the use of macro-algae farms to accelerate tidal currents). The drag force of kelp was parameterised into an idealised channel domain and the increase in tidal currents was explored using (1) a steady-state conservation of mass model and (2) a 3D, dynamically coupled wave-tide model (COAWST). Predictions of enhanced flow speeds and increased tidal energy resource were significant, with large increases (>100%) in the resource found for lower flow conditions. Moreover, we find MRE devices can significantly reduce physical conditions, such as wave energy devices reducing local wave height by more than 25%. Including the potential hydrodynamic interactions of MRE and aquaculture sites, potential locations in the North-western European Shelf Seas increased by ~97% (with ~22% and 16% utilising wave and hydrokinetic energy technologies already available). Our research therefore shows marine renewable energy and offshore aquaculture can be co-located, especially when future changes to design and biophysical interactions between these two "Blue Economy industries" are considered; however, improved biophysical ocean modelling is now needed to reduce uncertainty in aquaculture resilience.

DYNAMIC CHANGES OF THE PROTEOME IN THREE PERCIFORM FISH DURING EARLY DEVELOPMENTAL STAGES

L. Li*[1], L. Anjos[1], D.M. Power[1, 2*]

[1]Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[2]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China
Email: dpower@ualg.pt

Introduction

Proteomes unlike transcriptomes can give direct insight into changes in structure and function during development as shown by a recent study of zebrafish embryos (Purushothaman, Das et al. 2019). For this reason, studies of the global proteome during early development of fish species may contribute to identify the most appropriate aquaculture management practices linked to species specific characteristics. One challenge when working with proteins is their instability and this is a particularly acute problem when sampling in an industrial context. In the present study comparative quantitative proteomics was used to identify conserved global processes and species-specific processes during embryogenesis of three aquaculture species, the white sea bream (*Diplodus sargus*), the meagre (*Argyrosomus regius*) and the Gilthead sea bream (*Sparus aurata*). To assess if proprietary solutions for conservation (e.g. RNAlater©) can be used as an alternative to rapid freezing (-80ºC) a comparative study of the proteome of Gilthead sea bream eggs conserved with the two methods was made.

Materials and methods

Eggs from broodstock of the white sea bream, the meagre (*Argyrosomus regius*) and the Gilthead sea bream maintained in captivity (IPMA, Olhão, Portugal) were collected. Pools of eggs (n = 6 per timepoint) were sampled 24h before hatch and at hatch and then snap frozen at -80ºC. For comparison of the effect of the conservation method on the proteome, eggs from the same spawn of Gilthead sea bream were simultaneously collected into RNAlater© or snap frozen (-80ºC until extraction). Eggs were analyzed by SDS-PAGE and SWATH-MS (Sequential Window data independent Acquisition of the Total High-resolution-Mass Spectra) and the constituent proteins and their relative quantity was determined. SPSS v23 (IBM) was used to detect statistically significant differences between the egg proteomes generated.

Results

The molecular weight range of extracted egg proteins from white sea bream, Gilthead sea bream and meagre was quite similar except for the samples conserved in RNAlater©. The SWATH-MS analysis yielded a library of 2376 identified proteins when all the samples were considered as a single group. For each of the three species analyzed over 900 quantified proteins were identified in the frozen eggs (Table 1) and this was also the case for the Gilthead sea bream eggs conserved in RNAlater©.

Discussion and conclusion

The comparison of the egg proteome in the three Mediterranean fish species revealed a high level of conservation of the proteins identified in eggs. High and very similar protein dynamic changes were found between eggs 24h before hatch and at hatch for the three species analyzed, although species specific patterns of change were also identified. The results indicate RNAlater© is a suitable method for sample preservation for proteomics in situations where there are limited facilities for storage at -80ºC. The results of our study provide the foundation for understanding key conserved processes in fish early development.

Table 1. An overview of the total number of identified and quantified proteins and the difference in their expression in eggs 24 h before hatch (BH) and at hatch (H) for each of the species analyzed.

	Samples frozen in liquid nitrogen (24h BH versus H)			RNA later (24h BH)
	White sea bream	Meagre	Gilthead sea bream	Gilthead sea bream
Identified proteins	2376	2376	2376	1096
Quantified proteins	1149	922	960	961

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Acknowledgments:
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Reference

Purushothaman, K., P. P. Das, C. Presslauer, T. K. Lim, S. D. Johansen, Q. Lin and I. Babiak (2019). "Proteomics Analysis of Early Developmental Stages of Zebrafish Embryos." Int J Mol Sci 20(24).

AQUAPONICS IN FINLAND – CURRENT STATE AND THE FINNISH AQUAPONICS SOCIETY (FAPS)

Tobias Lipsewers

Finnish Aquaponics Society (FAPS), Sunilanpolku 10 A 4, 00720 Helsinki, Finland
Email: tobias.lipsewers@gmail.com

Introduction

The current state of Aquaponics (AP)-related activities in Finland and networking results (so far) are presented. Furthermore, a new organization - the Finnish Aquaponics Society (FAPS) - will be introduced to the European fish-farming community. The motivation of FAPS is to improve and coordinate the education, research, and communication on this topic in Finland. The main aims are to raise the public awareness about the advantages of aquaponic food production and develop ideas and innovative solutions to overcome obstacles, especially minding the challenges for this alternative food production method in the Northern hemisphere. The FAPS is a non-profit organization including like-minded researchers and entrepreneurs with a common goal, the start of a research and production facility in Finland, investigating the potential and optimization of AP and related systems.

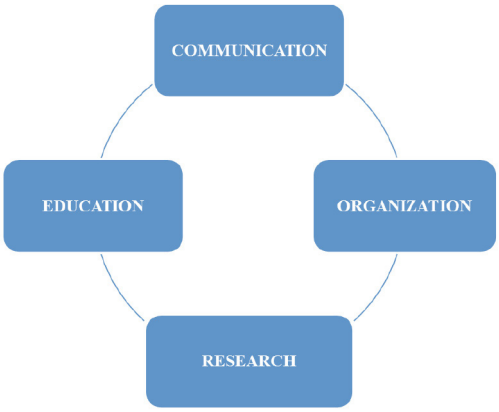
Material & Methods

Literature research and internet search with common search engines was carried out. As published scientific articles on AP in Finland are scarce, the presented facts are partially based on an unpublished case study (Ceder 2020, Master's thesis), networking (personal and Email communication), and other web pages (for example, website of the European Union [EU]).

Results

To the best of the author's knowledge, AP-related activities were very limited in Finland (up to date: February 2021) and can be summarized as follows:
- One company is selling different AP-produced lettuces and herbs (Kalaatti, Saarijärvi)
- One company is manufacturing decorative AP systems for recreation and education in a school, care homes, and shopping malls (SmartGrow, Helsinki)
- One researcher is using modeling tools to study AP (Natural Resources Institute Finland [LUKE], Jyväskylä)
- One research group is investigating AP using a small-scale system (University of Jyväskylä)

At the moment no alternative sources of animal protein (fish and other seafood) are produced in the country using this technique.



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Discussion

The major obstacles for AP are the energy costs, availability of renewable energy, and willingness of Finnish consumers to pay a higher price for higher quality products (Ceder 2020) and these factors are valid elswhere (Palm et al. 2018). In addition, the extensive literature research and interviews by Ceder (2020) did not reveal other entrepreneurs working with this technique at the moment. There were some attempts in the past but several projects were stopped due to different reasons. Firstly, the public awareness on AP products is rather low. Secondly, the fish products on Finnish markets are dominated by cheap Atlantic salmon (*Salmo salar*) originating from sea-based aquaculture systems in Norway. Thus, four key aspects need to be addressed in order to clear the way for the development and applications of AP:

Not only the education of potential end-consumers on AP itself but also the awareness on, for instance, food safety, sustainability, and animal rights has to improve, especially for products that are imported to Finland from outside the European Union (EU). Furthermore, the EU legislation on certified organic products should be revised. Currently, for example, an AP-produced lettuce cannot be certified organic because it was not grown in soil (Miličić et al. 2017). The advantage that plants could be grown without the use of pesticides and artificial fertilizers hydroponically (in coupled or decoupled AP systems), improving the health of humans and natural environments seems to be neglected. This is incomprehensible and needs to be rechecked. Furthermore, fish produced in recirculating aquaculture systems (RAS) can not be certified organic according to EU guidelines (Miličić et al. 2017). Certification as "organic" is no precondition to ensure healthy and safe food but it could increase the popularity of AP farming and the trust of the consumer in the products.

Conclusion

Plenty of work is ahead on a national and EU level to make AP systems an acceptable and economic food production method. Only starting this mission will promote the use of AP systems, to ensure safe, healthy, and sustainable alternatives to, for example, meat consumption and fish that has not been produced according to EU standards but is imported to EU countries in massive amounts.

References

Ceder, M. C. (2020). Aquaponics as a Circular Economy Solution - Opportunities and Obstacles for Aquaponics Systems Analyzed in a Case Study on Finland. Master's thesis (unpublished), Department of Health Sciences, University of London, UK.

EU legislation, assessed on the 2nd of February 2021.
https://ec.europa.eu/info/food-farming-fisheries/farming/organic-farming/organic-production-and-products_en#rulesonwineaquacultureandhydroponics

Miličić, V., Thorarinsdottir, R., Santos, M. D., & Hančič, M. T. (2017). Commercial aquaponics approaching the European market: to consumers' perceptions of aquaponics products in Europe. Water, 9(2), 80.

Palm, H. W., Knaus, U., Appelbaum, S., Goddek, S., Strauch, S. M., Vermeulen, T., ... & Kotzen, B. (2018). Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. *Aquaculture International*, *26*(3), 813-842.

NEW AQUACULTURE FISH PRODUCTS FROM CONSUMER TO THE MARKET

R. Llorente*, I. Peral, M. Ibargüen, A.B. Naranjo

AZTI, Basque Research and Technology Alliance (BRTA), Spain
E-mail: rllorente@azti.es

Introduction
Aquaculture is one of sectors for seafood production with high potential of growth which allows the suitable maintenance of fish consumption in the world, representing in 2018 the 46% of the total fish production and 52% of fishes consumed by humans (FAO, 2020).

However, most of the aquaculture products are commercialized as whole fish or with a minimum processing (gutting, deboning or filleting). In Spain less than 20% of aquaculture products are processed (Strategic Plan for Aquaculture in Spain, 2015-2020).

Taking into account the current consumers demand and the lack of aquaculture seafood products in the market, the development of new fish aquaculture products would be an opportunity to increase the commercial value and profitability of Mediterranean Aquaculture value chain.

The aim of this study was to Design and Develop new aquaculture fish products concepts at pilot scale in the frame of H2020 MedAID in order to increase the overall competitiveness and sustainability of the Mediterranean marine fish-farming sector.

Material and methods
For this purpose, three Mediterranean aquaculture fish species raw materials: seabass, gilthead seabream and meagre were processed at AZTI´s pilot plant. The work carried out included the design of formulation matrix and specifications for product and processing parameters, selection and provisioning of raw materials (fish), ingredients, packaging materials and other consumables and finally developing production batches at pilot-scale equipments. Microbiological and nutritional analyses were performed for quality and safety assurance.

Results
Eight Mediterranean aquaculture fish product prototypes were designed and developed at AZTI's pilot plant in Derio (Spain) to meet three specific criteria; innovativeness, health and convenience. New value-added products, tailor-made to satisfy the needs of different consumer profiles (children, senior, gourmet/premium, ethnic etc.) in Spain, France and Germany, and adapted to the needs of diverse food and fish market channels.

The range of products developed included, raw/minimally processed (intermediate) and processed (ready-to-eat) products for chilled and frozen storage for fish processing industry, Horeca and retail.

Products developed showed high nutritional value; nutriscores from A to B and being source of or high in protein and w-3 fatty acids. Moreover, a shelf-life of 6 months for frozen and from 30 days to 3 months for chilled pasteurized concepts was achieved.

Of these eight, four (Grilled sea bass with lemon, Sea and mountain burger, Sea bream breaded bites and Organic sea bream with couscous) products were finally selected for validation studies with consumers in Spain, France and Germany.

Conclusions
Products developed, could be an interesting alternative to improve the commercial profitability of aquaculture fish species and the market success. They showed an excellent nutritional profile and high quality sensory properties due to their flavour, taste and texture properties. They also present convenience advantages (ready-to-eat, no bones) and shelf-life improvement that meet consumer needs. The results achieved are expected to contribute to product and process development in Mediterranean Aquaculture value chain.

References
FAO. (2020). The state of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.
Ministerio de Agricultura, Alimentación y Medio Ambiente (2015). Fundación Observatorio Español de Acuicultura, Madrid, España. Plan estratégico plurianual de la acuicultura Española 2014-2020.

USING INSECTS TO RE-VALORIZE WASTES FOR AQUATIC FOOD PRODUCTION – OPPORTUNITIES AND CHALLENGES

E.R. Lock, G. Radhakrishnan, N. Liland, J.D. Rasinger, I. Belghit

Institute of Marine Research, Nordnesgaten 50, 5005 Bergen, Norway
E-mail: Erik-Jan.Lock@hi.no

During the last two decades the percentage of fish oil and meal in aquafeed has been reduced considerably. Plant-based ingredients have taken more place in formulations and with much success in terms of fish performance. However, with increasing pressure on resources needed to produce plant ingredients, like arable land, freshwater and artificial fertilizers, the quest for less resource intensive alternatives has intensified during the past years. Among several alternatives proposed, insects have received much attention. Especially the larvae of Black Soldier Fly (*Hermetia illucens*; BSF) and Yellow Mealworm (*Tenebrio molitor*) have been studied. Both can live in low-value biomass and have the ability to re-valorise these substrates into a high quality animal protein. We will present the results of project AquaFly where the nutritional and safety aspects along the seaweed-insect-fish-food chain were studied. Performance of BSF meal in both freshwater and seawater salmon will be discussed and we will touch on the transfer of heavy metals and arsenic along the food chain.

The EU has approved 7 insect species for use in aquafeed. For regulatory purposes methods are needed to identify these species in feed. We will present our latest results on the development of analytical tools for identification of insects in feed. Finally, we will discuss what, in our view, the main hurdles are for insects to be used in large quantities in aquaeed and contribute to a more circular bioeconomy.

THE RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL EPITHELIAL CELL LINE RTgutGC IS PERMISSIVE TO IPNV, SAV3 AND ISAV

Guro Løkka*, Amr A. A. Gamil, Øystein Evensen, Trond M. Kortner

Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
E-mail: guro.lokka@nmbu.no

Introduction

For the aquaculture industry, viral infections represent a major challenge causing economic losses and having a negative impact on the fish welfare. For salmonid fish, despite developing genetic fish strains with high resistance against certain viruses and the attempt to develop and use vaccines as preventive measure, there are still several viruses threatening the industry and new viral diseases are emerging continuously. Developing better and more efficacious vaccines will require better understanding of the virus host interaction. One of the main entry ports for viruses into the host is the mucosal surface of the gastrointestinal tract (GIT). However, the interaction between the viruses and the GIT has not been well studied. One of the main factors behind this has been the lack of *in vitro* tools. We have recently characterized and used the rainbow trout (*Oncorhynchus mykiss*) intestinal epithelial cell line RTgutGC as an *in vitro* system to evaluate the effects of functional feed ingredients on gut health conditions (Wang et al., 2019). In the current study, we have assessed the permissiveness of the RTgutGC cells to three of the important fish viruses; infectious pancreatic necrosis virus (IPNV), salmonid alphavirus 3 (SAV3) and infectious salmonid anemia virus (ISAV). The aim was to assess the permissiveness and to establish an *in vitro* method for investigating the interaction of the different viruses with the gut cells of salmonid fish.

Materials and methods

RTgutGC cells were infected with the different viruses using different multiplicity of infection (moi =10, 1 or 0.1). The permissiveness of the viruses was assessed by monitoring the development of cytopathic effect (CPE) and by measuring virus replication in the cells using real-time PCR. Immunofluorescence antibody technique was applied for visualizing virus inside the cells. Cell-virus interaction was studied by assessing the expression of the pro-inflammatory cytokine TNF-α and several anti-viral genes, namely Mx, IFN-α, PKR and IRF9, using real-time PCR. In addition, viral effects on epithelial barrier permeability were studied using an albumin translocation assay (Wang et al., 2019) performed in a well system where cells are grown to confluence on a permeable membrane creating an apical and a basolateral compartment.

Results and conclusions

We demonstrated that the RTgutGC cell line is permissive to all the three tested viruses, namely IPNV, SAV3 and ISAV (Figure 1 and 2). However, the infection profiles of the three viruses were notably different. IPNV infection had an early onset and rapid progression and the increase in the moi used for infection was positively correlated with CPE, virus yield and expression of pro-inflammatory and anti-viral genes. In contrast, SAV3 and ISAV infection had a delayed onset of CPE and more slow progress. For IPNV and SAV3, the highest virus yield was obtained following infection with 10 moi while for ISAV the highest yield was obtained using 0.1 moi. Expression of the tested anti-viral genes were induced following infection with all three viruses. For IPNV, the highest expression of the pro-inflammatory and anti-viral genes were detected following infection with 10 moi, while for SAV3 it was detected following infection with 0.1 moi. In contrast, for ISAV, there was no obvious correlation between the induced immune responses and the moi used for infection, except for TNF-α and IFN-α where the highest expression was detected in cells infected with 0.1 moi.

To our knowledge, this is the first study investigating the replication and interaction of viruses with the RTgutGC cells. The presented data showed that the RTgutGC cells can be used as an *in vitro* system to study virus interaction with the gut cells.

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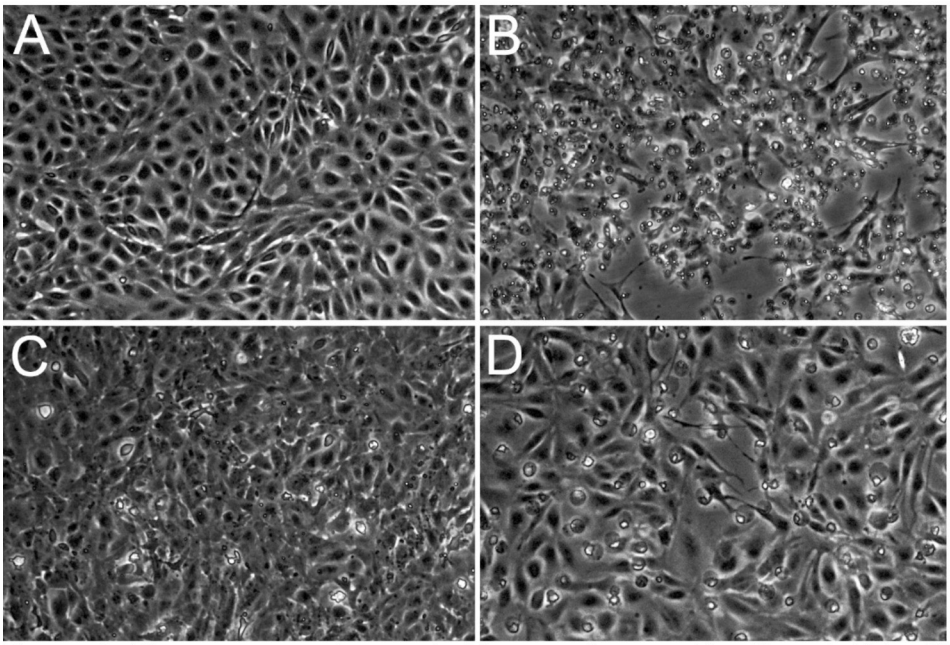

Figure 1. Phase contrast microscopical images of cytopathic effects in RTgutGC cells. A) Uninfected cells. (B-D) Cells infected with IPNV, SAV3 and ISAV, respectively, at multiplicity of infection=1. For IPNV, image was captured at 3 days post infection (dpi), while for SAV3 and ISAV, images were captured at 5dpi.

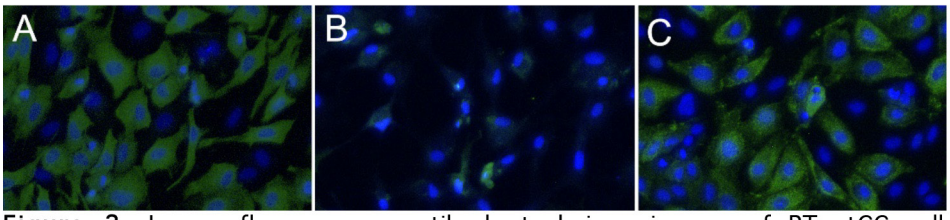

Figure 2. Immunofluorescence antibody technique images of RTgutGC cells infected with (A) IPNV, (B) SAV3 and (C) ISAV where virus particles are visualized with green color and cell nuclei are stained blue.

Reference

Wang, J., Lei, P., Gamil, A. A. A., Lagos, L., Yue, Y., Schirmer, K., . . . Kortner, T. M. (2019). Rainbow trout (*Oncorhynchus mykiss*) intestinal epithelial cells as a model for studying gut immune function and effects of functional feed ingredients. *Frontiers in Immunology, 10*(152). doi:10.3389/fimmu.2019.00152

NEW FISH PRODUCT IDEAS GENERATED BY EUROPEAN CONSUMERS

L. López-Mas[1,*], A. Claret[1], V. Stancu[2], K. Brunsø[2], I. Peral[3], E. Santa Cruz[3], A. Krystallis[4], & L. Guerrero[1]

[1]Institute of Agrifood Research and Technology (IRTA), Monells (Spain)
[2]Aarhus University, Aarhus (Denmark)
[3]AZTI-Tecnalia, Bizkaia (Spain), [4]Hellenic Centre for Marine Research, Athens (Greece)
*E-mail: laura.lopezm@irta.es

Introduction

Food lifestyles are changing; people have less time to spend on food purchase and preparation, therefore leading to increasing demand for new food products. However, around 76% of new food products launched in the market fail within the first year (Nielsen, 2014). One of the most effective ways to enhance new products' success in the market is by incorporating consumers' opinions and needs during the New Product Development (NPD) process (Moon *et al*., 2018).

This study aimed to explore the usefulness of a qualitative technique, focus groups, to generate new aquaculture fish product ideas as well as to identify the most relevant product dimensions affecting consumers' potential acceptance.

Materials and methods

Two focus groups were carried out in three EU countries (i.e. France, Germany, Spain). Six 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, as well as fish consumers. Fish consumption was used to split participants into two focus groups per country, one of them with 'high fish consumers' and, the other one, with 'low fish consumers'.

Focus group discussion sessions had two main sections: a) Creative techniques, to elicit ideas of new aquaculture fish products and to obtain the participants' acceptability of each new idea on a scale from 1 (I very much dislike this fish product idea) to 10 (I like this fish product idea); and b) Projective techniques, to gain a deeper understanding of consumers' perceptions and opinions about new fish products. To conduct the analysis, focus groups were audio and video recorded. Sessions were carried out in the national languages, translated into English and verbatim transcribed for further analysis. The textual analysis focused on keyword frequencies, co-occurrence, and context meaning.

Results

Creative techniques used during the focus groups sessions engaged participants to generate a pool of 112 new aquaculture product ideas. The mean acceptability score for every generated idea was used to set up a prioritisation list of them within each focus group. Table 1 shows an example from the Spanish focus group with high fish consumers.

The textual analysis, through a pseudo-triangulation process with three researchers, allowed the identification of the most relevant dimensions contained in the individual's discourses. These dimensions were: health, process/preparation, sensory, quality, price, familiarity, natural, food product, variety, convenience, ethical, and occasion.

A panel of eight experts from the food industry evaluated each of the 112 ideas generated to determine which dimensions were contained within each idea and to what extent. The experts scored the 12 dimensions of each idea in a continuous scale from 0 'this dimension is not contained at all in this product idea' to 10 'this dimension is fully contained in this product idea'.

By combining the score given to each idea by the participants (mean acceptability score) and the corresponding intensity of each dimension given by experts, a preference map was obtained. The dimensions that had a higher influence on the acceptability scores for high fish consumers from Spain were convenience, process/preparation, occasion, and variety. Spanish low fish consumers attributed greater importance to ethical issues. French and German participants had some dimensions in common. While high fish consumers from France gave more importance to health, quality, and natural, German participants focused on price, quality, natural, and ethical dimensions. Low fish consumers, both from France and Germany, attributed more importance to ethical and price dimensions.

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Table 1. Ideas generated in the focus group with Spanish high fish consumers as well as the most relevant dimensions and the participants' acceptability of the five ideas with higher mean acceptability score.

Idea	Dimension				Mean value	
	Process /Preparation	Variety	Convenience	Occasion	Dimension	Acceptability
Octopus surimi	3.8	7.4	5.4	3.1	4.9	9.50
Fish with dressing in a separate bag (fine herbs, olive oil, mustard, garlic, *etc.*)	8.5	5.1	7.1	4.5	6.3	9.50
Seabass, seabream, or meagre, totally clean, maintaining flavour (cubes or flakes)	5.8	7.3	7.8	5.4	6.5	9.33
Fish that do not lose flavour after cooking	2.9	2.4	1.7	3.4	2.6	9.17
Fish burgers	6.9	7.1	7.8	4.4	6.5	8.50

The ideas with higher potential to be well accepted in the market were those with a high score for the mean acceptability and containing the relevant dimensions for consumers. As shown in Table 1 for Spanish participants, two ideas had the highest mean acceptability, 9.5. However, when looking at the mean value of the relevant dimensions for these consumers, one idea got a score of 4.9 and, the other one, 6.3. Therefore, it would be more appropriate to select the idea with the highest mean dimension value, 'Fish with dressing in a separate bag (fine herbs, olive oil, mustard, garlic, *etc.*)'. However, the final selection of ideas also depends on other aspects, such as economic and technical feasibility, company values, or target market, among others.

Conclusions

Focus groups have been proven to be a useful technique to allow consumers generating new aquaculture product ideas and to identify the most relevant dimensions for them. This research also provides some hints to select the most promising ideas to scale in the NPD process (i.e. product concept testing, product development, market launching, *etc.*).

References

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.

Nielsen, 2014. Nielsen breakthrough innovation report: European edition - September 2014. New York (United States).

GENETIC ARCHITECTURE OF MORPHOLOGICAL ABNORMALITIES TRAIT IN GILTHEAD SEABREAM

A. Lorenzo-Felipe*[1], F. Naya-Català[2], S. León-Bernabeu[3], H.S. Shin[1], J. Calduch-Giner[2], E. Haro[3], M.J. Zamorano[1], J. Pérez-Sánchez[2], J.M. Afonso[1]

[1] Aquaculture Research Group (GIA), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de las Palmas de Gran Canaria (ULPGC), Carretera de Taliarte, s/n, 35214 Telde, Spain
alvaro_lorenzofelipe@hotmail.com
[2]Nutrigenomics and Fish Growth Endocrinology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain. [3]Aquanaria S.L., Calle Bentejui, s/n, 35107 San Bartolomé de Tirajana, Spain

Introduction

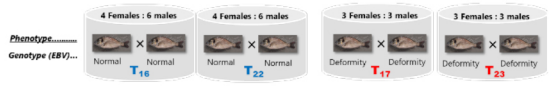
Despite the development and consolidation of the gilthead seabream aquaculture industry, it is estimated that the presence of skeletal deformities can affect up to 30% of production. Skeletal deformities have been associated to genetic basis (Lee-Montero et al., 2015; García-Celdrán et al., 2016; Fragkoulis et al., 2020). Quantitative trait loci (QTL) have been reported for severe skeletal deformities (Negrín-Báez et al., 2016). Riera-Heredia et al. (2019) described expression differential patterns (eQTL) in gilthead seabream with lordosis and LSK deformities versus normal ones, in genes related to bone extracellular matrix maturation and mineralization, and other involved in bone resorption.

The aim of this research was to study the genetic architecture of morphological abnormalities trait in gilthead seabream, contributing with new criteria to the European Certificate of Juvenile Quality (ECJQ), from a methodology integrating *Quantitative* (divergent genetic selection) and *Molecular Strategy* (RNAseq analysis).

Materials and methods

In this experiment, 2,100 alive breeders of the Spanish National Breeding Program (PROGENSA®) (Afonso et al., 2012) were evaluated by BLUP for presence-absence of any severe deformity trait (EBV_{def}). Two genetic crosses were established with 32 selected breeders according their EBV_{def}, (Fig.1). Eggs and larvae of genetic crosses were produced and cultured according to Fernández-Palacios et al. (2011). Daily, biological samples were stored for osteological and gene expression analysis. Biological samples from day 35 to day 71 DPH were stained for bone and cartilage using double-staining of fish staining protocols with some modifications (Potthoff, 1984; Taylor and Van Dyke, 1985), and skeletal deformities quantified.

Total RNA of selected samples was extracted using the *MagMAX™-96 RNA isolation kit*. RNA-seq libraries were prepared using the Illumina *NEBNextUltra™RNALibrary Prep Kit* and filtered with *FastQC v0.11.7* and *Prinseq v0.20.4*. Libraries were mapped and annotated using *HISAT2 v2.05*, and the CSIC gilthead seabream draft genome as reference (Pérez-Sánchez et al., 2019). Read numbers mapped over each gene were counted by *FeatureCounts v1.5.0-p3*. Differentially expressed genes (DEGs) were retrieved with normalized RPKM values using *DESEQ2 v1.20.0* at an adjusted FDR of 0.05. A less stringent statistical procedure was made by discriminant analysis (PLS-DA) and hierarchical clustering of filtered



a	0.2532	0.2461	0.2829	0.2777
$EBV_{deformity}$	- 0.0596	- 0.0596	+ 0.0536	+ 0.0553
Biomass	8,000 g	8,235 g	8,665 g	8,065 g
♀ Weight	3,490 g	3,375 g	4,130 g	3,710 g
♂ Weight	4,510 g	4,860 g	4,535 g	4,355 g

Fig 1. Example of mating scheme for two tanks within type of crossing (DxD, Deformity; NxN, Normal), with indication of additive relationship coefficient (a), estimated breeding value (EBV), total biomass, sex biomass, sex ratio (Red colour, deformed breeder; Blue colour, normal breeder).

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genes by ANOVA (P< 0.05). Variant calling and differential allelic frequencies were established by using sequentially *SAMtools v1.6, Freebayes v1.0.2, Popoolation2 v1.20,* and *SnpEff v4.3*.

Results and conclusions

The incidence of severe deformities was linked to the genetic deformity cross (DxD) in different development windows, being highly significant at the end of mineralization process (days 67-71 DPH) with a change in the slope prevalence at days 45-47 DPH. For RNAseq analysis, larvae from days 35-37 DPH (beginning of mineralization process) and days 5-6 DPH (swim bladder pre-inflation) were used to identify skeletal deformities-associated genes. From PLS-DA, 1,868 genes were of discriminant value (VIP ≥1) driving the separation between normal and deformity groups (1,398 unique gene descriptions). Among them, the 27.8% were located on super-scaffolds 20, 12, 17, 7 and 21, and GO-BP analysis disclosed 6 different enriched processes corresponding to nervous system development, biological adhesion, regulation of signaling, cellular developmental process, organic substance transport, and small molecule metabolic process.

In total, 64 discriminant genes were polymorphic and related to enriched GO-BPs, which strongly associated DEGs with different metabolic routes and gene-associated variants of skeletal deformities, becoming this trait a complex hub with diverse levels. These results were consistent with previous deformity-associated studies (Negrín-Báez et al., 2016), where a total of 22 QTLs were linked to this complex trait. These QTL markers displayed 33 overlapping gilthead seabream genes. Among them, 8 genes were up-regulated in the stringent FDR comparison between DxD35 (log2FC > 2.5) and NxN35 groups. This shortened list of genes included a varying number of genetic variants that ranged between 1 and 27: *egfl7* (27), *meis1* (13), *supt20h* (5), *kcnt1* (5), *asrgl1* (5), *mycp2* (2), *psma2* (2) and *palm* (1).

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References

Afonso et al. (2012). Proceedings of AQUA 2012.
Fernández-Palacios et al. (2011). In: Larval Fish Nutrition, pp. 151-181.
Fragkoulis et al. (2020). Aquaculture 497, 226-233.
García-Celdrán et al. (2016). Aquaculture 464, 601-608.
Lee-Montero et al. (2015). Animal Genetics 46, 164-174.
Negrín-Báez et al. (2016). Animal Genetics 47, 230-233.
Pérez-Sánchez et al. (2019). Frontiers in Marine Science 6,760.
Potthoff, T. 1984. In: Onotgeny and Systematics of Fishes, pp. 35-37.
Riera-Heredia et al. (2019). PLoS ONE, 14:e0215926.
Taylor and Van Dyke. 1985. Cybium, 9, 107-120.

TEMPORAL DYNAMICS OF THE GUT MICROBIOME IN ATLANTIC SALMON DURING SMOLTING IN AN INDUSTRIAL RECIRCULATING AQUACULTURE SYSTEM AND FOLLOWING SW TRANSFER

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
Aquaculture of teleost species, such as Atlantic salmon (*Salmo salar*), is becoming increasingly important to provide a sufficient source of protein for a continually expanding world population. The increase in demand has driven the expansion of land-based smolt production in recirculating aquaculture systems (RAS). Success of smolt production determines growth, osmotic tolerance and health following transfer to sea. Host-associated microbiota are an important component of fish health which have become of increasing interest in recent years, particularly in RAS where microbes are also a key component in maintaining water quality.

Materials and methods
In this study, we analysed the temporal dynamics of the microbial community associated with the gut in Atlantic salmon during smoltification in a commercial RAS facility and following transfer to a commercial sea site. Distal intestine and contents were sampled from 6 fish from triplicate tanks at 4 timepoints in FW (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 et al. 2016) was used to determine microbial composition in distal intestine, water and diet samples at the level of amplicon sequence variants (ASVs). Functionality was inferred using Piphillin (Iwai et al. 2016).

Results
Microbial diversity and richness showed significant associations with time ($p<0.001$) and increasing temporal trends in both were observed during FW production. Diversity ($p<0.001$) and richness ($p=0.002$) then declined significantly 1-week post-SWT before re-establishing with a completely different community structure after 4 weeks. In FW, microbial communities were dominated by *Proteobacteria* and *Firmicutes*, but post-SWT the community was dominated by the *Proteobacteria* family *Vibrionaceae*.
Core microbial taxa (present in >80% of samples) in the distal intestine were identified which could be assigned to 3 distinct categories: (1) omnipresent, (2) salinity specific or (3) transient. By incorporating diet and water data, true core taxa associated with the host alone were identified alongside those associated with diet, and those associated with tank water, which tended to be transient in nature.

Functional inference identified metabolic pathways associated with microbial communities in FW and SW. A shift in the relative contribution of 6 metabolic categories was observed pre- and post-SWT, including higher contribution of 'Xenobiotics biodegradation and metabolism' at FW4 ($p=0.012$) and of 'Glycan biosynthesis and metabolism' at SW1 ($p=0.012$).

Discussion and conclusions
A rising trend in microbial richness was observed in the distal intestine of Atlantic salmon reared in a FW RAS, during the parr – smolt transformation. Following transfer to SW, microbial diversity and richness declined and a distinct, less diverse, community structure was established, dominated by the *Vibrionaceae* family. Dominance was potentially a result of higher-than-average seawater temperatures observed in 2019 (Hatje et al. 2014).

Previous studies have reported a decline in diversity in the gut of Atlantic salmon as life history proceeds (Dehler et al. 2017, Lokesh et al. 2019). These studies covered a range of life history stages, while sampling intervals in the current study were designed to target a narrow window of smolt development, and the trend may be influenced by cycling of organic matter within the RAS.

Analysing diet and water samples supported the identification of 'true' core taxa associated with the host alone and not environment. Functional analysis suggested modulation of metabolic pathways post-SWT, but downstream impacts on fish growth and health in a commercial setting remain to be elucidated.

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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.

DEHLER, C.E., SECOMBES, C.J. and MARTIN, S.A.M., 2017. Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (Salmo salar L.). *Scientific Reports,* **7**(1), pp. 13877.

HATJE, E., NEUMAN, C., STEVENSON, H., BOWMAN, J.P. and KATOULI, M., 2014. Population dynamics of Vibrio and Pseudomonas species isolated from farmed Tasmanian Atlantic salmon (Salmo salar L.): a seasonal study. *Microbial ecology,* **68**(4), pp. 679-687.

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.

LOKESH, J., KIRON, V., SIPKEMA, D., FERNANDES, J.M.O. and MOUM, T., 2019. Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (Salmo salar) reveals stage-specific microbial signatures. *Microbiology Open,* **8**(4), pp. e00672.

CHANGES OF THE MAJOR FISH ALLERGEN PARVALBUMIN IN SEA BASS (*Dicentrarchus labrax)* HIGH PRESSURED FILLETS

Liliana Anjos[1, a, *], Arsenius Z. Loukissas[1, a], Theofania Tsironi[2], Deborah M. Power[1]

[1]Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[2]National Technical University of Athens, School of Chemical Engineering, Laboratory of Food Chemistry and Technology, Greece
Email: lanjos@ualg.pt a: both first authors

Introduction

Fresh fish is a valuable source of essential nutrients in the human diet, however, are highly perishable and their short shelf-live has a negative impact on aquaculture sustainability. For this reason, novel processing technologies, such as high-pressure processing (HPP) are being investigated as a means to extend the shelf life and improve the quality and safety of fish. Although HPP treatment of fish has a relatively small effect on sensory parameters, studies have shown a significant modification in the total proteome of HPP treated sea bass (*Dicentrarchus labrax*) (Tsironi et al., 2019). A further constraint to increased consumption of fish and shellfish is that they are strongly linked to human allergic reactions, caused by IgE-mediated immune reaction triggered by proteins such as the major allergen parvalbumin. Parvalbumins are sarcoplasmic calcium-binding proteins, highly conserved between fish species that are resistant to high temperatures, denaturing agents, and proteolytic activity. Calcium-binding by these proteins is of critical importance for the conformation of the IgE epitopes (Kuehn et al., 2014). The present study explored the effect of HPP on proteins and specifically **β**-parvalbumins present in the muscle of sea bass, one of the most farmed species in the Mediterranean.

Materials and methods

The effect of HPP was assessed on raw fillets of sea bass from aquaculture. All fish fillets were maintained on ice and handled in the same way until processing. In the control fillets (n = 8) no treatment was administered during the experiment and they were held at 2ºC. In the treated fillets (n = 8/ treatment) at three different pressures (300, 450, 600Mpa) and two pressure times (2, 5min) were tested using laboratory pilot scale Food Pressure Unit FPU 1.01. Control and HP treated fillets were stored under controlled isothermal conditions (2°C) for 12 days after HPP. Total soluble sarcoplasmic protein was extracted and quantified. 1-D SDS and native-PAGE were carried out to analyze the effect of HP on the general proteome profile of the control and treated samples. Semi-quantitative Western blot analysis, using a monoclonal anti-parvalbumin clone PARV-19 (mouse ascites fluid) specific for allergenic epitope detection, was carried out to detect sea bass **β**-Parvalbumin in the protein extracts derived from fillets exposed to different HPP conditions. Finally, the effect of Ca^{2+} on PARV-19 binding to sea bass **β**-parvalbumin was investigated using a gel shift assay, with protein extracts from control and HPP treated fillets in the presence or absence of $CaCl_2$ and in the presence of chelating agent, EGTA.

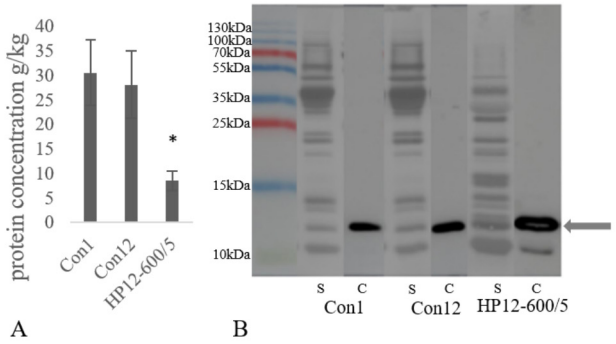

Figure 1: Effects of HPP and storage time on (A) protein solubility, (B) total protein profiles (S) and β-parvalbumin (C, black arrow). Sea bass fillet sarcoplasmic protein from Con – control samples (1 or 12 days of storage) or fillets processed at 600Mpa/5 min and stored for 12 days (HP12-600/5). A) mean ± SEM of n = 8 individuals per treatment (*, $p<0.05$). B) Western blot under native conditions detecting sea bass β-parvalbumin using the antibody PARV-19 (C). kDa (protein MW).

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Results

The results of protein quantification and SDS-PAGE indicated that sea bass white muscle sarcoplasmic protein solubility (Fig. 1 A) and protein profiles were affected by HPP treatment. The control samples revealed that storage time had no effect (Fig. 1 A, B). The major allergen in fish muscle, **β**-parvalbumin (Fig. 1 B) was identified by Western blot and revealed an immunoreactive protein of the expected size 12kDa, in all experimental conditions. Western blot results indicated that chemiluminescence signal intensity of **β**-parvalbumin was different between the control and HP processed fillets and revealed an additional **β**-parvalbumin isoform.

Discussion and conclusion

HPP caused a decrease in sarcoplasmic protein solubility and the proteome profile of protein extracts from sea bass fillets. Storage time at 2 ° C did not significantly affect the solubility of sarcoplasmic proteins in non-processed control fillets, as was previously reported (Tsironi et al., 2019). A specific immunoreactive **β**-parvalbumin protein signal with a MW of the expected size was detected in sea bass sarcoplasmic extracts. This indicates that the monoclonal antibody PARV-19, recognized the allergen epitope in sea bass β-parvalbumin as expected taking into consideration the high conservation of the allergens epitope between species (Kuehn et al., 2014). Our results indicate that HPP may affect the availability of the IgE β-parvalbumin epitope, which contrasts with its remarkable resistance to high temperatures, denaturing agents, and proteolytic activity.

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References

Kuehn, A., Swoboda, I., Arumugam, K., Hilger, C., & Hentges, F. (2014). Fish allergens at a glance: Variable allergenicity of parvalbumins, the major fish allergens. Frontiers in Immunology, 5(APR).

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. Journal of Food Engineering, 262(February), 83–91.

IDENTIFICATION OF *Photobacterium damselae subsp. piscicida* DIRECTLY FROM TISSUES IN SEABASS (*Dicentrarchus labrax*)

José Maria Ferreira[1], Florbela Soares[2], Marisa Barata[2], Pedro Pousão-Ferreira[2], Fernando Afonso[1] and Cátia Lourenço Marques[2,*]

[1] Faculty of Veterinary Medicine, Lisbon University
[2] Portuguese Institute for the Ocean and Atmosphere (IPMA)/Aquaculture Research Station of Olhão (EPPO)
e-mail: catia.marques@ipma.pt

Introduction

Photobacterium damselae subsp. *piscicida* is the causative agent of Photobacteriosis, a septicemic disease that is responsible for high mortality and morbidity in marine fish, highlighting the need for studies about the two subspecies (*P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae*) in the aquaculture context (Essam *et al*., 2016). The present study aimed to use molecular detection techniques, both directly in tissues or with previous bacterial growth, as a faster and more sensitive substitute for the conventional biochemical techniques. The former usually follow the steps mentioned in Bergey's Manual of Determinative Bacteriology (Holt, 1994) and, using the API 20NE galleries (bioMerieux), has a medium time of detection of about 6 days (counting with the bacteria growth time) and often requires additional confirmation by other means. The use of molecular techniques as either in association or as substitute of the traditional techniques will allow a faster and more precise diagnostics that, associated with earlier effective treatment, could decrease both mortality and morbidity which has great economic importance for the aquaculture sector.

Materials and Methods

The primers used targeted the genes: CPS (Eissa, et al., 2018) and 16SRNA (Osorio, et al., 2000), common to both subspecies, and UreC (Osorio et al., 2000), specific for *P. damselae* subsp. *damselae*. The growth of the bacteria isolates was done directly in a liquid medium (TSB) for 12 hours at 23ºC without agitation. To collect the bacteria, 1 mL of the medium was centrifuged, during 3 min at 6000G. Afterwards, the supernatant was removed, and the pellet resuspended in 50 μL of ultrapure water. A direct amplification from bacteria grown in solid medium (TSA) was also performed, by using an individualized colony resuspended in 50 μL ultrapure water. The DNA extraction step, in the grown bacteria, was performed by direct boiling of the pellet in the thermocycler, and, in the tissues, by using the Qiagen® QIAamp DNA micro kit. The PCR steps were: 94ºC for 5min, followed by 30 cycles of 45s at 94ºC, then 30s at 55ºC and 1min at 72ºC. A final extension step of 10min at 72ºC was performed.

The naturally infected fish (*Dicentrarchus labrax*) came from EPPO and other national fish farms.

Results and Discussion

It was possible to amplify a fragment of 267 bp (fig. 1), corresponding to the gene 16SRNA, using both the TSB and TSA mediums, with no significant change on the efficiency, which allows an important decrease on the culture time (from 24 to 12h). Additionally, the shortening of the DNA extraction step (boiling in the thermocycler), allowed a faster result, without the need for any kit or laborious protocols with the handling of toxic substances such as phenol and chloroform.

The confirmation of the infection by *P. piscicida s*ubsp. *damselae* was possible by performing PCRs both in cryopreserved bacterial isolates (both in *D. labrax* and *Sparus aurata*) and in tissues (spleen) of fish naturally infected (only in *D. labrax*) with chronic symptoms, from which there was no bacterial growth in the culture medium. The positive results of the extraction from tissues were from fish that exhibited macroscopic lesions, especially in the spleen, and of which there was no bacteria growth in appropriate mediums.

Comparing with the initial 6 days that the biochemical techniques require to have results, this molecular approach allows a time reduction to 2 days with the bacteria growth or to 1 and a half day using the kit tissue extraction, resulting in a faster and precise diagnostic associated with an earlier treatment.

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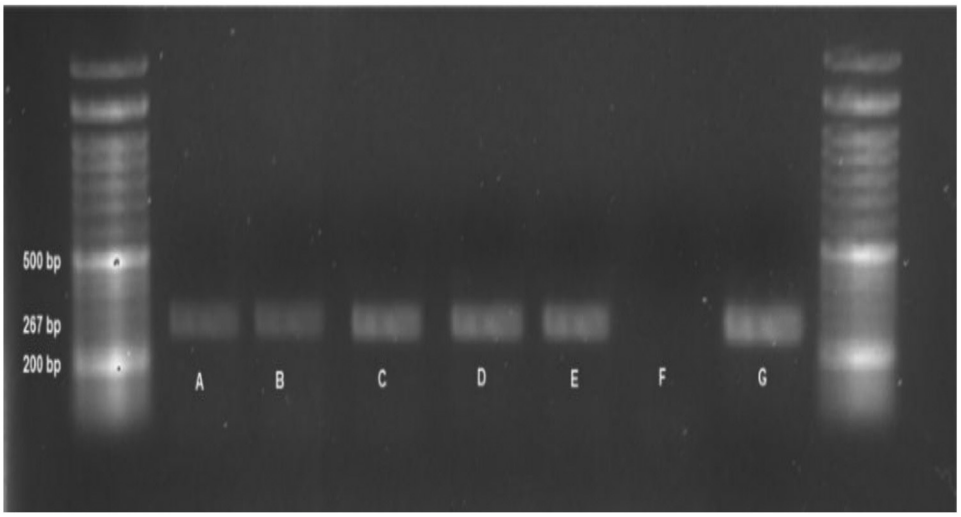
Fig. 1: NZYTECH ladder VI; A, B, C dilutions of gDNA extracted with the kit (1:10, 1:25, 1:50); D positive control;, E reamplification of a 1:10 dilution from the bacteria cultured on TSB medium; F negative control; G amplification from the TSB cultured bacteria (not diluted). The primer used in these reactions targeted the 16SRNA gene.



Conclusion

Bacterial identifications, such as *P. damselae* subsp. *piscicida*, using molecular techniques can be faster, easier, and with a higher degree of sensitivity than the traditional biochemical techniques. In addition, it allows the bacterial identification without the need of a growth in culture, decreasing the possibilities of contaminations. The much-needed prompt response to aquaculture producers is an important benefit of molecular identification which results in better prevention, diagnosis, and, consequently, treatment for *P. damselae* subesp. *piscicida* infection and should be explored for other pathogenic bacteria.

Acknowledgements

This work was financed by the projects DIVERSIAQUA II (MAR2020-P02M01-0656P) and SAUDE&AQUA (MAR-02.05.01-FEAMP-0009).

References

Eissa, I. A., Derwa, H. I., Ismail, M., El-lamie, M., Dessouki, A. A., Elsheshtawy, H., & Bayoumy, E. M. (2018). Molecular and phenotypic characterization of Photobacterium damselae among some marine fishes in Lake Temsah. *Microbial Pathogenesis*, 315-322.

Essam, H. M., Abdellrazeq, G. S., Tayel, S. I., Torky, H. A., & Fadel, A. H. (2016). Pathogenesis of Photobacterium damselae subspecies infections in seabass and seabream. *Microbial Pathogenesis*, 41-50.

Holt, J. (1994). *Bergey's Manual of Determinative Bacteriology.*

Osorio, C. R., Toranzo, A. E., Romalde, J. L., & Barja, J. L. (2000). Multiplex PCR assay for ureC and 16s rRNA genes clearly discriminates between both subspecies of Photobacterium damselae. *DISEASES OF AQUATIC ORGANISMS*, 177-180.

INCREASING OMEGA-3 PUFA DIETARY CONTENT IMPROVES GROWTH AND OSSIFICATION IN MEAGRE LARVAE WITHOUT AFFECTING THE INCIDENCE OF SKELETAL ANOMALIES

Leticia Luján*, Narcisa Bandarra, Paulo Gavaia, Catia Marques, Pedro Pousão, Laura Ribeiro

Instituto Português do Mar e da Atmosfera (IPMA) - Estacao Piloto De Piscicultura De Olhao.Av. Parque Natural da Ria Formosa, 8700-194, Olhão
Email: Leticia.lujan@ipma.pt

Introduction

Due to its high growth rate and feed efficiency, *Argyrosomus regius* is one of the most promising species for diversification of aquaculture in the south of Europe. To achieve profitable production of this species, we still need to improve our knowledge in formulating diets that maximize growth, while helping to minimize and control the incidence of deformities. Omega-3 fatty acids, especially Docosahexaenoic acid (DHA, 22:6n-3) and Eicosapentaenoic acid (EPA, 20:5n-3), are essential for growth and bone development in marine fish larvae and must be supplemented in the diet (Izquierdo., 2005). Furthermore, meagre theoretically presents a higher requirement of these nutrients due to its very fast growth. However, little information is available on optimal supplementation levels in this species. The present study examines the effects on growth and skeletogenesis of 3 different levels of ω3 fatty acids supplementation, with the aim of setting the most adequate levels of supplementation for the larval culture of this species, minimizing enrichment and its cost to the strictly necessary.

Material and methods

Fish larvae of *A. regius* from the same spawn were distributed by 9 rearing tanks of 300 L from 0 DAH to 42 DAH covering the period from the beginning of skeletogenesis, until the end of skeletal ossification. Fish larvae were fed with three experimental diets (live preys and inert microdiets) with increasing levels of n-3 PUFA (LD=26 mg/g; MD=34 mg/g and HD=42 mg/g). Briefly, at first feeding meagre larvae were fed with enriched rotifers (*Brachionus sp.*) until 10 DAH, followed by enriched *Artemia Instar II* introduced at 8 DAH and maintained until 25 DAH. Microdiets were introduced at 8 DAH and maintained until the end of the trial. Fish larvae were periodically sampled (at 12, 22, 32 and 42 DAH) to assess larval growth and ossification progress. Fish larvae sampled for ossification and to evaluate the frequency of vertebral anomalies, were double stained with Alcian Blue and Alizarin S for cartilage and bone, respectively (Walker and Kimmel., 2007). Fish were individually photographed, measured (total length) with a stereomicroscope (Leica MZ9.0) equipped with a F-View camera and analysed two independent times for ossification studies and detection of skeletal anomalies. All the remaining larvae at the end of the trial were counted to determine survival rate.

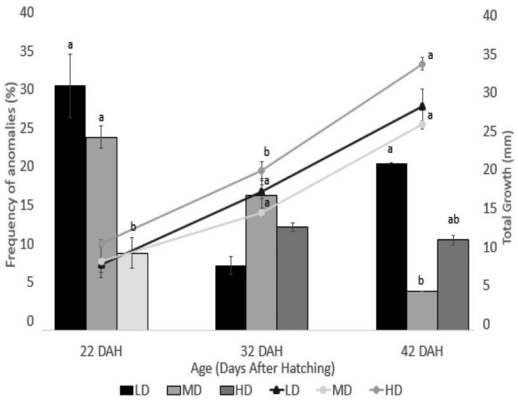
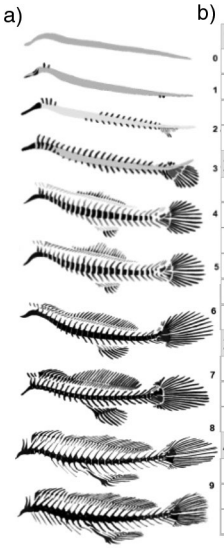


Figure 1: Frequency of malformations (bars) and Total Growth (lines) per treatment at 22,32 and 42 DAH (p>0.05)



12DAH	LD	MD	HD
0	3	35	0
1	7	25	3
2	34	30	25
3	56	10	72
22DAH	**LD**	**MD**	**HD**
2	0	3	0
3	0	8	0
4	15	28	0
5	45	40	7
6	35	23	65
7	5	0	28
32DAH	**LD**	**MD**	**HD**
6	2	3	0
7	88	95	70
8	10	3	30
42DAH	**LD**	**MD**	**HD**
7	3	8	0
8	30	43	13
9	67	50	87

Figure 2: a) Stages of skeletal development in *A.regius* larvae b) Percentages of larvae in each stage of ossification at 12, 22 ,32 and 42DAH in

(Continued on next page)

Results and Discussion

Similar survival rates were obtained among treatments ($p>0.05$), regardless the level of ω3 fatty acids of diets. However, meagre larvae fed HD diet exhibited higher growth ($p<0.05$) when compared to other experimental groups reaching 3,5 ± 0,48cm at 42 DAH. But, no linearity was observed between growth and ω3 dietary level since meagre larvae fed MD level exhibited the lower growth at 42 DAH (2.7 ± 0,52cm).

Regarding the progress of ossification, the results reflect a similar tendency to growth, where the diet with the highest content of ω3 fatty acids presented the best and fastest results. The beneficial effect of the HD diet on the ossification was evident from the 22 DAH. As observed for growth, the MD presented the worst results in ossification. This fact may be related with the oil blend from MD diet that contained a higher content of terrestrial oils. These oils usually present higher content of fatty acids ω6, which are related to decrease bone formation and with a more elevated incidence of skeletal diseases (Mangano *et al*., 2013). However, beyond the slower ossification, no deleterious effects were observed on bone health. Therefore, based on the results of growth, ossification, and frequency of anomalies, we can conclude that the supplementation of high levels of Omega-3 fatty acids improved growth and accelerated larval skeletal development in this specie without increasing the incidence of bone malformations.

References

Izquierdo, M. Essential fatty acid requirements in Mediterranean fish species. *Cahiers Options Méditerranéennes*. 2005, 63, 91–102.

Walker, M. B.; Kimmel, C. B. A two-color acid-free cartilage and bone stain for zebrafish larvae. *Biotechnic & Histochemistry*, 2007, 82 (1), 23-28.

Mangano, K.M.; Sahni, S.; Kerstetter, J.E.; Kenny, A.M.; Hannan, M.T. Polyunsaturated fatty acids and their relation with bone and muscle health in adults. *Current osteoporosis reports,* 2013, 11 (3), 203–212.

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EFFECT OF WATER TEMPERATURE ON GROWTH AND SKELETAL DEVELOPMENT IN MEAGRE (*Argyrosomus regius*)

L. Luján*, S. Sousa e Brito, S. Castanho, C. Marques, L. Ribeiro, P. Pousão-Ferreira

Portuguese Institute for the Ocean and Atmosphere (IPMA), Estacão Piloto De Piscicultura De Olhao Av. Parque Natural da Ria Formosa N, 8700-194, Olhão (Portugal)
E-mail: Leticia.lujan@ipma.pt

Abstract

Temperature (T[a]) is one of the most important abiotic factors affecting larval culture enhancing their metabolic activity, feed intake, growth rate and development. The faster development as a result of higher temperature may accelerate bone formation, affecting negatively skeletal development. In fact, higher rearing temperature has been identified as the probable cause for spinal deformity in golden pompano[1]. However, the consequences of this rapid development have not been properly addressed in most of the commonly reared species. Therefore, it is critical to identify optimal rearing temperatures for each species that promote optimal production and diminishes the incidence of skeletal deformities, avoiding losses for the aquaculture sector. Specially, when information is scarce, as for *Argyrosomus regius* a promising species for aquaculture diversification. The objective of this study was to assess the effect of different rearing temperatures on the growth, skeletal development, and the incidence of skeletal anomalies on meagre larvae.

Material and Methods

Fish larvae of *A. regius* from the same spawn were distributed at hatching (0 DAH) by 9 rearing tanks of 300 L and reared until fish larvae reached 1 cm of total length at 18, 21 and 24ºC (3 replicates for each treatment). The larvae were fed with the same feeding protocol (rotifers + artemia + commercial inert diet, CAVIAR, Bernaqua™, Belgium). The dissolved oxygen was maintained over 90% and the photoperiod selected was 14 h of light and 10 h of darkness. Fish larvae were periodically sampled during the trial to assess larval growth, ossification progress, and frequency of vertebral anomalies with double staining with Alcian Blue and Alizarin S for cartilage and bone, respectively [2]. The collected larvae were analysed for ossification and incidence of anomalies at similar standard length to avoid discrepancies related to differences in growth.

Results and Discussion

The state of ossification in fish larvae is more related to the length than to the age. In fact, for the selected sampling points at similar standard length but different age, ossification was similar regardless the different rearing temperatures.

The results obtained in growth performance during the experiment were in line with other studies with meagre where at higher temperatures growth was accelerated[3]. This was evidenced in our trial, where the rate of growth for each treatment was different. The larvae that were reared at 24ºC reached the selected length to study skeletal anomalies at 19 DAH, while the larvae at 21ºC needed 21 days and the larvae at 18ºC needed 25 days.

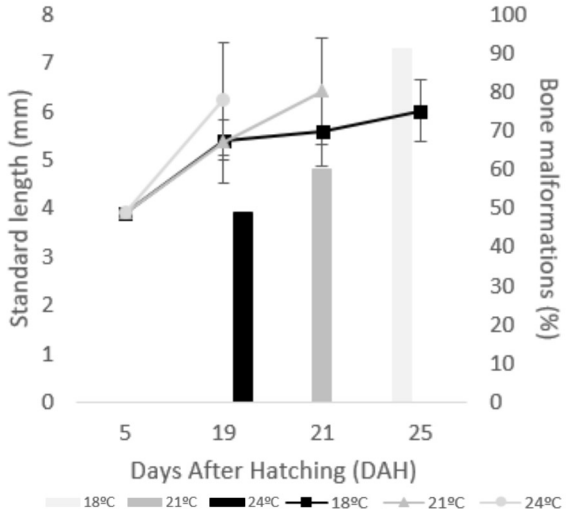

Figure 1: Growth (lines) and frequency of malformations at similar standard length (bars) at 18, 21 and 24ºC ($p>0.05$).

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Studies have reported high water temperature may cause an increase in the incidence of spinal deformities at the egg hatching stage, after hatching, or in the juvenile stage[1]. This knowledge is based on the insights that at high T^a, the larvae grow faster leading to a negative effect in skeletal development. This fact was not in agreement with the results of our study. For the same length, larvae reared at the high T^a (24ºC) presented a lower frequency of bone deformities when compared with the other treatments, whereas larvae reared at 18 ºC presented the higher frequency of anomalies ($p<0.05$), contrary to the expected. These results shown the importance of establishing rearing temperatures in a species specific way. Because, while higher temperatures can enhance malformations in some species as been reported,[4] may be required for the optimal growth in species with higher growth rate as in the case of meagre. In fact, *A.regius* seems to grow faster at higher T^a (24ºC) while maintains better skeletal development and present less deformities than larvae that grow slower at 18ºC or 21ºC.

References

1.Han, M., Luo, M., Yang, R., Qin, J. G. & Ma, Z. Impact of temperature on survival and spinal development of golden pompano *Trachinotus ovatus* (Linnaeus 1758). *Aquaculture Reports* 18, 100556 (2020).

2.Walker, M. B. & Kimmel, C. B. A two-color acid-free cartilage and bone stain for zebrafish larvae *Biotechnic & Histochemistry*. 81, 23–28 (2007).

3.Antonopoulou, E, Chatzigiannidou, I; Feidantsis, K, Kounna, C & Chatzifotis, S. Effect of water temperature on cellular stress responses in meagre *(Argyrosomus regius*). *Fish physiology and biochemistry*. 46, 1075-1091 (2020).

4.Georgakopoulou, E., Katharios, P., Divanach, P. & Koumoundouros, G. Effect of temperature on the development of skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758). *Aquaculture* (2010).

Acknowledgements

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INVESTIGATION OF THE MICROBIOLOGICAL QUALITY AND SAFETY OF FARMED EDIBLE SEAWEEDS *Alaria esculenta* AND *Saccharina latissima*, OBTAINED FROM SCOTLAND (UK) IN TWO DIFFERENT YEARS

A. Lytou *[1], E. Schoina[1], Y. Liu[2], N. Evangelopoulos[1], K. Michalek[3], M. Stanley[3], E. Panagou[1] and G-J. Nychas[1]

[1] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
[2]Department of Food Science and Engineering, Shandong Agricultural University, Shandong, China
[3]Scottish Association for Marine Science (SAMS)
E-mail: alytou@gmail.com

Introduction

Although seaweeds are considered to be a very popular food commodity in Asian countries since elder times, the farming of seaweeds has recently experienced interest in Western countries. Seaweeds are gaining popularity in the Western world due to their marketing and perception as 'superfood', the increased interest in healthier diets as well as due to the preference for more sustainable food sources and production procedures (Sa Monteiro et al., 2019 (EFSA)). The investigation of quality and safety of seaweeds is of great importance for both fresh and dried products since there is limited information about both categories. Moreover, the fact that rehydrated products are usually consumed without any further processing (just after a rehydration period) renders seaweed a foodstuff of high risk for raising public health issues.

Methods

Fresh *Alaria esculenta* and *Saccharina latissima* samples, grown at the west coast of Scotland, cultivated and harvested by SAMS research group in 2019 (June) and 2020 (May). The collected samples were stored at -20°C for a few days and were subsequently sent to the Laboratory of Food Microbiology and Biotechnology (Agricultural University of Athens, Greece) for microbiological quality assessment. The received samples were thawed at 0°C, separated into 50g portions and stored at different temperatures (5, 15°C – 2019 and 0, 5, 10, 15°C - 2020) for specific time intervals. Microbiological analysis was performed on the day of their arrival at the lab and at certain days of storage, for the estimation of Total Viable Counts (TVC), *Pseudomonas* spp., Lactic acid bacteria, Enterobacteriaceae, *Bacillus* spp., *Vibrio* spp., *Aeromonas* spp., yeast and moulds, *E. coli*, *Salmonella* and *Staphylococcus* spp. Along with frozen samples, dried samples (10-12% moisture) were also received, stored at 15 and 25°C for a 6-month period and examined both for the presence of pathogens (on their arrival at the lab) as well as for the estimation of several spoilage bacteria level throughout storage. The second part of the experimental design included the rehydration of dried samples harvested in 2019, in an attempt to simulate a common consumer practice for seaweed consumption. A specific quantity of dried samples (30-40 g) was soaked into sterile water (200 mL H_20/10 g of dried seaweed) for 5 min, removed, allowed to drain off, placed in polystyrene trays and stored for 7 days at 4 and 12°C. Four samples (n=4) were analysed for each experimental scenario (year, storage temperature, condition of the samples).

Results and Discussion

The total aerobes population (TVC) of fresh samples, harvested in two different years is presented in Figures 1 and 2. Differentiated microbial levels and growth evolution can be observed between the 2019 and 2020 samples, while a similar pattern was also observed in the populations of specific microorganisms, which were in much lower levels (or even absent) in the 2020-samples. However, the initial population in both years was within the range reported in literature for these two species (Blikra et al., 2019).

The level of TVC in dried products was 3-4 log units higher than in the fresh ones (ca. 7.0 log CFU/g) in 2019 samples while 2020 products were of higher microbiological quality due to the lower initial population as well as due to more appropriate practices followed after harvest.

As far as the rehydration process is concerned, the initial population of samples after rehydration was 6.0 and 4.9 log CFU/g in *Alaria* and *Saccharina*, respectively, while the microbial level of the dried samples that were used for rehydration was 1.0 log higher in both species.

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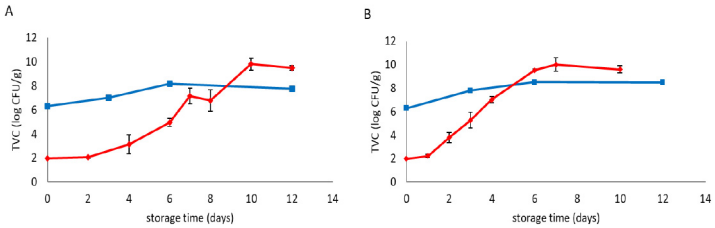

Fig 1. Total aerobes population (TVC) of fresh *Alaria esculenta* throughout storage at 5 (A) and 15 °C (B)

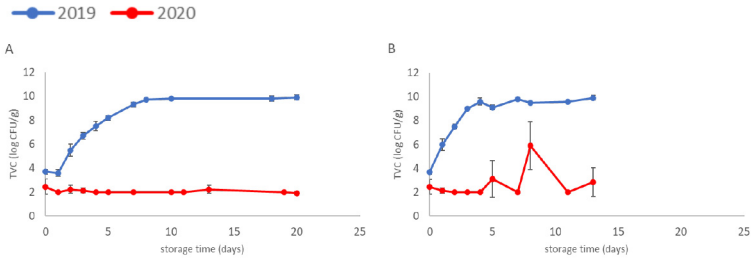

Fig 2. Total aerobes population (TVC) of fresh *Saccharina latissima* throughout storage at 5 (A) and 15 °C (B)

Acknowledgement

This work has been supported by project ‘’IMPAQT’’ (EU H2020 research and innovation programme under Grant Agreement No 774109).

References

Blikra, M. J., Løvdal, T., Vaka, M. R., Roiha, I. S., Lunestad, B. T., Lind-seth, C., Skipnes, D. (2019). Assessment of food quality and microbial safety of brown macroalgae (*Alaria esculenta* and *Saccharina latissima*). Journal of the Science of Food and Agriculture, 99(3),1198–1206.

Sa Monteiro, M., Sloth, J., Holdt, S. and Hansen M. (2019). Analysis and Risk Assessment of Seaweed. EFSA Journal 17(S2):170915

MICROBIOLOGICAL QUALITY AND SAFETY OF FARMED SEAWEED *Alaria esculenta* AND SALMON *Salmo salar* CO-CULTURED IN AN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM

A. Lytou *[1], D. Lymperi[1], J. Casserly[2], F. Kane[2], E. Panagou[1] and G-J. Nychas[1]

[1] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
[2] Marine Institute, Ireland
E-mail: alytou@gmail.com

Introduction

Although advantages and limitations of Integrated Multi-trophic Aquaculture (IMTA) have been widely reported, few studies have focused on potential effects of IMTA on the microbiological quality and safety of the end products (Califano et al., 2020). In the current study, the spoilage potential as well as the presence of pathogenic bacteria in two different species (salmon *Salmo salar* and seaweed *Alaria esculenta*) cultured in IMTA system were investigated. In addition, several nutritional parameters were evaluated so as to provide a preliminary view about the nutritional quality of such products.

Methods

Alaria esculenta and Atlantic salmon were co-cultivated at a pilot scale IMTA site, Lehanagh Pool, operated by the Marine Institute in the west coast of Ireland. Both species were harvested in May 2020. The collected samples were subsequently packaged and sent to the Laboratory of Food Microbiology and Biotechnology (Agricultural University of Athens, Greece) for microbial testing. The received samples were placed in polystyrene trays and stored at different temperatures (0, 5, 10, 15^{o}C for the seaweed, and 0 and 4^{o}C for the salmon) for specific time intervals. Microbiological analysis was performed on the day of their arrival at the lab and at certain days of storage, for the estimation of Total Viable Counts (TVC), *Pseudomonas* spp., Lactic acid bacteria, Enterobacteriaceae, *Bacillus* spp., *B. thermosphacta*, *Vibrio* spp., *Aeromonas* spp., yeast and moulds, *E. coli*, *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus*. Nutritional analyses, including the determination of protein, fat, carbohydrate, ash and moisture content, were performed on the day the samples arrived at the lab.

Results and Discussion

Representative microbial populations of salmon samples (0 and 4^{o}C) are presented in Fig. 1. Products were of acceptable microbial quality (TVC < 7.0 log CFU/g) and safe for human consumption for up to 12 days stored at 0^{o}C and 8 days stored at 4^{o}C. The time period for seaweed samples (Fig. 2) was 8 and 4 days for 0 and 5^{o}C, respectively, while initial microbial load was in accordance with findings reported by Blikra et al. (2019). It should be noted that bacteria of Enterobacteriaceae family which are considered as hygiene indicator, were at low levels - even below enumeration limit - in both species, while all the examined pathogenic bacteria were absent both at the beginning and at the end of storage. Although further research is intended regarding quality and safety of IMTA products, for example, the detection of antibiotic resistant microbial strains, these preliminary findings provide significant information about the overall microbiological quality of IMTA products. As far as the nutritional quality of the tested samples is concerned, specific nutritional parameters (such as protein, fat and carbohydrate content) were found to be similar to reference values for the same species (non-IMTA).

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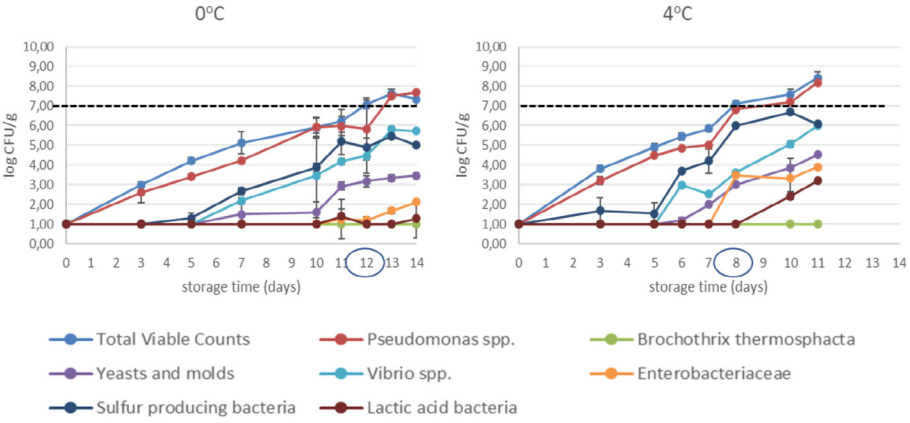

Fig 1. Microbial populations of salmon throughout storage at 0 and 4 °C

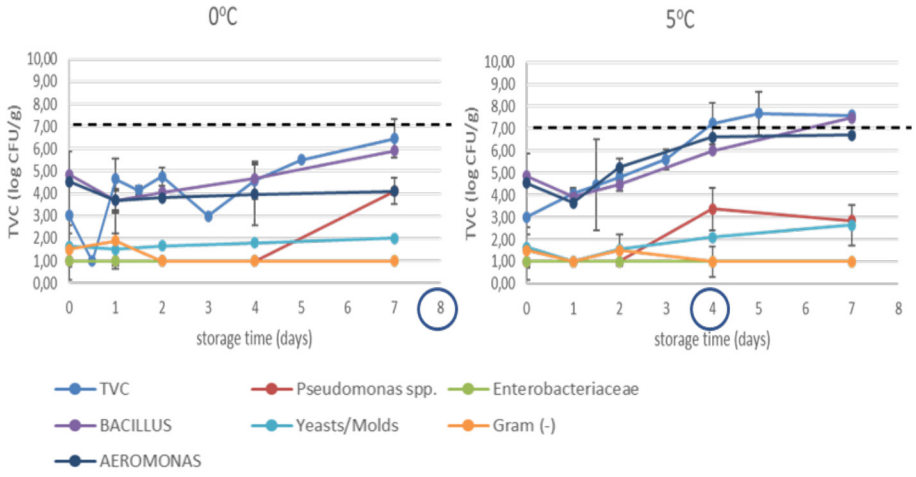

Fig 2. Microbial populations of seaweed *Alaria esculenta* cultivated in IMTA and stored at 0 and 5 °C

Acknowledgement

This work has been supported by project ‘’IMPAQT’’ (EU H2020 research and innovation programme under Grant Agreement No 774109).

References

Blikra, M. J., Løvdal, T., Vaka, M. R., Roiha, I. S., Lunestad, B. T., Lind-seth, C., Skipnes, D. (2019). Assessment of food quality and microbial safety of brown macroalgae (*Alaria esculenta* and *Saccharina latissima*). Journal of the Science of Food and Agriculture, 99, 3,1198–1206.

Califano G., Kwantes M., Abreu M.H., Rodrigo, R., Wichard T. (2020). Cultivating the Macroalgal Holobiont: Effects of Integrated Multi-Trophic Aquaculture on the Microbiome of *Ulva rigida* (Chlorophyta). Frontiers in Marine Science, 7, 52.

QUANTIFICATION OF *Flavobacterium psychrophilum* IN IMMERSION CHALLENGED ATLANTIC SALMON (*Salmo salar*) SELECTED FOR DISEASE RESISTANCE

Macchia, V.*[1], Grammes, F.[2], Davie, A.[1], Reeve A.[3], Moen, T.[2,] Adams, A.[1], Desbois, A.[1], Hoare, R.[1]

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
[2]AquaGen, 7462 Trondheim, Norway. [3]AquaGen Scotland Ltd, 16a Stirling University Innovation Park, Stirling, Scotland, FK9 4NF, UK
E-mail: valeria.macchia@stir.ac.uk

Introduction

*Flavobacterium psychrophilu*m is the causative agent of rainbow trout fry syndrome (RTFS), which has caused substantial losses in the rainbow trout (*Oncorhynchus mykiss*) industry globally, including the UK, for decades. The disease is widespread and can cause high mortality in fry and larger fish in freshwater hatcheries and on-growing sites. Antibiotics are often used to treat affected stock and currently no vaccines are available in the UK. Recently, *F. psychrophilum* has also been isolated from Atlantic salmon fry (*Salmo salar*) in Scotland causing concern for the industry. Using a newly developed immersion challenge model we conducted a challenge trial to search for markers of resistance to *F. psychrophilum* in a 2018 cohort. A subsequent GWAS (genome-wide association study) of the immersion challenged salmon fry (n=3060) revealed a strong quantitative trait loci (QTL) located on chromosome 9.

Aim & Materials and Methods

The aim of the current study was to test if the bacterial load was correlated to the alleles at the QTL. In order to test this hypothesis, the immersion challenge was repeated (2019 cohort) in small scale (n=400) using a different *F. psychrophilum* strain. Salmon fry (n=50) were sampled at day 3, 6 and 10 post challenge. DNA was extracted from head kidney tissue (n=70) and subsequently used to i) determine the genotype of each individual fish, using a high-density SNP chip (70,000 markers) and ii) measure the bacterial load using a qPCR assay for *rpoC* – a *F. psychrophilum* characteristic gene, adapted for use with SYBR Green. The elongation factor 1-alpha gene (*ef1a*) was used to normalise the amount of host DNA relative to bacterial DNA.

Results

The SYBR Green based qPCR assay developed in this study suggests that this method is rapid, sensitive and reproducible for the specific detection and quantification of *F. psychrophilum* load in fish tissue. Therefore, this tool can be used for diagnostic testing and surveillance programmes. Additionally, this assay helped in determining the correlation between bacterial load in salmon head kidney tissues and their genotypes. Finally, the selection of resistance to *F. psychrophilum* in Atlantic salmon is a promising alternative strategy to the use of a restricted number of antibiotics to control RTFS.

CREATION OF A NOVEL TOOL FOR THE AQUACULTURE INDUSTRY TO DELIVER HEALTHY AND SUSTAINABLE SEAFOOD

Alan MacDonald*, Natalia Serpetti and Sofia C. Franco

Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, PA37 1QA
Email: alan.macdonald@sams.ac.uk

Introduction

The fast-growing contribution of aquaculture to global seafood production in recent years has increased scrutiny of the long-term environmental and socio-economic sustainability of the industry. Environmental and ecological concerns include the sustainability of aquaculture feeds, the management of water quality and benthic impacts, health and welfare challenges, biosecurity and escapees, amongst others. Addressing these has become critical to unlock the development of the industry, both in established segments, such as Atlantic salmon (*Salmo salar*) or blue mussels (*Mytilus* spp.), and novel species, such as sugar kelp (*Saccharina latissima*) farming.

Social acceptance and environmentally sustainable production of Atlantic salmon in Scotland have been aided by effective operational changes, such as the replacement of wild-caught fish in feeds by terrestrial ingredients, as well as trials to explore the potential of integrated multi-trophic aquaculture (IMTA) to reduce the local footprint of fish farms. However, this decrease of wild-caught fish in aquaculture feed has reduced the poly-unsaturated fatty acid (PUFA) content in the final product (Sprague et al. 2016), affecting the key marketable attribute and, thus, the health value of oily fish. Furthermore, though feed composition has been linked to the products' nutritional value, it is unclear how changes in feed composition translate to changes in the farm-level environmental footprint of production, a crucial issue to the sector's regulation and social licence to operate.

In practice, though past trials (i.e. IMTA) aimed to reduce the local footprint of fish farms and real operational changes (i.e. ingredient substitution in feed) target a reduction in the global impacts of fish farming, no study has looked at the health-environmental synergies and conflicts that result from changes in operational choices. Several questions arise from this: How does changing fish feed affect the health value of aquaculture products and the farm-level environmental footprint? How sensitive is the farm-level environment to changes in the fish feed? And, if sensitive, how can negative impacts be minimised? Does this change in monoculture and IMTA contexts?

We propose that the nutritional quality of final products and the local environmental impacts at farm-level should not be assessed in isolation. Here we present a new application for industry to quantify the environmental, economic and nutritional output of aquaculture, in both monoculture and IMTA, given multiple operational changes.

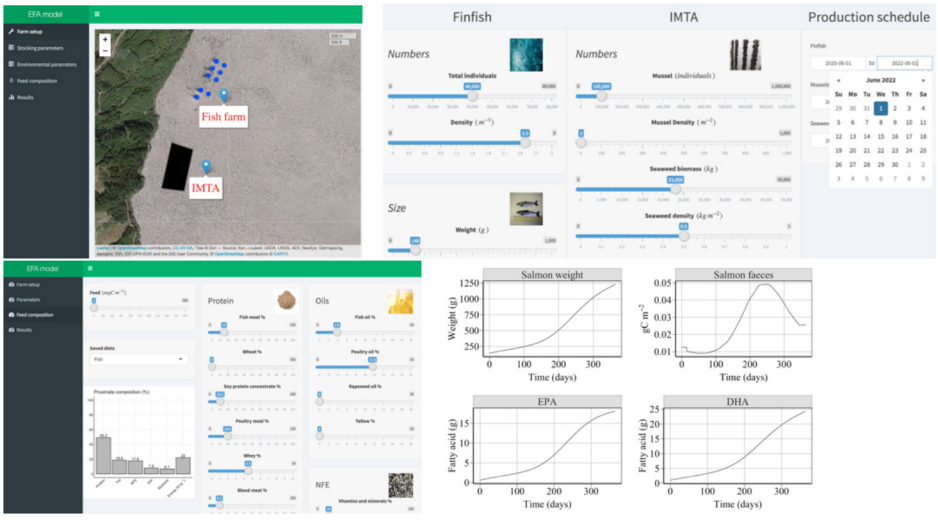

Fig. 1. An example of setting up a farm (upper left), selection of production variables (upper right) and feed ingredients (lower left) and output simulation of changes in salmon weight, total faeces and fatty acid profile (shown for EPA and DHA only) (lower right).

(Continued on next page)

Material and methods

An existing ecosystem model (Ren et al., 2012) was coupled to new models of fatty acid dynamics for Atlantic salmon, blue mussels and sugar kelp. The removal of salmon faeces and uneaten feed by mussels was modelled using a new size-dependent consumption model. A graphical user interface (GUI) allows the user to create a virtual Atlantic salmon monoculture and IMTA (Atlantic salmon, blue mussels, sugar kelp) farm and predict the consequence of operational changes to the local environment and nutritional output.

The user can vary farm location, the abundance of cultured and co-cultured species and the proximate composition of the fish (salmon) feed. Values selected by the user are fed into the underlying coupled ecosystem and PUFA model, driven by temperature and proximate composition of the fish feed. Modelled output is species abundance and fatty acid content, particulate organic carbon and nitrogen, dissolved organic nitrogen, ammonium and nitrate concentrations in the water column, total organic carbon and nitrogen concentrations on the sediment. The concentration of the latter nutrients can be used as a proxy for the farm-level environmental footprint.

Results

We demonstrate the model with results from an IMTA farm given a representative feed. Figure 1 shows an example of how results are viewed in the GUI for salmon fatty acids and daily faecal waste from the salmon stock (the latter is a large contributor to total organic waste of the farm). Further outputs, such as fatty acid content in mussels and seaweed, and nutrient concentrations will be presented in full at the conference.

Discussion and conclusion

The FYNE model was designed for business use and allows to hit targets for fatty acid nutritional quality and harvestable weight, while helping to comply with regulatory frameworks on farm discharges and environmental impact. Current work is being done on model validation and parameterisation of the ecosystem component and will be presented at the conference. Future model development will focus on the economics of monoculture and IMTA farms so that, given a choice of farm setup, the user is presented with a timeline of running costs and profit. User testing of the GUI and discussion with businesses will identify features to be improved for commercial use. These additions will ensure the GUI offers a holistic view of a farm to optimise trade-offs between profit, nutritional value of the products and local environmental impacts during grow-out stage.

References

Chang et al. 2019, shiny: Web Application Framework for R, R Package Version 1.4.0. Available at http://CRAN. R-project. org/package= shiny.

Ren et al. 2012. Ecological Modelling, 246, 34-46.

Sawyer et al. 2016. Ecological Modelling, 323, 96-105.

Sprague et al. 2016. 2006–2015. Scientific reports, 6(1), 1-9.

METHIONINE AND TRYPTOPHAN PLAY DIFFERENT MODULATORY ROLES IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*) INNATE IMMUNE RESPONSE AND APOPTOSIS SIGNALLING – AN *IN VITRO* STUDY

M. Machado[1,2,3,4*], C. Serra[1], A. Oliva-Teles[1,5], N. M. dos Santos[3,4] and B. Costas[1]

*mcasimiro@ciimar.up.pt
[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR),Portugal
[2] Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Portugal
[3] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Portugal
[4] Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal
[5] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto (FCUP), Portugal

Introduction: The range of metabolic pathways that are dependent on a proper supply of specific amino acids (AA) unveils their importance in the support of animal's metabolism and health. AA play central roles in vital pathways for immune support and specific AA supplementation has shown to be able to modulate fish immunity. Even though *in vivo* trials are an important tool to evaluate the immunomodulatory role of AA at the organism and tissue level, *in vitro* studies allow a high screening capability to unveil relevant information at a cellular level. The present *in vitro* approach was conceived to evaluate methionine and tryptophan role in immune-related mechanisms aiming to understand their direct effect in leucocyte functioning and AA pathways.

Material and methods: Primary cultures of head-kidney leucocytes were established and kept in AA (methionine or tryptophan) supplemented cultured media and the effect on cell viability was assessed. Also, nitric oxide, ATP, total antioxidant capacity and immune-related genes were evaluated in response to lipopolysaccharides extracted from *Photobacterium damselae* subsp. *piscicida* or UV-inactivated bacteria (UV*Phdp*). Moreover, caspase-3 activity and apoptosis-related gene expression were evaluated in response to the apoptosis-inducing protein, AIP56.

Results and discussion: Methionine and tryptophan showed a distinct role in the leucocyte's immune response, with different and contrasting outcomes through the modulation of specific key pathways. On one hand, methionine surplus improved leucocytes viability and increased polyamine production and methionine-related gene expression in response to inflammation. It also induced lower signals of apoptosis by AIP56 induction, with lower caspase 3 activity and higher *nfk*β expression. On the other hand, cells cultured in tryptophan-supplemented medium presented several signals of an attenuated inflammation, with decreased ATP production and enhanced expression of anti-inflammatory genes. In response to AIP56, leucocytes cultured in tryptophan-rich medium presented lower resilience to the toxin with higher caspase 3 activity, higher expression of caspase 8, and lower expression of *nfk*β. In conclusion, the *in vitro* studies showed the ability of methionine surplus to improve cell response to an inflammatory insult and to lower the signals of apoptosis signals by AIP56 induction, while tryptophan attenuated several cellular signals of inflammatory response to UV*Phdp* and lower cell resilience to AIP56.

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EU-CONEXUS- EUROPEAN UNIVERSITY FOR SMART URBAN COASTAL SUSTAINABILITY: EDUCATION AND RESEARCH

E. Malandrakis*[1], A. Tsopelakos[1], E. Chatzoglou[1], I. Baer-Eiselt[3], N. Ntalamagka[2], L. Delvaux[3], F. Baltaretu[4], V. Charitou[1], E. Flemetakis[1], S. Kintzios[1], A. De Luis[2], A. Margineanu[4], G. Moschopoulou[1], N. Narbutiene[2], A. Sancho[2], P. Vidal[2], H. Miliou[1]

[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
Email: emalandrak@aua.gr

Introduction

EU-CONEXUS was created in the framework of the European universities' initiative, led by the European Commission, with the aim to strengthen strategic partnerships across Europe and develop international competitiveness of European universities. EU-CONEXUS, addresses global and local challenges by tackling coastal environmental, technical, economic and societal needs. The EU-CONEXUS consortium includes six universities partners from all European geographical regions who have close relationship to coastal environment, face common and shared socio-economic and intellectual challenges: La Rochelle Université (France), Universidad Católica de Valencia (Spain), Zadar University (Croatia), Agricultural University of Athene (Greece), Technical University of Civil Engineering of Bucharest (Romania) and the University of Klaipeda (Lithuania) and 3 Associated Partners: Waterford Institute of Technology (Ireland), Rostock University (Germany) and Frederick University (Cyprus). EU-CONEXUS has focused on urban and semi-urban coastlines as increasingly densely populated areas and very important for trade, aquaculture and fisheries, energy, tourism. At the same time, these coastlines are the most vulnerable areas, with regard to the consequences of climate change. Created around the theme of Smart Urban Coastal Sustainability (SUCS), the university aims to create joint study and research programs. The EU-CONEXUS Joint Research Area aims to brings together researchers and students from the different partner institutions around common fields of interest. The final EU-CONEXUS goal is to become the global leader in higher education and research in the area of SUCS, with a perspective of expansion beyond the size of its founding consortium.

Materials and Methods

To identify international skills and competencies that employees are expected to have, EU-CONEXUS has developed a list of stakeholders from public and private field, active in the field of: agriculture, food processing industry, aquaculture, fisheries, marine biotechnology, construction, coastal management, energy, data science, environmental and digital law, ports, tourism, to which a first survey was submitted. The survey results are to be used for the development of the EU-CONEXUS study offer and activities for students. Two Minor programmes have been organized and launched as interdisciplinary set of courses in the same or different field of studies, that can be supplementary to main Study Programme. Courses can be chosen freely and make up to 30 ECTS throughout studies. A questionnaire for the Scientific mapping has been developed in order to record Research units and the Research teams from all participant Universities. A Joint Research Steering Committee (JRSC) has been created by the collaboration of all partners in order to manage a network of Joint Research Institutes (JRIs). Four JRIS have been created in order to bring together researchers and students from the different partner institutions around common fields of interest. Joint Standard Operating Procedures (JSOPs) has been developed for the JRIs, unifying and harmonizing common research protocols and processes between laboratories. In order to support the development of the EU-CONEXUS Joint Research Area, a call for research staff mobility among the alliance has been launched. Research staff can visit another partner or associated partner university, its research units/laboratories or to participate to a research conference in-situ. EU-CONEXUS supports the implementation of joint research projects in the framework by creating a Project Development Fund. Eligibility and Funding criteria, Selection Rules, Dissemination Process and Application documents have been prepared.

Results

As a result of successful cooperation, EU-CONEXUS reached one of its first achievement. On the 15th of January, the European University, launched its first joint educational offer of two Minors, to which 220 students were enrolled. The Minor in Coastal Development and Sustainable Maritime Tourism provides with competences and professional skills in five key sectors: Biodiversity and Coastal Zone Management, Environmental Education. Human Geography, Sustainable Tourism, Coastal Engineering. The Minor in Blue Economy and Growth is related to the main industrial and service sectors

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of the blue economy: Aquaculture and Fisheries, Marine Biotechnology, Ocean Energy, Transport and Shipbuilding, Coastal and Maritime Tourism. The developed skills map will help define the needs of economic actors in an urban coastal environment, in regard to the professionalizing education and vocational training. The continuous involvement of the regional economic communities will allow the constant adaptation of the studies offered to their evolving needs. In the field of Research, has given an overview of EU-CONEXUS research characteristics on the level of research units and research teams, encompassing all faculties and disciplines of the partner universities, providing with a solid information base of the research activities in EU-CONEXUS. The results of the Scientific mapping showed that Life Sciences and Biotechnology Institute consists of 21 research units and 61 research teams; Environmental Sciences and Biodiversity Institute consists of 11 research units and 43 research teams; Coastal Engineering Institute consists of 32 research units and 81 research teams; Social, Culture and Human Sciences Institute consists of 41 research units and 127 research teams. The call for Project Development Fund as well as the Research Staff Mobility call have been launched, in order to develop collaborative research and innovation projects. It is expected for researchers to collaborate together, to be trained on certain protocols and processes or impulse new joint research projects on the smart urban coastal sustainability topic.

Conclusions

The Minor programs bring a lot of advantages to students. They will access a multi- and transdisciplinary curriculum that will allow them to shape their studies according to their future professional needs. They will gain from an international environment as they will have access to various resources and experts in order to develop their knowledge and intercultural competencies. They will interact with students and teachers from 5 other countries, raising their cultural awareness and social skills. Enhancing international cooperation in joint research activities, EU-CONEXUS Joint Institutes and research teams represent a multicultural, multilingual and multidisciplinary environment for conducting excellent research and innovation projects. Researchers and students from the different partner institutions collaborate on interdisciplinary research challenges, sharing common equipment, research outputs and methodology. The Project Development Fund supports EU-CONEXUS partners for the preparation of research and innovation projects, thus enhancing submission to international calls. The call for Research Staff Mobility gives researchers the opportunity to scientific knowledge exchange, life-long training and job shadowing on SUCS topics. In conclusion, EU-CONEXUS based on the decade-long expertise of the partner universities, will be able to cover SUCS from a global point of view, by cross disciplinary based approaches, vocational training, professionalizing education and innovative research in the field of blue growth.

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ACTIVITY BASED RESOURCES ALLOCATION (ABRA) MODEL ON ASSESSING COST-EFFECTIVENESS OF IMTA INSTALLATIONS

G. Malindretos[1*], P. Kinanlis[1], J.Casserly[2], F. Kane[2]

[1] Harokopio University, Athens, Greece
email [1*] gmal@hua.gr
[2] Marine Institute, Galway, Ireland

Introduction
For the purposes of IMPAQT project (H2020, GA 774109), the research team assigned to assess the cost effectiveness of Integrated Multi-Trophic Aquaculture (IMTA), exploring methodologies to evaluate IMTA against mono-culture installations, being the previous state on Marine Institute site, the leading project's pilot partner. Combining existing literature and actual day-to-day observations on the pilot site provided by both managerial and research executives, Harokopio University and Marine Institute research teams worked on the activity-based resources allocation (ABRA) model to assess the cost-effectiveness of IMTA. The model provides allocation by both activity and species on site, in order to provide a clear before/after comparison.

Literature review
According to Krishnan (2006), activity-based costing (ABC) is a system that reduces the level of random cost allocations associated with the traditional costing systems. ABC improves decisions making, involving resource allocation, product mix, pricing and marketing (e.g. Mishra et al., 2017; Homburg et al., 2018).

Kumar and Mahto (2013) suggest ABC as a costing methodology that identifies activities in an organization and assigns the cost of each activity to all products and services according to the actual consumption.Analysis usescost drivers, factors that relate to a change in the cost of every business activity. Due to this, a cost driver is a measure of the amount of resources consumed by an activity.

Porter (1985) suggests a cost driver can be used to optimize and coordinate the performance of activities. In Activity-Based Costing (ABC) a large number of diverse cost drivers may be used, between resources - activities and between activities - products. ABC allows an in-depth product analysis by explaining the relationships between the products and activities.

Methodological steps
ABRA model focuses on cost-effectiveness assessment on the finfish pilot Marine Institute, a research centered non-profit organization in Ireland, which added shellfish and seaweed to the existing Atlantic salmon in the framework of the IMPAQT project. After separating installation costs, MI focused on the day-to-day expenses and resources, before and after IMPAQT system, which includes both IMTA and the IADAS IT system providing real-time data, integral for production monitoring (refereed as "D" cost category and associated with two activities, namely "infrastructure maintenance" and "routine inspections", table 2).

The following tables present the results of the ABRA model application in Marine coastal site. For both conditions before and after the deployment of the new system, observations and measurements have been applied in order to estimate the allocation of labor's effort to the various activities. Specific activities, such as stock harvesting , that do not take place every month , have been converted on a monthly rate.

Results by applying ABRA on the project's pilots
ABRA model resulted the following total costs per species and platform maintenance.

It is noticeable, that although total labor cost increases, the cost allocated to species A is reduced by almost 7 % (from 9000 to 8,405). This is a very significant outcome that constitutes a part of the overall cost effectiveness analysis which further links cost of resources with the anticipated monetary benefits derived from the implementation of the new system. In other words ABRA model provides a first indication of the new system's prospects based on the expected savings due to the new nature of the activities engaged in IMTA operations. In addition, it identifies the real nature of cost behavior, allowing the monitoring of the resources used to the various activities, contributing to their effective re-engineering and continuous improvement.

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Table 1 - Allocation of labor's effort before the deployment of IMTA & IADAS system

PROCESS : HARVEST MONOCULTURE		ACTIVITIES	Stock deployment	Fish feeding	Mortality removal and disposal	Stock Management	Stock harvesting	Environmental monitoring	Record keeping	Biosecurity	Routine inspections	Stock maintenance	Boat maintenance	Infrastructure maintenance	total
SPECIES : Atlantic Salmon															
RESOURCES	number	COST/month (€)	1,2%	27,2%	3,9%	3,6%	1,2%	4,9%	4,9%	9,7%	9,7%	4,8%	3,9%	25,1%	100%
Personnel	2	9000	107,62	2448,36	349,74	322,83	107,62	437,22	437,22	874,44	874,44	430,20	349,78	2260,09	9000

Table 2 - Allocation of labor's effort after the deployment of IMTA & IADAS system

A	B	C	D
89%	4%	3%	4%
8405	362	320	383

A -Salmon in an IMTA Scenario
B - Unfed species, Shellfish
C - Plants, Seaweed
D - IMPAQT Platform

Table 3 -Total cost per species (after)

References

Homburg, C., Nasev, J., & Plank, P. (2018). The impact of cost allocation errors on price and product-mix decisions. Review of Quantitative Finance and Accounting, 51(2), 497-527.

Krishnan, A. (2006). An application of activity based costing in higher learning institution: A local case study. Contemporary management research, 2(2), 75

Kumar, N. &Mahto, D. (2013). Current Trends of Application of Activity Based Costing (ABC)

Mishra, B. K., Prasad, A., Srinivasan, D., &ElHafsi, M. (2017). Pricing and capacity planning for product-line expansion and reduction. International Journal of Production Research, 55(18), 5502-5519

Porter, M.E., (1985). Competitive Advantage, The Free Press, New York.

SPERM QUALITY IN CAPTIVE-BRED BARRAMUNDI *Lates calcarifer*: EFFECT ON SPAWNING PERFORMANCE AND PATERNAL CONTRIBUTION

A.F. Marc[ab*], J.L. Guppy[b], J. Goldsbury[b], D. Rudd[a], H.S. Cate[c], D.B.B.P. Paris[ab], and D.R. Jerry[b]

[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
[c] Mainstream Aquaculture Group Pty Ltd, Werribee, VIC 3030, Australia
E-mail: adrien.marc@my.jcu.edu.au

Introduction
Barramundi (*Lates calcarifer*) is a species with a mass-spawning reproductive strategy, whereby adults synchronously release their gametes into the water column. In male barramundi, this spawning strategy usually results in differential broodstock contributions to progeny cohorts, creating skews in family size that hinder the effectiveness of selective breeding programs (Domingos et al., 2014; Frost et al., 2006; Loughnan et al., 2013). As sperm quality is known to influence fertilization success and early larval development (Herráez et al., 2017), the relationship between sperm quality of captive-bred broodstock and paternal contribution following mass-spawning events was investigated.

Materials and methods
The fertility of male barramundi (n = 22) from three different breeding cohorts were assessed. The physical condition of broodstock were recorded including body weight, total length and condition factor (K). Milt samples were collected through testicular cannulation and sperm volume, concentration and total count were determined. Sperm quality assessment was performed, including sperm motility using computer-assisted sperm analysis and sperm integrity (i.e. viability assay and TUNEL DNA fragmentation assay) using flow cytometry (Marc et al., 2021). Mass-spawning events were induced using intramuscular injection of luteinizing hormone-releasing analogue. Broodstock spawned on two consecutive nights after the injection. Eggs at 2.5 h and 12 h post-fertilization (hpf), and larvae at 24 h and 48 h post-hatch (hph) were collected to assess spawning success and survival. Offspring collected at 2.5 hpf and 24 hph were also genotyped using microsatellites (Domingos et al., 2014) to determine their parentage and to examine the relationship between sperm quality of individual males and offspring survival.

Results
Firstly, it was found that male physical condition and sperm quality were highly variable within each of the breeding cohorts. Males with a lower condition factor showed lower sperm motility, whereas males with a higher condition factor showed higher sperm motility and higher levels of sperm DNA damage. Secondly, highly skewed paternal and maternal contributions were observed in all spawns resulting in loss of genetic diversity and high inbreeding rates in offspring (5.9 to 24.4%). The total number of eggs released and the rate of fertilization was variable between spawns and cohorts. Offspring mortality occurred mainly during embryonic development between 2.5 hpf and hatching at 12 hpf, where about 25% of fertilized embryos had arrested development. Mortality of larvae continued over the next 24 h of larval development where an additional ~25% of larvae died before larvae survival rates between 24 and 48 hph stabilized across all spawns (≤ 90.9%). Lastly, the analysis of the relationships between male fertility and spawning performance showed that while all males demonstrated fertilization capability, paternal contribution was dominated by males with a lower condition and high sperm concentration in offspring collected at 2.5 hpf. However, while the paternal contribution of offspring collected at 24 hpf was also associated with males with a lower condition, a strong relationship between sperm DNA damage and larvae survival ($r(22) = .66, P < .001$) was observed. Interestingly, this relationship between sperm DNA damage and larvae survival rate was only significant in offspring generated during the first spawning night. Overall, it was determined that male fertility accounted for up to 33% of the total variation observed in mass-spawning events.

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Discussion and conclusion

In this study, the outcome of mass-spawning events using captive-bred barramundi broodstock was similar to previous reports including high skewed paternal contribution, resulting in high inbreeding rates (Domingos et al., 2014; Frost et al., 2006; Loughnan et al., 2013). The investigation of male barramundi fertility revealed for the first time the presence of a high variation in physical condition, milt characteristics and sperm quality between male broodstock. Although sperm motility is used as a common indicator of fertility (Valdebenito et al., 2015), in this study, sperm concentration was the only pre-spawning assessment measure found to be significantly correlated to the fertilization rate. In line with recent studies (Herráez et al., 2017), the direct negative relationship between sperm DNA damage and larval survival rate of the first night of spawning provides evidence of an important paternal effect on offspring development in barramundi. However, the absence of a relationship between sperm DNA damage and the larval survival rate of the second spawning night indicates that sperm DNA damage level measured before spawning does not reflect the level of spermatozoa released during the second night. It is likely, DNA damage occurs in mature spermatozoa that had been stored for an extended time in the sperm duct and not during spermatogenesis. Lastly, while male fertility accounted for up to 33% of the total variation observed, the effect of male fertility on paternal contribution seemed overshadowed by additional variables such as spawning behaviour, social hierarchy and potential stress associated with the spawning induction. Due to the complex dynamics occurring during mass-spawning events, development of artificial reproductive technology is recommended to generate offspring using artificial fertilization to gain control over parental genetic contribution and improve the rate of genetic gain in selective breeding programs.

References

Domingos, J.A., Smith-Keune, C., Jerry, D.R., 2014. Fate of genetic diversity within and between generations and implications for DNA parentage analysis in selective breeding of mass spawners: A case study of commercially farmed barramundi, *Lates calcarifer*. Aquaculture 424–425, 174–182.

Frost, L.A., Evans, B.S., Jerry, D.R., 2006. Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). Aquaculture 261, 1056–1064.

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.

Loughnan, S.R., Domingos, J.A., Smith-keune, C., Forrester, J.P., Jerry, D.R., Beheregaray, L.B., Robinson, N.A., 2013. Broodstock contribution after mass spawning and size grading in barramundi (*Lates calcarifer*, Bloch). Aquaculture 404–405, 139–149.

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.

Valdebenito, I.I., Gallegos, P.C., Effer, B.R., 2015. Gamete quality in fish: Evaluation parameters and determining factors. Zygote 23, 177–197.

DO CONSUMERS WANT INFORMATION ABOUT AQUACULTURE ?

Catherine Mariojouls* (1), Terhi Latvala (2), Rafael Gines (3), Laurent Muller (4)

(1) AgroParisTech, UMR SAD-APT, University Paris-Saclay, France
catherine.mariojouls@agroparistech.fr
(2) LUKE, Finland
(3) University Las Palmas de Gran Canaria, Spain
(4) INRAE, UMR GAEL, France

Introduction
The question of whether fish consumers want information about aquaculture is complex and little studied. In AquaImpact European research programme, WP4 is dedicated to communication, including consumers' studies which first step is a qualitative study through consumers'focus-groups.

Methodology
In Finland, France and Spain, a series of five consumers' focus groups was organized during spring and summer 2019. Each focus-group gathered about 10 participants, all being fish consumers, with diversified profiles as for gender, age, professional. In the three countries, the focus-groups were organized similarly, with a moderator following a guide, asking questions and facilitating answers and discussion within the group. All focus-groups were fully recorded and fully transcripted before analysis. Each focus-groups lasted from 1,5 to 2 hours.

The discussion was organized in three main themes: 1 : Fish consumption habits and choices ; 2 : About fish farming : information existing, needs for information ; 3 : About receiving a set of information on fish farming… Theme 3 included two phases : we tested the reactions of consumers after being shown a basic information package (including a brief presentation of world aquaculture, then 6 slides about European salmon farming, as farmed salmon is imported and consumed in the three countries), then we asked the consumers their opinion about disseminating information about aquaculture to the public in the future.

Main results
Unsurprisingly, the consumed fish species and their presentation vary among the three countries, but showed no contradiction compared to the results of previous surveys. The answers show that in the three countries, some people choose strictly wild fish, because they prefer their taste or because they reject farmed fish, while others buy both wild and farmed fish, some without paying attention to the production mode. Many other criteria than 'wild' or 'farmed' have been quoted as playing a role in the buying act, notably price is important.

Information received today by consumers about fish farming is considered scarce but not actively searched (in France, and by some participants in Finland and Spain), while considered abundant by some others, in Finland and Spain. Many sources of information are quoted, from the vendor to mass media, notably TV reports being mentioned as especially important in France but bringing mainly negative information, while in Finland the contents of TV reports and social media are questioned.

Consumers expressed doubts and questions about fish farming, mostly in France, also in Finland, less in Spain where fish farming is perceived more positively. The main topics being questioned in the three countries are feed content and use of antibiotics and drugs, to a lesser extent fish welfare, while concerns are expressed about pollutants and controls.

After being shown basic information on aquaculture in eight slides, most participants declared they are satisfied to receive information, but did not consider it the same way. Asked to score the novelness of information from 1 (min) to 5 (max), compared to what they knew before, in Finland and Spain participants scored it from 3 to 5, with very high scores (4 and 5) frequently attributed, while in France the average scoring was lower (3 to 4), with rare score 5 and some very low (2 and 1). Beyond « novelness », some participants, especially in France and also in Finland, question the content of the discourse, as for reliability and style.

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For the future, disseminating information to all public is unanimously accepted as good and necessary, because consumers want to have complete information and transparency. They call for information on a wide range of topics from farming conditions to regulation and fish health, and through many suggested channels. Credibility of information sources was discussed, without showing unanimous preferences for some sources. Some consumers in France and Spain mention that certification of farmed fish may bring overall reassurance and may reduce the need to search information from multiple sources.

Beyond the information needs, there are some ethical concerns expressed by some consumers, especially in France, about intensive fish farming. In the three countries, consumers made references to other sectors of animal production (cattle, poultry, pigs), and we noticed some common features with general trends about perception of agriculture production and food consumption.

Conclusion and perspectives

This first phase in our consumers'studies show that being brought information on aquaculture is seen positively in the three countries, on a wide range of subjects, but our results also unveil many questions and, from some participants, criticism about intensive fish farming.

The results of this qualitative work have been used to build a quantitative survey on a wide panel, whose results are under analysis.

MOLECULAR AND FUNCTIONAL CHARACTERISATION OF *fads2*, *elovl5* AND *elovl2* GENES IN GRASS CARP (*Ctenopharyngodon idella)*

M. Marrero[1*], Ó. Monroig[2], J.C. Navarro[2], A. Ribes-Navarro[2], A. Galindo[1], J.A. Pérez[1], C. Rodríguez[1]

[1]Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, S/C de Tenerife, Spain
[2]Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), Castellón, Spain
*E-mail: mmarrera@ull.edu.es

Introduction

Long-chain ($\geq C_{20}$) n-3 and n-6 polyunsaturated fatty acids (LC-PUFAs) are essential nutrients involved in critical biological processes ensuring normal growth and development of vertebrates including fish. Current aquafeeds have high inclusion levels of vegetable oils (VOs), devoid of LC-PUFAs but typically containing C18 polyunsaturated fatty acids (PUFA) such as α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). Consequently, there is a growing interest to understand the capacity of farmed fish species to biosynthesise the physiologically important LC-PUFAs arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from the C18 PUFAs precursors available in the diet. In fish, the LC-PUFA biosynthesis pathways involve sequential desaturation and elongation reactions from ALA and LA, catalysed by fatty acyl desaturases (Fads) and elongation of very long-chain fatty acids (Elovl) proteins. In particular, the Fads2 is a key enzyme that catalyses the introduction of new double bonds between an existing one and the carboxylic group of the fatty acyl chain (Castro et al., 2016). Moreover, Elovl2 and Elovl5 have demonstrated to play pivotal roles in PUFA elongation (Castro et al., 2016). The aim of this work was the molecular cloning and functional characterisation of three genes (*fads2*, *elovl5* and *elovl2*) encoding enzymes involved in LC-PUFA biosynthesis pathway in the grass carp (*Ctenopharyngodon idella*), an herbivorous freshwater fish with a high market value and reputation for its meat quality in Asian countries.

Material and methods

To obtain full-length *fads2*, *elovl5* and *elovl2* sequences, blast searches were carried out on the Transcriptome Shotgun Assembly (TSA), and the archive used was *Ctenopharyngodon idella*; taxid: 7959. Partial TSA sequences were subsequently assembled with the online tool cap3. The open reading frames (ORFs) of the corresponding target genes were identified and then isolated by PCR from *C. idella* liver and brain (1:1) complementary DNA (cDNA) as template and primers with specific restriction sites for further cloning into pYES2 vector. The *C. idella* Fads2, Elovl5 and Elovl2 were functionally characterized by heterologous expression in yeast. Transgenic yeast expressing the *C. idella fads2* were grown in the presence of a series of exogenously supplemented PUFA substrates to test the Δ6 (18:3n-3; 18:2n-6; 24:5n-3), Δ8 (20:3n-3; 20:2n-6), Δ5 (20:4n-3; 20:3n-6) and Δ4 (22:5n-3; 22:4n-6) desaturase capabilities. Additionally, yeast expressing the *C. idella elovl5* and *elovl2* were grown in the presence of different PUFA substrates to test the C18 → C20 (18:3n-3; 18:2n-6; 18:4n-3; 18:3n-6), C20 → C22 (20:5n-3; 20:4n-6) and C22 → C24 (22:5n-3; 22:4n-6) elongase activities. After 48 h of incubation, yeast were harvested and washed. Total lipids extracted from yeast were used to prepare fatty acyl methyl esters that were analysed by gas chromatography. Conversions of PUFA substrates to the corresponding products were calculated according to the formula: [individual product area/(all products areas + substrate area)] x 100.

Results

The results showed that *C. idella* Fads2 has Δ5, Δ6 and Δ8 activities. Moreover, the Elovl5 presented higher conversion rates in C18 substrates than Elovl2, which presents higher values in those of C20 and C22 (Table 1).

Discussion and conclusions

Our results confirm the data recently published (Xie et al., 2020) reporting on the Δ6 activity of the *C. idella* Fads2. However, our study further show that this enzyme has also the Δ5 and Δ8 desaturation capabilities and hence enabling this species to perform all the desaturation reactions (Δ5, Δ6 and Δ8) required for the biosynthesis of the biologically active ARA and EPA from C_{18} precursors. Moreover, the *C. idella* Fads2 was able to desaturate 24:5n-3 to 24:6n-3, a Δ6 desaturation reaction required for the biosynthesis of DHA through the so-called "Sprecher pathway" (Tocher et al., 2003). Moreover, functional assays of the *C. idella* elongases showed that Elovl5 was able to elongate C_{18} and C_{20} PUFA substrates whereas Elovl2 exhibited elongation ability towards all PUFA assayed, with particularly high activity towards C_{20} and C_{22} substrates. Overall, the three enzymes studied herein showed preferences by n-3 substrates in agreement with other teleost species (Galindo et al., 2020). In conclusion, the present study shows that all the desaturase and elongase activities required to convert C_{18} PUFA into ARA, EPA and DHA are present in *C. idella*.

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Table 1. Functional characterisation of the *C. idella* Fads2, Elovl5 and Elovl2: conversions (%) of PUFA substrates.

	Desaturase				Elongases			
	Conversion (%)				Conversion (%)			
Substrate	Product	Fads2	Activity	Substrate	Product	Elovl5	Elovl2	Activity
18:3n-3	18:4n-3	3.6	Δ6	18:3n-3	20:3n-3	17.74	9.25	C18 → C20
18:2n-6	18:3n-6	0.9	Δ6	18:2n-6	20:2n-6	13.17	1.20	C18 → C20
20:3n-3	20:4n-3	2.0 [a]	Δ8	18:4n-3	20:4n-3	68.69	22.64	C18 → C20
20:2n-6	20:3n-6	0.2 [a]	Δ8	18:3n-6	20:3n-6	58.04	10.04	C18 → C20
20:4n-3	20:5n-3	1.3	Δ5	20:5n-3	22:5n-3	45.14	71.08	C20 → C22
20:3n-6	20:4n-6	0.8	Δ5	20:4n-6	22:4n-6	31.32	42.99	C20 → C22
22:5n-3	22:6n-3	nd	Δ4	22:5n-3	24:5n-3	0.56	19.84	C22 → C24
22:4n-6	22:5n-6	nd	Δ4	22:4n-6	24:4n-6	nd	19.41	C22 → C24
24:5n-3	24:6n-3	0.8	Δ6					

[a] Conversions of substrates 20:3n-3 and 20:2n-6 by Fads2 include stepwise reactions due to multifunctional desaturation abilities. Thus, the conversions of the *C. idella* Fads2 on 20:3n-3 and 20:2n-6 include the Δ8 desaturation toward 20:4n-3 and 20:3n-6, respectively, and their subsequent Δ5 desaturation to 20:5n-3 and 20:4n-6, respectively. For the elongases, all product areas include those of the initial elongation products, as well as those from stepwise elongations occurring subsequently. Not detected (nd).

References

Castro et al., 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire. Prog. Lipid Res., 62, 25-40.

Delgado-Lista et al., 2012. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. Br. J. Nutr. 107, S201–S213.

Galindo et al. 2020. Polyunsaturated fatty acid metabolism in three fish species with different trophic level. Aquaculture, 530, 735761.

Laviano et al., 2013. Omega-3 fatty acids in cancer. Curr. Opin. Clin. Nutr. Met. C. 16, 156–161.

Tocher et al., 2003. Biochemical and molecular studies of the polyunsaturated fatty acid desaturation pathway in fish. Fishlarvae.com, pp. 211-228.

Xie et al., 2020. Comparsion of activities of fatty acyl desaturases and elongases among six teleosts with different feeding and ecological habits. Front. Mar. Sci. 7:117.

EXPLORING DIFFERENT DIETS AND FEEDING FRECUENCY IN *OCTOPUS VULGARIS* JUVENILES REARED IN CAPTIVITY

M. Martínez-Pérez[1], A. Castro-Bugallo[1], A. Roura*[1,2]

[1] Estación de Ciencias Marinas de Toralla, CIM, Universidade de Vigo.
[2] Instituto de Investigaciones Marinas de Vigo (IIM-CSIC), Eduardo Cabello 6, Vigo
*Corresponding author email: chiquipulpi@gmail.com

Introduction

Octopus vulgaris is a good candidate for the diversification of the aquaculture industry by several reasons: high growth rate, high percentage of protein, high fertility, good feed conversion, good commercial value and an increasing extension of its market. This fact has generated a wide field of study, where researchers, and more recently, private companies have committed resources to develop viable cultivation techniques for industrial production. An essential objective in this long-term goal, is the achievement of viable feeding methods. There are several fattening studies testing fresh/unfrozen ingredients (Chapela et al. 2006; Estefanell et al. 2011), and even formulated feeds (Cerezo-Valverde et al. 2017; Morillo-Velarde et al. 2015) to grow sub-adults >0.5kg collected from the wild. However, there is a complete lack of studies evaluating the acceptance of different diets in early juveniles. In this work, we carried out a series of short feeding trials evaluating feeding rhythms (daily feeding and intermittent fasting) and food sources (6 natural foods and 5 formulated moist diets) in juveniles of *O. vulgaris* reared in captivity.

Methods

The specimens used in this study were grown in captivity within the project OCTOBLUE at Estación de Ciencias Marinas de Toralla (ECIMAT). Eight one-week experiments were carried out using two treatments with three replicates and one individual per replicate. During the first three weeks, two feeding frequencies were tested: daily intake (DI) and intermittent fasting (IF,one fasting day every two) using three natural defrosted foods: galatheids (*Munida gregaria*), hoki (*Macruronus magellanicus*) and sea bass (*Dicentrarchus labrax*). After that, five experiments were carried out using intermittent fasting testing natural food - hoki, prawn heads (*Pleoticus muelleri*), live mussels (*Mytilus galloprovincialis*) and fresh opened mussels - against five formulated moist diets.

Six *O. vulgaris* juveniles (13 to 25.8 g) were placed in 6 rectangular 10-liter plexiglass tanks. An open flow (50 renewals / day) of filtered seawater (5 µm) was used: Tª 18º ± 0.3ºC., S 35 ‰. An equinoctial photoperiod was applied, using a led lamp (Fluval Marine Led of 59 w) oriented to the roof to avoid direct light. Oxygen concentration and salinity were eventually checked, temperature was measured by continuous recording. Routinely tasks carried were: a) weighing and addition of food, b) cleaning, removal and weighing of food excess, c) weighing all individuals once a week. The food

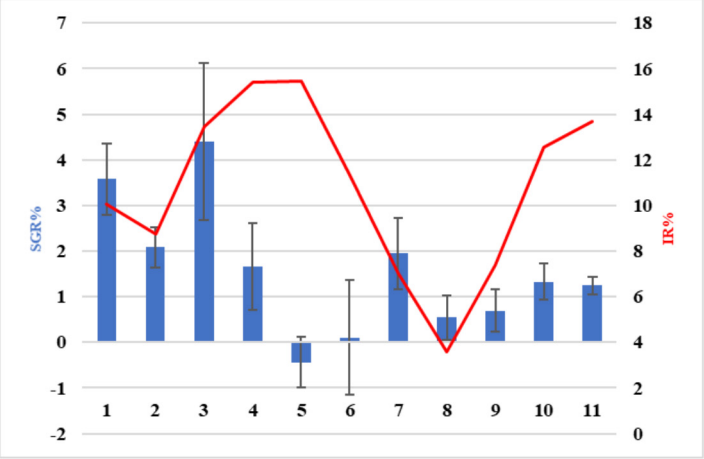

Figure 1. Average IR(%) and SGR(%) for the different food evaluated in the intermittent fasting trials. SGR error bars correspond to standard deviation. The horizontal axis numbers correspond to: 1) seabass, 2) hoki, 3) galatheids, 4) prawn heads, 5) live mussel, 6) fresh opened mussel, 7-11) formulated diets 1 to 5.

EXPLORING DIFFERENT DIETS AND FEEDING FRECUENCY IN *Octopus vulgaris* JUVENILES REARED IN CAPTIVITY

M. Martínez-Pérez[1], A. Castro-Bugallo[1], A. Roura*[1,2]

[1] Estación de Ciencias Marinas de Toralla, CIM, Universidade de Vigo.
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*Corresponding author email: chiquipulpi@gmail.com

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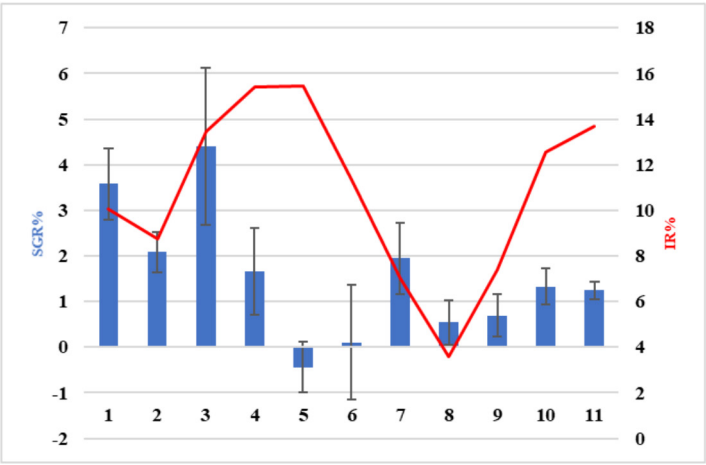

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Results

At the end of the study the specimens ranged from 63.5 to 115.4 g and no mortality was registered.

Feeding period

Juveniles subjected to intermittent fasting ate significantly more (IR_{IF} = 10.76±3.57%) than those with daily intake (IR_{DI} = 6.21±2.49%, $p<0.05$), but no significant differences were observed in SGR, despite being higher for the IF (SGR_{IF} = 3.35±1.41%) than those with DI (SGR_{DI} = 2.93±0.85%). FCR values were similar for both feeding periods (FCR_{DI} = 2.23±0.27, FCR_{IF} = 2.26±0.27). Independently of the feeding period, galatheids were the food that promoted better growth (3.9-4.4%) followed by seabass (2.46-3.58%) and hoki (1.35-2.41%).

Differences between foods with intermittent fasting

The best daily growths were obtained using galatheids (4.40±1.72%) and seabass (3.58±0.69%), despite not being the most ingested food (Fig. 1). Shrimp heads were consumed in abundance (IR above 15%) but unproductively (FCR=6.97±3.92). Mussels were easily opened by the juveniles and ingested with avidity, but it was the only food that promoted negative growth, even when administered opened to reduce octopus's energy expenditure. All formulated diets were accepted by the juveniles (IR on average between 3.6 and 13.7%) and serve as a basis for improvement. The first formulated diet reached very acceptable SGR (1.94±0.77%), the highest ever recorded for a formulated diet in

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References

Cerezo-Valverde, J., García, B.G. 2017. High feeding and growth rates in common octopus (*Octopus vulgaris*) fed formulated feeds with an improved amino acid profile and mixture of binders. *Aquaculture Research*. 48:3308–19.

Chapela, A., González, Á.F., Dawe, E.G. 2006. Growth of common octopus (*Octopus vulgaris*) in cages suspended from rafts. Scientia Marina, 70(1), 121–129.

Estefanell, J., Socorro, J., Tuya, F., Izquierdo, M., Roo, J. 2011. Growth, protein retention and biochemical composition in *Octopus vulgaris* fed on different diets based on crustaceans and aquaculture by-products. *Aquaculture*. 322–323:91–8.

Morillo-Velarde, P. S., Valverde, J. C., García-García, B. 2015. A simple format feed to test the acceptability of ingredients for common octopus (*Octopus vulgaris* Cuvier, 1797). Aquaculture Research, 46(4), 994–1000.

CULTIVATION OF THE SEAWEED *Ulva* spp. WITH EFFLUENT FROM A SHRIMP BIOFLOC REARING SYSTEM: DIFFERENT SPECIES AND STOCKING DENSITY

Mateus A. Martins[1,*], Vitor F. da Silva[1], Patricio R. Tarapuez[2], Leila Hayashi[3], Felipe do N. Vieira[1].

[1] Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Laboratório de Camarões Marinhos – LCM. Rua dos Coroas, 503, Barra da Lagoa, CEP: 88061-600, Florianópolis, SC, Brazil
[2] Universidad de Nariño, Facultad de Ciencias Pecuarias, Programa de Ingeniería em Producción Acuícola, Calle 18 carrera 50, Bloque 2, Piso 4, Sede Torobajo, Pasto, Nariño, Colombia.
[3] Universidade Federal de Santa Catarina - UFSC, Departamento de Aquicultura, Seção de Macroalgas do Laboratório de Camarões Marinhos - LCM. Rua dos Coroas, 503, Barra da Lagoa, CEP: 88061-600, Florianópolis, SC, Brazil.
*E-mail: m.aranha.martins@gmail.com.

Introduction

Integrated Multitrophic Aquaculture (AMTI) is a promising approach to deal with the low recovery of nutrients observed in many production systems, including biofloc technology (BFT) (Poli et al., 2019). In this regard, seaweeds, such as *Ulva* spp. can be used to take advantage of the dissolved inorganic substances accumulating in the rearing units, considering their economic importance and the subsequent uses of the harvested algae (Silva et al., 2013). However, studies focused on seaweed growth performance when cultivated in a biofloc environment or using biofloc-rich water are lacking. Therefore, this study aimed to evaluate two aspects of the cultivation of *Ulva* using effluent from biofloc shrimp rearing: different species and stocking density.

Material and methods

An experiment was conducted in a completely randomized design in triplicate from 4 June 2019 to 25 June 2019 to evaluate the cultivation of *Ulva fasciata* and *U. ohnoi* collected from the wild in the city of Florianópolis, SC, Brazil. After proper acclimation, they were stocked in semi-cylindrical 60 L tanks equipped with heating and aeration systems and located inside a greenhouse under natural irradiance. Initially, 15 L of water from a shrimp biofloc rearing unit was filtered through a bag type filter and mixed with 45 L of seawater in each of the tanks. Weekly, the water was discarded and the same procedure was performed. In addition, every week the algae were weighed and screened for adhered organisms.

The best performing algae, *U. ohnoi*, was then subjected to an experiment lasting from 26 September 2019 to 16 October 2019 to evaluate two stocking densities, 2 and 4 g L^{-1}, in a completely randomized design in quadruplicate. Newly collected algae were cultivated using the same experimental units as mentioned in the previous paragraph.

Results

When evaluating different macroalgae species, *U. ohnoi* performed significantly better than *U. fasciata* for all variables evaluated (Table 1). In fact, there was an average decrease in *U. fasciata* biomass throughout the three-week experiment. In the assessment of the different stocking densities of *U. ohnoi*, although the highest stocking density resulted in a significantly higher final biomass, the lower density resulted in a significantly greater specific growth rate (Table 1).

Discussion

Reasons for the better growth performance of *U. ohnoi* when compared to *U. fasciata* could be that the algae were more adapted to culture conditions due to the fact that they were collected in lagoons instead of the beach intertidal zone for the other species. It has been shown that wild algae growing under different environmental conditions exhibit different morphologies, which can then affect nutrient uptake rates and location in the water column (Raven and Taylor, 2003), factors that affect their growth performance.

The significantly lower specific growth rate found for the highest stocking density of *U. ohnoi* was likely caused by self-shading due to an excessive algae biomass, being a common occurrence when algae stocking densities are increased (Shin et al., 2020).

Conclusion

U. ohnoi performed significantly better than *U. fasciata* and its cultivation was more efficient under the 2 g L^{-1} density, due to the highest specific growth rate achieved. Overall, this research highlights the importance of species selection and optimisation of culture conditions for macroalgae cultivated in integrated systems using BFT.

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Table 1: Growth performance of seaweeds assessed throughout two three-week experiments evaluating algae species (*Ulva fasciata* and *Ulva ohnoi*) and algae density in the culture of *U. ohnoi* when employing water from a biofloc system as fertilizer.

Variable	Seaweed species		*p*-value	Different densities (*U. ohnoi*)		*p*-value
	U. fasciata	*U. ohnoi*		2 g L^{-1}	4 g L^{-1}	
Final biomass (g)	189.0±74.6	379.6±51.7*	0.022	301.9±24.8	378.8±53.0*	0.039
Change in biomass (g)	-13.1±75.3	182.5±50.3	0.020	181.5±24.9	137.9±53.1	0.188
SGR (% day^{-1})	-†	3.0±0.6	-	4.3±0.4	2.7±0.7*	0.006
Yield (g m^{-3} day^{-1})	-†	138.3±38.1	-	137.5±18.8	104.5±40.2	0.188

Data presented as mean±standard deviation. *Statistically significant. Student´s t-test used in all instances. SGR: Specific Growth Rate. †There was no SGR and no yield.

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References

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.

Raven, J., Taylor, R., 2003. Macroalgal growth in nutrient-enriched estuaries: a biogeochemical and evolutionary perspective. Water, Air & Soil Pollution Focus, 3: 7-26.

Shin, S.K., Kim, S.K., Kim, J.H., Han, T., Yarish, C., Kim, J., 2020. Effects of stocking density on the productivity and nutrient removal of *Agarophyton vermiculophyllum* in *Paralichthys olivaceus* biofloc effluent. Journal of Applied Phycology, 32: 2605-2614.

Silva, M., Vieira, L., Almeida, A.P., Kijjoa, A., 2013. The marine macroalgae of the genus *Ulva*: chemistry, biological activities and potential applications. Journal of Oceanography and Marine Research, 1(1): 1-6.

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 238.2 thousand tons in 2018.

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 231.0 thousand tons in 2018, 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 km^2. 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 2018; 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.

INVESTIGATING FACTORS INFLUENCING THE EFFICACY OF NANOFILTERED HYPOSALINE WATER TO CONTROL SEA LICE ON A COMMERCIAL ATLANTIC SALMON (*Salmo salar)* FARM

Tom Mc Dermott[a]*, Jack D'Arcy[a], Suzanne Kelly[a], Robert F. Kerr[b], Damien O'Keefe[b], Michael Ó Ceallacháin[b], Felix Scholz[c], Neil M. Ruane[a]

[a] Marine Institute, Rinville, Oranmore, H91 R673 Co. Galway, Ireland
[b] Bradán Beo Teoranta, Kilkieran, Co. Galway, Ireland
[c] FishVet Group, Oranmore, Galway, Ireland

Corresponding author at: Marine Institute, Rinville, Oranmore, Co. Galway H91 R673 Ireland. Tel.: +353 91 387200
E-mail address: tom.mcdermott@marine.ie

Introduction

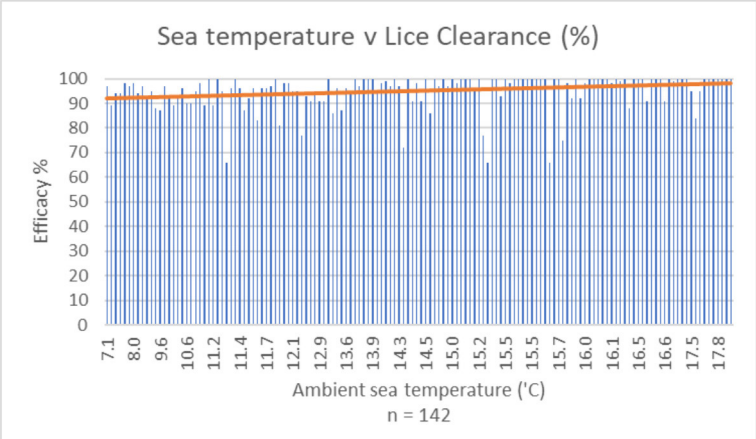
Sea lice are currently a significant threat to the Atlantic salmon aquaculture industry in Ireland. Concerns of anthropogenic climate forcing and a move away from chemotherapeutants to meet stringent organic standards has prompted farms at this latitude to re-examine their approach to control this threat by investigating host-parasite life histories, especially *in situ* developmental response to novel abiotic conditions (Borchel et al., 2021; Cerbule et al., 2020; Bui et al., 2019). Soft freshwater, a proven success in treating AGD was also shown to reduce sea lice numbers (Powell et al., 2015). In 2019, a novel technique to bath treat salmon for sea lice with freshwater produced from the sea by nanofiltration was trialled by a commercial organic farm in Kilkieran Bay on the West coast of Ireland.

Materials and methods

Taking advantage of improvements in the production of soft freshwater at sea, floating nanofiltration plants were deployed with capacity to treat salmon for sea lice and AGD throughout the production cycle. Selectively removing bivalent and monovalent ions a 90 m tarpaulin-lined pen was filled with 1,934 ML of 5ppt freshwater. Salmon were pumped, dewatered, and moved to the treatment pen and retained for 4.5 h. After treatment the salmon were pumped back to the original pen in their grid. Sea lice were monitored by the Marine Institute pre- and post-treatment, and throughout 2019 and 2020 in keeping with the National Sea Lice Monitoring Programme (O'Donohoe et al., 2020; O'Donohoe et al., 2021*in press*). Analysis of treatments (n = 142) included additional sampling by the farm at their sites in Kilkieran Bay with all results collated to investigate factors likely to influence efficacy.

Fig. 1

Efficacy as ambient sea temperatures ('C) increased



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Results

By treating both AGD and sea lice in a single operation it was possible to manipulate retention time, hyposalinity, pH and pumping time during tarp baths to achieve an annual > 90+% efficacy for sea lice at both sites in 2019 and 2020 with equivalent AGD control to that achieved by comparable freshwater derived from a terrestrial source. High efficacy helped both farms maintain sea lice levels with just 2 notices to treat (NTT). In addition, efficacy was not impeded at high ambient temperatures (Fig. 1), and concerns relating to heavy metal toxicity (Al^{3+}) were addressed while maintaining biosecurity.
Fig. 1

References

Borchel, A., Heggland, E. I. & Nilsen, F. (2021). The transcriptomic response of adult salmon lice (*Lepeophtheirus salmonis*) to reduced salinity, Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, Volume 37. doi.org/10.1016/j.cbd.2020.100778

Bui, S., Oppedal, F., Sievers, M., Dempster, T. 2019. Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. Rev. Aquacult. 11, 168-186. https://doi.org/10.1111/raq.12232

Cerbule, K., Godfroid, J. 2020. Salmon louse (*Lepeophtheirus salmonis* (Krøyer)) control methods and efficacy in Atlantic salmon *(Salmo salar* (Linnaeus)) aquaculture: a literature review. Fishes 5, 11. https://doi.org/10.3390/fishes502001

O'Donohoe, P., Kane, F., Kelly, S., McDermott, T., D'Arcy, J., Casserly, J., Downes, J., McLoughlin, S., Ruane, N., Jackson, D. 2020. National survey of sea lice (*Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann) on fish farms in Ireland – 2019. Irish Fisheries Bulletin No. 50. Marine Institute. http://hdl.handle.net/10793/1590

O'Donohoe, P., Kane, F., Kelly, S., McDermott, T., D'Arcy, J., Casserly, J., Downes, J., McLoughlin, S., Ruane, N. 2021. National survey of sea lice (*Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann) on fish farms in Ireland – 2020. Irish Fisheries Bulletin No.52. Marine Institute. *In press*

Powell, M.D., Reynolds, P., Kristensen, T. 2015. Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: considerations of water chemistry and fish welfare in Norway. Aquaculture 448, 18-28. https://doi.org/10.1016/j.aquaculture.2015.05.027

SALMON FROM THE NORTH: INSIGHT INTO THE CARBON FOOTPRINT OF SALMON FROM FAROE ISLANDS AND LOW EMISSION TRANSPORT TO THE MARKET

Shraddha Mehta[1], Jon Gregersen[2]

[1] SINTEF Ocean, Brattørkaia 17C, 7010 Trondheim, Norway
email: shraddha.mehta@sintef.no
[2] HiddenFjord Salmon, (PFLuna), Faroe Islands

Introduction

Farmed salmonoids production in The Faroe Islands was close to 80 000 tons in 2018 which is more than 40% share of all export goods[1]. The Faroese aquaculture industry has evolved since the 1980s and today consists of a rather small number of vertically integrated companies that are highly committed to sustainable growth[2]. Given the stringent environmental regulations since 2003 and a comparatively lower average of feed factor the Faroese producers claim their products to be environmentally sustainable[3,4]. However, the environmental footprint of the aquaculture activities in the Faroe Islands has not been investigated before and has not been compared with its neighbouring countries like Norway and Iceland. The Faroe Islands have a well-developed trade infrastructure and are connected to the global markets by effective logistics making it possible to export fresh fish[5]. A significant volume of salmon produced in the Faroes is exported to global market and the assessment of the carbon footprint of the different transport modes is necessary. The goal of this study is to highlight the factors and the different parameters that affect the GHG emissions from farmed salmon production in the Faroe Islands, its transport to the international market and present some solutions to reduce emissions. A company specific case study will be presented to demonstrate results from the emission reduction measures.

Material and Methods

This study adopted a holistic approach from raw material extraction to consumer market known as 'cradle-to-retailer' based on ISO standard 14040:2006 on principles and framework for Life Cycle Assessment (ISO, 2006)[6]. Primary data for the life cycle assessment has been obtained from the production of HiddenFjord salmon and its export to the international markets. The SimaPro software and the NTMcalc advanced tool has been used to calculate the GHG emissions from the production and transport of salmon.

Results

The GHG emissions of the different modes of transport from Faroes are calculated and the factors such as load factor, type of vehicle, return logistics that have a significant impact on the total emissions are evaluated. The percentage contribution of the different production stages to total the carbon footprint of salmon is calculated. The percentage reduction in GHG emissions from moving air transport to sea transport to specific markets has also been estimated. The findings of this study will be compared with the results of the carbon footprint of Norwegian salmon published by SINTEF[7].

Discussion and Conclusion

The carbon footprint assessment of the aquaculture production in the Faroe Islands gives an insight to the significant contributing factors to total carbon footprint and highlight the opportunities to reduce the footprint. This study will make it possible to compare differences and similarities in the production practices, technology, and emission reduction strategies between the Norwegian and the HiddenFjord salmon. Future opportunities for low emission transport and the consumer feedback and quality preferences will also be discussed.

1. https://strandbunadur.is/wp-content/uploads/2019/03/4.-Niels-nytt.pdf
2. https://sjomatnorge.no/wp-content/uploads/2018/06/ISFA-Report-2018-FINAL-FOR-WEB.pdf
2. http://salmon-from-the-faroe-islands.com/environment.html
4. https://www.faroeseseafood.com/species/faroe-islands-salmon/
5. https://www.faroeseseafood.com/fishery-aquaculture/sustainable-aquaculture/
6. ISO (2006). Environmental management – life cycle assessment – principles and framework. ISO14040. *Geneva: International Organization for Standardization,* ISO 14040:2006(E); 2006a

INSECT-BASED DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*) AND ITS IMPACT ON GUT HEALTH AND PROTEIN UTILIZATION

Melenchón F.[1*], Larrán A.M.[1], de Mercado E.[1], Weththasinghe P.[2], Lagos L.[2], Mercado L.[3], Cortés M.S.[4], Tomás-Almenar C.[1]

Aquaculture Research Centre, Agro-Technological Institute of Castilla y León, Segovia, Spain
*melramfe@itacyl.es
Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway
Molecular Immunology and Genetics Laboratory, Biology Institute, Pontifical Catholic University of Valparaíso, Valparaíso, Chile
Laboratory of Immunology, Center of Aquatic Biotechnology, Department of Biology, University of Santiago of Chile, Chile.

Introduction

The search for protein alternatives to fishmeal in fish feed is still one of the main challenge of aquaculture. As such, some of the most promising alternatives are insect meals, due to reasons like the high value of their protein or the low environmental impact of their production. Insects have already proven their efficiency in fish growth on lower inclusion levels (Dumas, 2018; Melenchón, 2020). The interest in insect meals has increased over the last years, and this work provides insight on the effect of the inclusion of two different insect meals on gut health and their efficiency in the use of protein.

Material and Methods

Three isoproteic (43%) and isolipidic (17.5%) diets were tested by replacing 50% of fishmeal (18% feed inclusion) with two different insect meals: *Hermetia illucens* (HI50), and *Tenebrio molitor* (TM50), against a control diet (C; 0% fishmeal replacement). 360 rainbow trouts (*Oncorhynchus mykiss*) with an initial body weight of 14.5 g were cultivated in a RAS system, in optimal and controlled conditions during 77 days, until they reached a final body weight of 75.9 g. At the end of the growth trial, distal intestine histomorphology and concentration of TNF-α, together with efficiency in the use of protein, were analysed.

Table I. Effect on protein utilization and gut health

Growth performance	C	HI50	TM50	SEM
Weight Gain (%)[1]	415.03^{a}	354.74^{b}	438.84^{a}	12.43
Feed Conversion Ratio[1]	0.90^{b}	0.98^{a}	0.88^{b}	0.02
Protein utilization				
$ADC_{Protein}$ (%)[1]	92.58^{a}	81.01^{b}	91.17^{a}	1.05
Protein Efficiency Ratio	2.49^{ab}	2.34^{b}	2.58^{a}	0.05
Gut health				
Villi height (μm)	287.52^{b}	247.92^{c}	335.82^{a}	11.83
Villi Width (μm)	50.47^{a}	46.78^{b}	51.31^{a}	1.19
Lamina Propria width (μm)	10.50^{a}	8.54^{b}	10.20^{a}	0.44
TNFα-DI (ng/ L)	0.43^{ab}	0.33^{b}	0.58^{a}	0.06

[1]Data shown as reference (Melenchón *et al*., 2019). HI50: 50% fishmeal replacement with *Hermetia illucens*; TM50: 50% fishmeal replacement with *Tenebrio molitor*; $ADC_{Protein}$: apparent digestibility coefficient of the protein; TNFα-DI: Tumor Necrosis Factor alpha in distal intestine; $^{a, b}$ Indicate significant differences ($P<0.05$) between diets. Data are expressed as lsmeans ± SEM (standard error of the mean).

(Continued on next page)

Results and discussion

Fish fed with C and TM50 had longer and wider villi than HI50 in distal intestine (Table I). As consequence of lower villi height, the absorption surface was reduced, and fish fed with HI50 showed a poor protein utilization and growth performance (Table I and Melenchón *et al*., 2019). The lower villi width could be related to the decrease of lamina propria size due to a lower cellular infiltration (20 % in HI50 vs. 40 % in C and TM50), corroborated by a lower level of TNFα in distal intestine (Table I). Other authors showed that high levels of HI can lead to these same villi alterations in fish (Dumas *et al*., 2018; Vargas-Abúndez *et al*., 2019). HI has a higher amount of chitin than TM in its composition (7.5 *vs*. 5.9 %), which could be one possible explanation for the villi shortening, as well as for the lower digestibility of the protein (Kroeckel *et al*., 2012). This could also be related to the lower level of TNFα in distal intestine, since it has been described that chitin might have an anti-inflammatory effect (Lopez-Santamarina *et al*., 2020). Nevertheless, since TM50 showed the best growth performance and no detrimental effects on health, the higher cellular infiltration was considered within physiological levels; as shown in a previous experience (Melenchón *et al*., 2020), there is a safety margin for the inclusion of chitin and HI meal in rainbow trout feeds.

Bibliography

Dumas A., Raggi T., Barkhouse J., Lewis E., Weltzien E. 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, 492: 24–34.

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, 364–365: 345–352.

Lopez-Santamarina A., del Carmen A.M., Lamas A., Miranda J.M., Franco C.M., Cepeda A. 2020. Animal-Origin Prebiotics Based on Chitin: An Alternative for the Future? A Critical Review. Foods, 9: 782.

Melenchón F., Larrán A., Fernández I.V., de Mercado E., Sánchez-Muros M.J., Fabrikov D., Hidalgo M.C., Cardenete G., Lourenço H.M., Tomás-Almenar C. 2019. Aquaculture Europe 2019. Our future. Growing from water (EAS2019), pp. 903-904.

Melenchón F., Larrán A.M., de Mercado E., Hidalgo M.C., Cardenete G., Barroso F., Fabrikov D., Lourenço H.M., Pessoa M.F., Tomás-Almenar C. 2020. Potential use of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) insectmeals in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, early view, **https://doi.org/10.1111/anu.13201**

Vargas-Abúndez A.J., Randazzo B., Foddai M., Sanchini L., Truzzi C., Giorgini E., Gasco L., Olivotto I. 2019. Insect meal based diets for clownfish: Biometric, histological, spectroscopic, biochemical and molecular implications. Aquaculture, 498: 1–11.

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EXPLORING THE LIVER FUNCTIONALITY ON RAINBOW TROUT *(Oncorhynchus mykiss)* FED INSECT-BASED DIETS

Melenchón F.[1*], Larrán A.M.[1], de Mercado E.[1], Hidalgo M.C.[2], Morales A.E.[2], Sánchez-Muros M.J.[3], Fabrikov D.[3], Tomás-Almenar C.[1]

Aquaculture Research Centre, Agro-Technological Institute of Castilla y León, Segovia, Spain
*melramfe@itacyl.es
Department of Zoology, University of Granada, Granada, Spain
Department of Biology and Geology, University of Almería, Almería, Spain

Introduction

Due to reasons like the value of their protein, or to the low environmental impact of their production, insectmeals are some of the most promising alternatives to fishmeal. The inclusion of insects in fish feed formulations have already proven their efficiency in fish growth on lower inclusion levels (Melenchón, 2020). This work provides insight on the effect of the inclusion of two insect meals on liver functionality.

Material and Methods

A total of 360 rainbow trouts (*Oncorhynchus mykiss*) with an initial body weight of 14.5 g were cultivated in a RAS system during 77 days, up to a final body weight of 75.9 g. Three isoproteic (43%) and isolipidic (17.5%) diets were tested by replacing 50% of fishmeal (18% feed inclusion) with two different insect meals: *Hermetia illucens* (diet HI50), and *Tenebrio molitor* (diet TM50), against a control diet (C; no fishmeal replacement). After the trial, somatic indices, liver histomorphology and the activity of intermediary metabolism enzymes in liver were analysed.

Results and discussion

Concerning liver histomorphology, the sizes of hepatocyte cytoplasms and nuclei, as well as a qualitative analysis focused on tissue necrosis and cellular vacuolization, did not show any significant differences ($P>0.05$). Even though the growth of HI50 showed a worse performance than C and TM50, this supports the idea that the status of HI50 fish was within healthy margins, which is close to the results of a previous experience with a lower level of fishmeal replacement (Melenchón *et al*., 2020). However, HI50 fish had the highest viscerosomatic index (VSI) over C and TM50. The work of Mikołajczak *et al*. (2020) showed differences in VSI between a diet with *Zophobas morio* and another one with *Tenebrio molitor*; this could had been due either to the different levels of saturated fatty acids between both insects, which could lead to lipid accumulation in viscera, or to the lower digestibility levels of *Zophobas morio* when compared with *Tenebrio molitor* (Fontes et al., 2019), since low digestibilities might increase intestinal length (German and Thorn, 2006). However, there is very few literature about this last point, so it would remain as speculative.

Talking about intermediary metabolism enzymes in liver, no differences were found between C and TM50, which agrees with the results of Mikołajczak *et al*. (2020) and Chemello *et al*. (2020). However, HI50 showed significant differences for glutamate oxaloacetate transaminase (GOT; differences with TM50). Since this enzyme is known as an indicator of good availability and use of the protein, this result would agree with the lower protein digestibility and weight gain observed in these fish. In conclusion, the insect-based diets did not alter the structure and functionality of the liver, while it is true that HI50 had worse efficiency in the use of protein and, in consequence, growth performance.

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Table I. Effect of insectmeals on growth and liver functionality

Growth performance[1]	**C**	**HI50**	**TM50**	**SEM**
Weight gain (%)	415.03^{a}	354.74^{b}	438.84^{a}	12.43
Feed Conversion Ratio	0.90^{b}	0.98^{a}	0.88^{b}	0.02
$ADC_{Protein}$ (%)	92.58^{a}	81.01^{b}	91.17^{a}	1.05
Somatic indices				
Viscerosomatic index	14.35^{b}	15.92^{a}	14.53^{b}	0.45
Hepatosomatic index	1.27	1.44	1.29	0.07
Liver Intermediary Metabolism (U/mg protein)				
Fructose 1,6-biphosphatase (FBPase)	26.63	22.77	27.72	3.9
Pyruvate kinase (PK)	55.1	50.16	64.87	7.22
Glucose−6-phosphate dehydrogenase (G6PDH)	39.34	33.12	38.89	3.36
Glutamate dehydrogenase (GDH)	574.55	379.37	522.96	58.23
Glutamate pyruvate transaminase (GPT)	329.30	371.52	333.51	35.43
Glutamate oxaloacetate transaminase (GOT)	270.25^{ab}	169.31^{b}	326.32^{a}	38.24
Liver Histomorphology (µm)				
Hepatocyte nucleus diameter	2.28	2.31	2.23	0.03
Hepatocyte diameter	4.58	4.65	4.60	0.06

[1]Growth performance data shown as reference (Melenchón *et al*., 2019); $ADC_{Protein}$: apparent digestibility coefficient of the protein. C: control diet; HI50: 50% fishmeal replacement with *Hermetia illucens*; TM50: 50% fishmeal replacement with *Tenebrio molitor*; U: unit of enzyme activity. $^{a, b}$ Indicate significant differences ($P<0.05$) between diets. Values expressed as mean ± SEM (standard error of the mean; n = 4 tanks per diet, 2 fish per tank).

Bibliography

Chemello, G., Renna, M., Caimi, C., Guerreiro, I., Oliva-Teles, A., Enes, P., Blasato I., Schiavone A., Gai F., Gasco, L. (2020). Partially defatted *Tenebrio molitor* larva meal in diets for grow-out rainbow trout, *Oncorhynchus mykiss* (Walbaum): Effects on growth performance, diet digestibility and metabolic responses. Animals, 10(2), 229.

Fontes T.V., Batista de Oliveira K.R.B., Gomes Almeida I.L., Orlando T.M., Rodrigues P.B., da Costa D.V., Rosa P.V. (2019). Digestibility of Insect Meals for Nile Tilapia Fingerlings. Animals, 9: 181.

German D.P., Horn M.H., 2006. Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Marine Biology, 148: 1123–1134.

Melenchón F., Larrán A., Fernández I.V., de Mercado E., Sánchez-Muros M.J., Fabrikov D., Hidalgo M.C., Cardenete G., Lourenço H.M., Tomás-Almenar C. 2019. Aquaculture Europe 2019. Our future growing from water (EAS2019), pp. 903-904.

Melenchón F., Larrán A.M., de Mercado E., Hidalgo M.C., Cardenete G., Barroso F., Fabrikov D., Lourenço H.M., Pessoa M.F., Tomás-Almenar C. 2020. Potential use of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) insectmeals in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, early view, https://doi.org/10.1111/anu.13201

Mikołajczak, Z., Rawski, M., Mazurkiewicz, J., Kieronczyk, B., & Józefiak, D. (2020). The effect of hydrolyzed insect meals in sea trout fingerling (*Salmo trutta* m. *trutta*) diets on growth performance, microbiota and biochemical blood parameters. Animals, 10(6), 1031.

Acknowledgements: study supported by INIA (ref: RTA 2015-00021-C03), FEDER, Agencia Estatal de Investigación (Ministry of Science, Innovation and Universities, ref: BES2017-080567) and FSE funds; feeds elaborated by LifeBIOENCAPSULATION S.L.

BRANCHIAL NITROGEN CYCLE SYMBIONTS 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

Recirculating aquaculture systems (RAS) present an environmentally sustainable method to culture fish. Due to the reuse of water and high fish density, accumulation of waste excreted by the fish can be problematic. Teleost fish produce ammonia as their main nitrogenous waste and excrete most of it via their gills. Since ammonia has a negative effect on fish health and water quality, reducing water ammonia concentrations is of key interest in RAS.

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. This project aims to investigate the fundamental characteristics of this novel symbiosis and determine whether it can be used in aquaculture to decrease ammonia stress of 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.

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 A gene (a functional marker for ammonia oxidizing bacteria). Additionally, 16S rRNA amplicon sequencing was used to obtain an overview of bacteria present in fish gill samples.

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

Based on molecular methods, we confirmed the presence of *Nitrosomonas* ammonia-oxidizers in carp and zebrafish gills (fig. 1). Additionally, microscopy indicated that these bacteria seem to be located intracellularly.

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 a higher proportion of protein in their diet (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.

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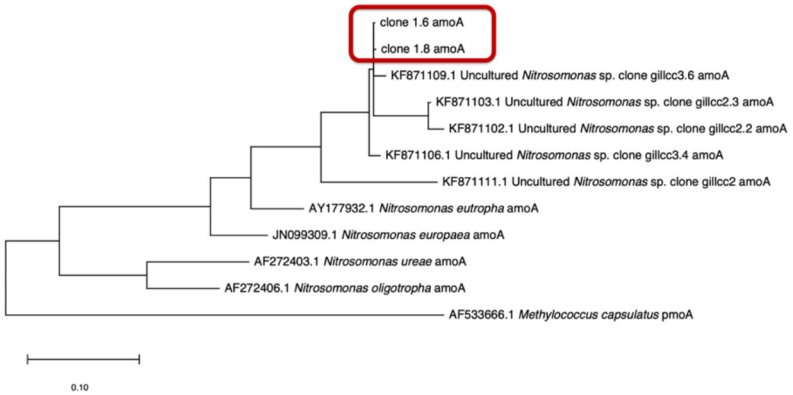

Fig. 1: Phylogenetic tree of the ammonia monooxygenase A gene clones identified in carp gill tissue.

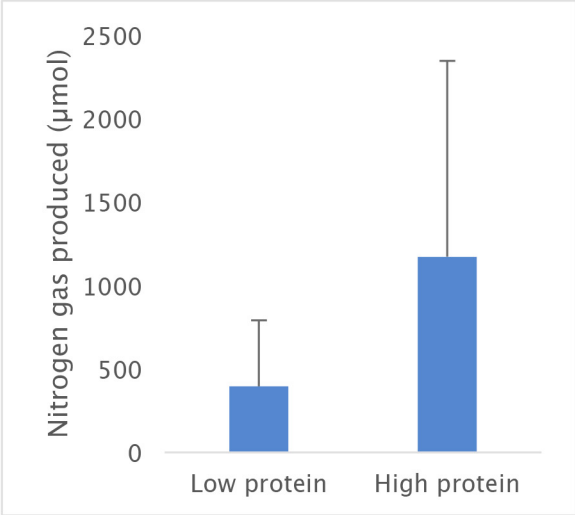

Fig. 2: Production of dinitrogen gas by carp fed by hand or ad libitum through a pendulum feeder. Mean ± SD, n=6.

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 the bacteria are transmitted, as well as the moment of colonization of the gills using germ-free zebrafish. We will also explore how widespread this symbiosis between teleost fish and nitrogen cycle bacteria is.

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.

PROPOLIS INHIBITS LIFE STAGES OF AQUACULTURALLY IMPORTANT OOMYCETE PATHOGENS *Aphanomyces astaci* AND *Saprolegnia parasitica*

A. Miljanović[1]*, M. Dent[1], D. Pavić[1], D. Grbin[1], I. Jerković[2], Z. Marijanović[2], S. Pedisić[3], I. Maguire[4], A. Bielen[1]

[1]Faculty of Food Technology and Biotechnology, Department for Biochemical Engineering, Zagreb, Croatia
[2]Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21 000 Split, Croatia
[3]Faculty of Food Technology and Biotechnology, Department of Food Engineering, Prolaz Petra Kasandrića 3, 23 000 Zadar, Croatia
[4]Faculty of Science, Department of Biology, Rooseveltov trg 6, 10 000 Zagreb, Croatia
Email: amiljanovic@pbf.hr

Introduction

Saprolegnia parasitica and *Aphanomyces astaci* (Oomycetes) are pathogens with negative impact in freshwater aquaculture. *Saprolegnia parasitica* causes saprolegniosis, a disease affecting mostly salmonid fishes, while *A. astaci* causes crayfish plague. Chemicals harmful to humans and the environment are being used globally in aquaculture facilities to prevent the spread of these pathogens. Thus, the development of new, ecologically acceptable methods for their control is urgently needed.

The aim of this study was to examine whether propolis, known for its antimicrobial properties and stimulatory effect on the host immune system, can inhibit the life stages of *A. astaci* and *S. parasitica in vitro*.

Materials and methods

Two propolis formulations were used: P1 (pure propolis in ethanol), and P2 (propolis in ethanol with the addition of sage and pepper mint). Their chemical composition was determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography/mass spectrometry (GC-MS).

Two main life stages of the oomycete pathogens, namely mycelium and zoospores, were treated with propolis formulations and their main components, pinocembrin and chrysin. The effect of these compounds on mycelial growth was assessed using the disk diffusion assay and radial growth inhibition test, followed by determination of EC50 values. To test the inhibition of zoospore germination, sporulation was induced by washing the mycelium grown in liquid PG1 with natural water. Test compounds were added to the resulting zoospore suspension in a range of concentrations, and their effect was assessed by comparing the percentage of germinated zoospores with the results of control experiments. Malachite green, with known toxicity towards *A. astaci* and *S. parasitica*, was used as a positive control.

Results

Both propolis samples were rich in volatile and polyphenol compounds. As shown by UPLC-MS/MS analysis, chrysin was most abundant in both propolis samples (up to app. 50 μg/mL), followed by pinocembrin in P2 (4 μg/mL). Significantly higher number of different volatile components was found by GC-MS in P2 than in P1 propolis sample, probably because of addition of sage and pepper mint.

In case of *S. parasitica,* both propolis formulations moderately inhibited the mycelial growth: EC50 (P1) = 206.2 μg/mL, EC50 (P2) = 206.6 μg/mL for P2, when compared with a known inhibitory compound malachite green (EC50 = 0.1 μg/mL). In contrast, EC50 values for P1, P2, and malachite green for mycelial growth inhibition of *A. astaci* were up to 36 times lower (5.6, 8.6, and 0.02 μg/mL, respectively). Considering zoospore germination, the minimal concentration that caused complete inhibition of *S. parasitica* germination (minimum inhibitory concentration, MIC) was 62.0 and 39.0 μg/mL for P1 and P2 propolis samples, respectively, while for malachite green it was 0.08 μg/mL. Similar results were obtained for *Aphanomyces astaci* zoospores: MIC was 31.3 and 39.0 μg/mL for P1 and P2, respectively, while malachite green inhibited zoospore germination at a concentration of 0.04 μg/mL.

The inhibitory potential of main propolis components, chrysin and pinocembrin, was also tested but these compounds didn´t show significant anti-oomycete activity when applied in concentrations determined in propolis samples. This suggests that the observed anti-oomycete activity of propolis formulations was probably due to synergistic activity of a number of minor bioactive components.

Conclusions

Our results demonstrate the inhibitory activity of propolis towards life stages of pathogenic oomycetes *S. parasitica* and *A. astaci*. Interestingly, *S. parasitica* mycelium was relatively resistant to propolis in comparison to mycelium of *A. astaci*, but zoospores of both species (as main infection agents) were highly susceptible. Future *in vivo* testing is needed to explore the suggested host-protective effects of propolis during the infection process and to demonstrate its applicability as a feed additive in the fish farms.

ULTRA-SENSITIVE SINGLE-CELL-ICP-MS MEASUREMENTS FOR STUDYING Ag NANOPARTICLES INTERNALIZATION IN CELL LINES FROM SEA-BASS (*Dicentrarchus labrax*) AND CLAMS (*Ruditapes philippinarum*)

A. MOREDA-PIÑEIRO*[1], C. SUÁREZ-OUBIÑA[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
*antonio.moreda@usc.es

Introduction

Metal and metal oxide nanoparticles (NPs) have been widely used due to their exceptional physicochemical properties. Large-scale production and use will result in the release of these particles into the environment. One of the biggest threats of using NPs is the transfer and magnification of these particles in the trophic chain. Therefore, data are necessary for evaluating the environmental risk of these emerging pollutants, especially regarding the uptake and biological effects. *In vitro* assays can provide significant information on how these NPs are capable to be internalised in cells as a previous stage for elucidating the bioaccumulation in fish and molluscs and also the bioavailability in humans.

Single-cell-inductively coupled plasma – mass spectrometry (SC-ICP-MS) has opened a new area of research which allows the quantification of metals in single biological cells at ultra-low levels (attograms per cell), sensitive determination not provided by other instrumental techniques. Therefore, the aim of the current research has been to explore the possibilities of SC-ICP-MS to assess Ag NPs inside kidney cells from sea-bass (*Dicentrarchus labrax*) and clams (*Ruditapes philippinarum*) as a previous stage for performing bioaccumulation studies of Ag NPs in these cultured species.

Methods and results

Kidney sea-bass and clam cells were exposed to Ag NPs (15 and 100 nm) at different NPs concentrations and exposure times, and the internalization of Ag NPs in each single cell was assessed by SC-ICP-MS.

Several parameters regarding SC-ICP-MS were studied with the aim of developing a novel, high sensitivity and accurate method for assessing Ag NPs in single cells from aquaculture products. Properly cell concentration and dwell time were optimised for avoiding multi-cell coincidence. In addition, the effect of washing stages (1% PBS) was tested in order to remove non-internalised Ag NPs and/or Ag NPs adsorbed onto the cell's surface. This procedure helps to avoid high dissolved backgrounds, possible interferences, or excess particles that could mask the signal generated by the single cells.

Optimised SC-ICP-MS conditions were applied for assessing the rate of internalization of Ag NPs of different size distributions based on the concentration and exposure time. Results are now being used for designing bioaccumulation assays of Ag NPs in cultured sea-bass and clams.

Acknowledgments

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VEGETAL-BASED DIETS INDUCED MOLECULAR DYSREGULATION IN CARNIVOROUS FISH: FOCUS ON AMINO ACID TRANSPORTERS IN RAINBOW TROUT (*Oncorhynchus mykiss*) CELL LINES

Guillaume Morin *, Karine Pinel, Karine Dias, Iban Seiliez and Florian Beaumatin

Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, NUMEA, Saint-Pée-sur-Nivelle, France
E-mail : florian.beaumatin@inrae.fr

Introduction

Carnivorous farmed fish such as rainbow trout (RT) has high dietary protein requirements fulfilled in the past by fishmeal (FM). Due to marine resources preservation, FM production is limited and dietary amino acids (AA) must be provided by alternative protein sources such as plant proteins to sustain aquaculture development. Although the use of plant proteins can effectively reduce FM inclusion in fish feeds, plant-based diets disturb fish physiology owing to multifactorial reasons such as antinutritional factors and imbalanced levels of minerals, fatty acids and essential AA. To offset AA deficiencies, AA can be added in crystalline form into plant-based diets to improve fish growth. However, even if this supplementation improves fish growth performances, it does not rescue totally the growth retardation observed when compared to fish fed with FM-based diets. The remaining question is therefore to determine if this growth retardation is still partially related to AA metabolism or if it relies on other causes aforementioned. In fact, we observed, through an in-depth analysis of transcriptional data from two previous studies[1,2], that fish fed with plant-based diets supplemented with free AA overexpress some target genes of the general nonderepressible 2 (GCN2) pathway[1,2]. Since GCN2 pathway was shown to be activated by lack of AA, inhibiting on one hand general protein synthesis and overexpressing on the other hand genes involved in AA metabolism (synthesis, transport…), overexpression of these genes suggests that AA metabolism is dysregulated by plant-based diets in spite of the addition of free AA. Moreover, some amino acid transporters (AAT) were shown to be also dysregulated in fish fed plant-based diets. Since GCN2 is known to promote AAT upregulation when activated, we decided to focus our study on a sub-family of AAT that were shown dysregulated in both studies: the cationic amino acid transporters (CAAT). Therefore, our aim was to identify members of this sub-family of genes in RT genome, since, surprisingly, none of them received attention so far. Then, we determined their expression levels in RT tissues prior to assess their regulation mode by AA availability as well as by the GCN2 pathway by mean of an *in vitro* approach: the use of RT cell lines.

Materials and methods

Cell culture experiments were performed at 18°C. Considering the key role played by the liver in AA metabolism and that nutrients are first absorbed by intestine, we used two cell lines derived from hepatocytes, namely RTH-149 and RTL-W1 as well as the RTgutGC cell line derived from enterocytes. Together with cell lines experiments, CAAT expression in RT tissues were assessed by RT-qPCR. Two different pools of RNA samples extracted from intestine, liver, muscle, kidney and ovary , or from brain and hypophysis, from fish fed a "commercial like diet" were used to validate primers and identify CAAT expressed in trout tissues.

Results

Salmonids, including RT, underwent, during evolution, two more whole genome duplication events (WGD) compared to mammals leading to the possible existence of different paralogs corresponding to each human gene. Hence, we aimed to identify all ortholog gene sequences present in RT genome, for each known human CAAT. *In silico* analysis reveals that, for the 14 CAAT known in human, 47 ortholog genes exist in RT genome. As it was determined that about half of the duplicated genes have their expression retained after WGD in RT[3], we assessed CAAT expression in RT tissue pools. We show that 26 of the 47 identified CAAT genes in RT genome are expressed in these tissues. Interestingly, the proportion of CAAT found to be expressed in RT tissues match with the estimated 50% of expression loss of duplicated genes. Once we identified CAAT expressed in RT, we aimed to study the regulation of their expression by AA availability. In order to assess accurately and precisely the AA-dependant regulation of CAAT expression, we used RT cell lines, an *in vitro* approach recently validated for fish nutrition[4]aquaculture provides more than 50% of fish consumed worldwide but faces new issues that challenge its sustainability. One of them relies on the replacement of fish meal (FM. In the RTH-149, RTL-W1 and RTgutGC cell lines, we detected 15 out of the 26 CAAT whose expression was detected in RT tissues. Our results showed that, almost half of them including CAAT identified in *in vivo* studies, are up-regulated upon starvation. Interestingly, we observed that these up-regulations were significantly abolished when AA were supplemented

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to the starvation medium demonstrating the CAAT AA specific regulations. Then, to strengthen these results, we conducted the opposite experiment using HF, a pharmacological activator of GCN2 pathway, in order to mimic AA starvation while cells were grown in nutritive rich conditions (containing AA). Accordingly, we observed a specific upregulation of the same CAAT overexpressed by AA withdrawal, reinforcing the assumption of the implication of the GCN2 pathway in the regulation of CAAT expressions. Finally, we conducted experiments to assess if a single cationic AA deprivation (arginine (R) and/or lysine (K)) could be sufficient to upregulate the CAAT expression. Interestingly, we noticed that such starvation activates the GCN2 pathway and leads to upregulation of CAATs whose overexpression in starvation is AA dependant, suggesting an involvement of GCN2 in this effect.

Conclusion

Altogether, beyond the identification of CAAT sub-family of genes in trout genome as well as the validation of their expression in trout tissues, our work demonstrates that part of these transporters are specifically up-regulated following an AA but also R and K starvation. Moreover, we observed that GCN2 pathway is activated in the same conditions and pinpointed pharmacologically its involvement the regulation of CAAT expression upregulated by AA withdrawal. However, *in silico* analysis reveals that RT CAAT protein sequences are poorly conserved with their mammalian counterparts, making possible the emergence during evolution of functional divergences for these proteins. Therefore, we are assessing transport activities and roles on cell physiology of CAATs, using RT cell lines combined with new molecular technics such as gene invalidation. Since AAT are the main gate for AA absorption by organisms and because AA have a major role on RT physiology as metabolic fuel, protein building blocks but also signalling molecules, characterization of RT CAAT will increase our knowledge on AA metabolism dysregulations induced by plant-based diets. Finally, this study might help to adapt fish feed formulations to notably cope with the new stakes of aquaculture.

References

1. Balasubramanian, M. N. *et al.* Molecular pathways associated with the nutritional programming of plant-based diet acceptance in rainbow trout following an early feeding exposure. *BMC Genomics* 17, 449 (2016).
2. Callet, T. *et al.* Detection of new pathways involved in the acceptance and the utilisation of a plant-based diet in isogenic lines of rainbow trout fry. *PLOS ONE* 13, e0201462 (2018).
3. Berthelot, C. *et al.* The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. *Nat. Commun.* 5, 3657 (2014).
4. Morin, G., Pinel, K., Dias, K., Seiliez, I. & Beaumatin, F. RTH-149 Cell Line, a Useful Tool to Decipher Molecular Mechanisms Related to Fish Nutrition. *Cells* 9, 1754 (2020).

MICROBIOLOGICAL STATUS OF PARENCHYMAL ORGANS OF THE CARP FISHES AS DEPENDING ON THE WATER QUALITY

Marina A. Morozova*, Nina A. Abrosimova[1], Olga S. Chemisova[2]

*Azov-Black Sea Branch of VNIRO (AzNIIRKh), Rostov-on-Don, Russia
1 Don State Technical University, Rostov-on-Don, Russia
2 Rostov-on-Don Plague Control Research Institute of the Federal Service for Surveillance in the Sphere of Consumers Rights Protection and Human Welfare
E-mail: abrosimovana@yandex.ru

Pond aquaculture is the basis of commercial aquaculture in the South of Russia. One of the most important issues in the management of pond fish farming is the quality of water coming from open natural water bodies. In artificial reservoirs, productive qualities and viability of fish are in direct dependence on the hydrochemical mode and sanitary condition of water sources. The spread of distribution of sanitary indicator, pathogenic and potentially pathogenic bacteria by a water way is known to be rather wide. These facts together create the prerequisites for the active colonization of the fish organism by microorganisms, largely due to the water transfer factor.

If the composition of the gill and intestine microflora is, to a large extent, similar to the composition of the aquatic microflora, then as regards the bacteria availability in parenchymal organs, there are significant differences in the literature. Some researchers believe that parenchymal organs of healthy fish are almost always free from bacteria. Others point to the bacterial contamination of fish.

The aim of our study was to identify contamination of parenchymal organs of juvenile carp fish reared in ponds.

The research was carried out at three fish farms of Southern Russia (Krasnodar and Stavropol Regions). Liver and kidney samples of four species of the carp fish (carp, silver carp, grass carp and golden carp) were taken for the analysis. In total, there were examined 223 fish. By 15 or 30 fingerlings of each species were sampled. The studies were performed in summer (June-July) and autumn (September-November). The rivers Kuban and Beysug were the water source for the rearing ponds. The bacterial cultures were identified by using the Bruker Daltonics Autoflex speed III mass spectrometer (Germany) with the Biotyper system.

We found that the proportion of fish with kidney contamination averaged 23.5 % (3.3-53.3 %) and liver contamination was 10.3 % (0 – 17.8 %). *Aeromonas veronii*, and *Shewanella R. (Sh. putrefaciens, Sh. profunda*) prevailed in the microflora of those organs. The taxonomic composition of Aeromonas was represented by nine species: *Aeromonas hydrophila, A. veronii, A. ichthiosmia, A. sobria, A. eucrenophila, A. jandaei, A. caviae, A. bestiarum, A. media*. In parenchymal organs of the fish from the ponds fed by the river Beysug, there were discovered *Plesiomonas shigelloides, Corynebacterium striatum* and *Vibrio cholerae* (non O1 / non O139). Single isolates of *Staphylococcus haemolyticus, Citrobacter freundii, Proteus vulgaris* and *Pseudomonas putida* were found in the microflora of the fish from two farms with the water supplied by the Kuban river. Detection of these bacteria evidenced the sanitary problems of the fish habitat.

Taking into account the tense ecological state of the steppe rivers of the eastern Azov region, namely the Beisug and the Kuban rivers, caused by both natural factors, in particular low water level and poor flowage, and the anthropogenic ones, such as industrial wastewater discharge, shipping and agriculture, we believe that the main reason for such species succession in the microflora of parenchymal organs of fish is, primarily, the inflow of initially polluted waters into the ponds.

SHOULD WE GO OFFSHORE? A CASE STUDY OF OFFSHORE ATLANTIC SALMON AQUACULTURE IN FARMS OFF THE WEST COAST OF SCOTLAND

B. Morro*[1], L. Falconer[1], C. Díaz-Gil[2], T.P. Adams[3], D. Aleynik[3], S. Rey Planellas[1]

1 Institute of aquaculture, University of Stirling, Stirling, UK. FK9 4LA
2 Xelect, Saint Andrews, UK. KY16 9LB
3 The Scottish Association for Marine Science (SAMS), Oban, UK. PA37 1QA

Introduction

A number of factors make the planned expansion of the Atlantic salmon aquaculture industry unachievable in its current form, which is based primarily on sea cages in sheltered sea lochs. These limitations include the impact of sea lice infestations and amoebic gill disease (AGD) on fish health (Costello 2009, Van Geest *et al*. 2014), the cost and environmental impact of chemical treatments against the parasites (Aaen *et al*. 2015), planning issues, biomass limits per farm, and the increasing impact of harmful algal blooms (HABs), which can be particularly acute in restricted water exchange environments (Gowen *et al*. 2012). One strategy with the potential to reduce the impact of these limitations is the development of aquaculture in more dispersive "offshore" environments. However, it has challenges of its own. It requires the development of a new integrated health management plan and the adaptation of the farming planning and infrastructure. Improved, science-based evidence with direct relevance to the complex environment of the West coast of Scotland will allow planning and regulation of this offshore transition, informing on the most suitable locations and fish rearing protocols. The present work focuses on the fish health and welfare implications of moving cages offshore, within a framework that also studies physical oceanography, wave modelling, and sea lice/HAB modelling. Furthermore, stakeholders also offered their expertise and infrastructure, giving us access to real farm data.

Materials and methods

Weekly health historical data of 5 inshore and 3 offshore farms was supplied by the partner salmon farming company for a period corresponding to a full Atlantic salmon production cycle. Data was provided per pen and included mortalities (%), average weight (kg), fish density (kg/m^3), haematocrit (%, n = 8), sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) counts per fish (n = 20), and AGD gill score (0 - 3, n = 20). Records of anti-parasite treatments (e.g. SLICE and Thermolicer) were also shared, detailing the date and the pens that were treated. Finally, environmental data on each location was provided by farms (*e.g*. wind speed and direction, temperature, dissolved oxygen) and by a weather research forecast model. This data was analysed using both univariate and multivariate statistics, allowing us to quantify the effect of health (e.g. sea lice prevalence), anti-parasite treatments (e.g. Thermolicer) and weather (e.g. storms) variables on response variables like fish mortality.

Results and Discussion

After one year (1 full production cycle in seawater), starting with just deployed smolt fish of 90 to 110 g and ending up with harvest size fish of between 3 and 7 kg, the result on the potential advantages of offshore aquaculture of Atlantic salmon over the inshore counterpart are mixed. After deployment, mortalities of around 4% were registered during the first week, independently of the rearing location. As a general trend, mortality was not affected by the location of the farms. Similarly, AGD showed no significant differences. Despite the apparent increased difficulty in sea lice attachment due to high water velocity, offshore locations showed significantly higher counts of adult *L. salmonis* and *Caligus* spp., while the *L salmonis* chalimus counts were higher in inshore locations. However, these differences in sea lice prevalence might have more to do with the difficulty associated with carrying out anti-parasite treatments in offshore and less with the offshore situation of the farms. According to the data facilitated by the farms, the number of treatment events was markedly lower in offshore. This is likely due to the accessibility restrictions caused by bad weather, which also prevent routine fish health monitoring, and it highlights the logistical difficulties encountered by offshore farms.

References

Aaen, SM *et al*. (2015). *Trends in parasitology* 31.2: 72-81.
Balseiro, P *et al*. (2018). *Journal of fish biology*, 93(3), 567-579.
Costello, M (2009). *Journal of Fish Diseases*, 32:115–118.
Gowen R *et al*. (2012). *Oceanography and Marine Biology: An annual review*, 50: 65-126
Van Geest, J *et al*. (2014). *Aquaculture*, 434:233–240

EVALUATION OF DEFATTED BLACK SOLDIER FLY (*Hermetia illucens*) PREPUPAE LARVAE MEAL AS FISH MEAL REPLACEMENT IN DIETS FOR GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES – EFFECTS ON APPARENT DIGESTIBILITY COEFFICIENTS AND DIGESTIVE ENZYMES ACTIVITY

S. Moutinho[1,2]*, C. Castro[1], A. Oliva-Teles[1,2], S. Martínez-Llorens[3], H. Peres[1]

[1] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto
[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto
[3] Research Group of Aquaculture and Biodiversity, Institute of Animal Science and Technology, Universitat Politècnica de València

*smoutinho@ciimar.up.pt

Introduction

In order to support the predicted growth of aquaculture production, it is of uttermost importance to reduce the dependency on marine feed resources. Insects arise as a more sustainable alternative to fish meal as they have fast growth, require minimal land and water, and can convert low-quality waste materials into high-valuable ingredients, taking part a circular bioeconomy. Black soldier fly (*Hermetia illucens*) is one of the most promising insect species to be included in aquafeeds. *H. illucens* larvae meal (HM) has a high protein content, with an amino acid profile similar to fish meal, and is also a good source of vitamins and minerals. Although it is also a good source of lipids, its fatty acid profile is suboptimal for feed purposes of marine carnivorous fish species, and so, defatting has become a standard practice to obtain a more consistent and higher-quality protein product. Given its potential, this work aimed to evaluate the replacement of fish meal with defatted HM on diets digestibility and activity of digestive enzymes in gilthead seabream juveniles.

Materials and methods

Four experimental diets were formulated to include increasing levels of defatted HM: 0% (HM0), 15% (HM15), 30% (HM30), and 45% (HM45), replacing 22, 60, and 100% of total FM protein, respectively. Diets were randomly assigned to triplicate groups of fish (initial weight of 32g). After 67 days of feeding, the posterior and anterior intestines were sampled to determine digestive enzymes activity. To determine the apparent digestibility coefficients (ADCs), chromium oxide was included as the digestibility marker (0.5%) to the diets used previously. Fecal collection was performed for a total of 21 days and the content of dry matter, protein, lipid, and energy of feces and diets was determined.

Results and discussion

Preliminary results indicate that ADC of protein and lipids were not affected by the dietary treatments, while ADC of dry matter and energy were significantly improved with the presence of HM (Fig. 1). The inclusion of moderate levels of HM also led to an increase in the intestinal activity of proteolytic enzymes – trypsin, chymotrypsin, and total proteases – as well as of amylase (Fig. 2). Chitin, a biopolymer present in the exoskeleton of insects, has been linked to decreased nutrient availability and absorption in some fish species (Kroeckel et al. 2012; Dumas et al. 2018), which was not observed in this study. Other studies have demonstrated that chitin and its derivatives are involved in several biological mechanisms, including gut microbiota and intestinal morphology modulation (Wan et al. 2017). This prebiotic effect of chitin could, therefore, explain the increase in energy ADC through the production of short-chain fatty acid by gut bacteria and/or through the improvement of intestinal morphology, increasing nutrient absorption. Lauric acid represents most of the FA found in HM and is used primarily as an energy source. The contribution of lauric acid to the overall energy pool could also help to explain the observed results. This study indicates that defatted HM is well tolerated by gilthead seabream juveniles and is a good candidate for inclusion in aquafeeds. Further studies are required to fully understand the mechanisms between insect meals and chitin in nutrient absorption and utilization by fish.

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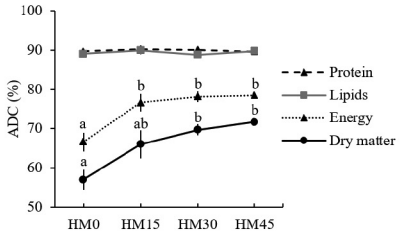

Fig 1: Apparent digestibility coefficients (ADC, %) of gilthead seabream fed the experimental diets. Values are presented as mean (n=3) and standard deviation. Different superscript letters indicate significant differences between treatments (one-way ANOVA, $p<0.05$)

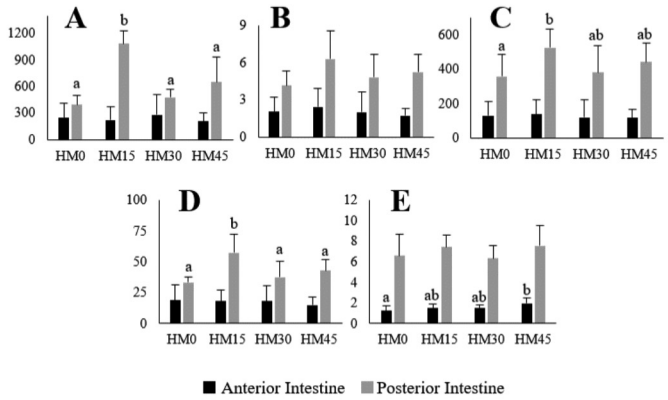

Fig 2. Digestive enzymes activity of the anterior and posterior intestine of gilthead seabream juveniles fed the experimental diets. A – Amylase (nmol/mg protein); B – Lipase (nmol/mg protein); C – Trypsin (nmol/mg protein); D – Chymotrypsin (μmol/mg protein); E – Total proteases (nmol/mg protein/min). Values are presented as mean (n=9) and standard deviation. Different superscript letters indicate significant differences between treatments for each portion (one-way ANOVA, $p<0.05$)

References

Kroeckel, S., Harjes, A., … & Schulz, C. 2012. Aquaculture, 364-365, 345-352. doi: 10.1016/j.aquaculture.2012.08.041

Dumas, A., Raggi, T., … & Weltzien, E. (2018). Aquaculture, 492, 24-34. doi: j.aquaculture.2018.03.038

Wan, J., Jiang, F., … & Jun, H. 2017. RSC Adv. 7: 9669. doi:10.1039/c7ra00142h

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DEVELOPMENT OF A SANDWICH ELISA SYSTEM, SPECIFIC FOR TRANSTHYRETIN IN GILTHEAD SEA BREAM (*Sparus aurata*)

Eleni A. Galliopoulou[a*], Anjos, Liliana[b], Arkadios Dimitroglou[c], Dimitris Barkas[c], Leonidas Papaharisis[c], Katerina A. Moutou[a], Deborah M. Power[b]

a Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece
email: galliopoulou@uth.gr
b Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8000 Faro, Portugal
c Nireus Aquaculture SA, Department of Research and Development, 34400 Pyrgoulaki, Evvoia, Greece

Introduction

Thyroid hormones (THs), thyroxine (T4) and triiodo-L-thyronine (T3), are synthesized in the thyroid follicles which in fish do not form a gland but are distributed in the pharyngeal region at the insertion of the gill bars. THs do not circulate in a free form but are bound to TH distributor proteins (THDP) that ensure there is an even distribution of the hormones throughout the body and contribute to their targeted delivery to specific tissues. Transthyretin (TTR) is a THDP, which in the gilthead sea bream (*Sparus aurata*) is a 130aa protein that shares 47–54% sequence similarity with other vertebrate TTRs (Santos and Power 1999), has a similar affinity for T3 and T4, and the major site of its synthesis is the liver (Morgado et al. 2008). In mammals TTR measurements in blood using ELISA or Western blot are used to monitor nutritional status. To assess if TTR can be used to indicate nutritional status in fish a robust ELISA protocol to determine TTR levels in gilthead sea bream blood was developed. The method was subsequently used to monitor the effects of different diet compositions on TTR circulating levels.

Materials and methods

For the development and the establishment of a sandwich ELISA, blood serum samples from immature sea bream were collected from three full sib families from the Nireus breeding program. 120 fish per family were distributed in 6 tanks: 3 tanks were the control group that was fed on a standard commercial diet (Feedus), and the other 3 tanks were the experimental group, fed on a diet rich in plant-based raw materials. The feeding rate (FR, %), specific growth rate (SGR, %/day) and feed conversion rate (FCR) were determined. Sampling was performed at four time points during the feed trial, i) at the start of the experiment, ii) at 2 months, iii) at 4 months, and iv) at 6 months. Optimization of the concentrations of sea bream recombinant TTR (sbrTTR) and specific sb TTR polyclonal (rabbit and chicken) antibodies were done by dot blot analysis and then used to develop a sandwich ELISA for sbTTR. The final antisera concentrations chosen gave adequate sensitivity of the sandwich ELISA for detection of sbTTR in blood, whilst giving a low nonspecific background reaction. Different buffers, sample dilutions, and incubation times were evaluated for ELISA optimization. The protein content of blood serum samples was determined by Bradford assay. The statistical analysis of the ELISA results and the blood serum protein was performed using an IBM SPSS® software platform.

Results

The titration of the primary and the secondary rabbit and chicken antibodies revealed the most appropriate dilutions that were combined with decreasing sbrTTR concentrations. Following preliminary trials the rabbit antibody was selected as the coating (capture) antibody (1:1000) and the chicken antibody (post immune egg, animal 2) was used for detection (1:2500) and quantification in the sandwich ELISA assay. Serial dilutions of sbrTTR (1:50-1:5,000,000) were used for the construction of the standard curve. A standard curve was generated in each ELISA reaction and used for the determination of sea bream TTR concentration (μg/mg plasma protein) in fish plasma samples (Figure 1).

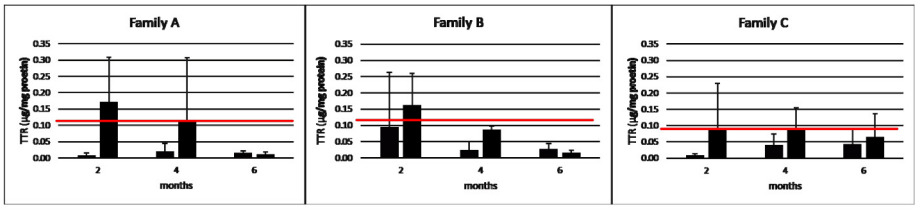

Fig. 1: Plasma TTR concentration in different families fed two different feeds (blue, control; green, plant) at 2, 4 and 6 months, respectively.

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Both sampling time (2, 4 and 6 months) and feed had a significant effect on plasma TTR concentration. Family, as genetic background, shaped a differentiated pattern of TTR fluctuations with diet and time. FCR of the plant diet was higher than that of the control diet in all families. However, a distinct temporal pattern of FCR and SGR was observed per family.

Overall, TTR levels were significantly and negatively correlated with SGR and positively with FCR. These correlations were very strong in fish fed on the control diet, yet they became very weak in the fish fed on the plant diet. The significance of correlations differentiates also with family, supporting a considerable effect of genetic background on the link of TTR with nutrition and growth parameters in gilthead sea bream.

Discussion

The advantage of sandwich ELISA is that no sample purification is required, and the assay can be very sensitive (up to 2 to 5 times more sensitive than the others, Aydin 2015). In view of the substantial use of the positive and negative controls to compare data from different ELISA plates (Terato et al. 2016), variable negative controls were evaluated in the present study. The highly specific and sensitive sandwich ELISA system that was developed for the measurement of TTR could be a useful approach for the links between feeding and growth in breeding programs for gilthead sea bream. A large-scale experiment is required to reach well-documented conclusions.

Acknowledgments

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References

Aydin, Suleyman. 2015. "A Short History, Principles, and Types of ELISA, and Our Laboratory Experience with Peptide/Protein Analyses Using ELISA." Peptides 72:4–15

Morgado, Isabel, Eduardo P. Melo, Erik Lundberg, Nídia L. Estrela, A. Elisabeth Sauer-Eriksson, and Deborah M. Power. 2008. "Hormone Affinity and Fibril Formation of Piscine Transthyretin: The Role of the N-Terminal." Molecular and Cellular Endocrinology 295(1–2):48–58

Santos, Cecilia R. A., Liliana Anjos, and Deborah M. Power. 2002. "Transthyretin in Fish: State of the Art." Clinical Chemistry and Laboratory Medicine 40(12):1244–49

Terato, Kuniaki, Christopher Do, Jessica Chang, and Takaki Waritani. 2016. "Preventing Further Misuse of the ELISA Technique and Misinterpretation of Serological Antibody Assay Data." Vaccine 34(39):4643–44.

EXAMINATION OF THE EFFECT OF ELEVATED NACL CONCENTRATIONS ON THE BURDEN OF THE ECTOPARASITES TRICHODINIDS AND DACTYLOGYRIDS ON PIKEPERCH (*Sander lucioperca*) MAINTAINED IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

H. Muhammad[ab], C. Naas[ab], A. Müller-Belecke[a], and W. Kloas[b]

[a]Institute of Inland Fisheries in Potsdam-Sacrow, Im Königswald 2, 14469 Potsdam, Germany
[b]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany
Email: harishmuh@gmail.com

Introduction

Pikeperch is a high valued species and one of the favorite food fishes in Europe. Declining wild catches and increasing demand have raised the popularity to intensify production techniques of this species using recirculating aquaculture systems (RAS). However, RAS possesses disadvantages, as high stocking densities increase the risk of disease transmission of pathogens and ectoparasites (Bregnballe, 2015; Németh et al., 2013). One of the most innocuous treatments for freshwater fish in a closed aquaculture system is using low concentrations of sodium chloride (NaCl) as prolonged immersion (Noga, 2010). This study was conducted to evaluate the effect of elevated NaCl concentrations on the burden of common ectoparasites in pikeperch, protozoan Trichodinids and monogenean Dactylogyrids, maintained in RAS.

Materials and Methods

Pikeperch (120.5±15.7 g) were brought from overwintering conditions in Lake Sacrow, Potsdam-Sacrow, Germany. The treatments consisted of a negative control stocked with uninfected pikeperch, a positive control stocked with infected pikeperch, and two treatment groups stocked with infected pikeperch and addition of NaCl 3 PSU and 6 PSU, respectively. Both controls were kept without NaCl application (0 PSU). All treatments were performed in three replicates in four units of RAS for 21 days. Each RAS consisted of three rearing tanks (320 L) and a filtration unit (120 L). Each RAS was stocked with 57 fish (19 fish/tank). On days 0, 7, and 21 of the experiment, four fish per tank were sacrificed (total 12 fish/treatment) for ectoparasite analysis. The mucus from several areas of skin around the dorsal fin, anal fin, ventral fin, pectoral fin was swabbed, and the second raker of the right gill was dissected for examination. The smear of mucus and gill samples were examined within six windows of observation at 100 fold magnification under a light microscope (window per view = 0.031 mm^2).

Results

Regarding performance parameters, there were no significant differences in specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), final biomass, and condition factor (CF) among all treatments ($p \leq 0.05$). At the beginning and day 7 of the experiment, ectoparasites Trichodinids and Dactylogyrids were found in the positive control (0 PSU), 3 PSU, and 6 PSU treatments. In contrast, there were no ectoparasites found in the negative control. At the end of the experiment, we observed a declining trend of prevalence and abundance of Trichodinids (by 83.3% and 98.15% in 3 PSU and both 100% in 6 PSU treatments) and Dactylogyrids (by 100% both in 3 PSU and 6 PSU). Oppositely, there was a stable trend of Trichodinids prevalence (100%) and an increasing trend of Dactylogyrids prevalence (up to 25%) in the positive control (0 PSU) from the beginning to the end of the experiment.

Discussion

Among all treatments, the application of 6 PSU NaCl was the most effective in controlling the ectoparasites, Trichodinids and Dactylogyrids. A relatively similar result based on Németh et al. (2013), the continuous application of 5 PSU NaCl was able to eliminate *Trichodina* sp. within two weeks of application. Based on this finding it might be possible that 6 PSU is sufficient to eliminate Trichodinids. Concentrations below these ranges (e.g. 3 PSU) are probably capable of reducing, not completely removing all Trichodinids on the host. A possible mechanism how NaCl can remove ectoparasites is initiating an osmotic pressure difference that is harmful (Kirschner, 2004) or removing an excess of mucus and of debris being associated with ectoparasite infestations (Noga, 2010).

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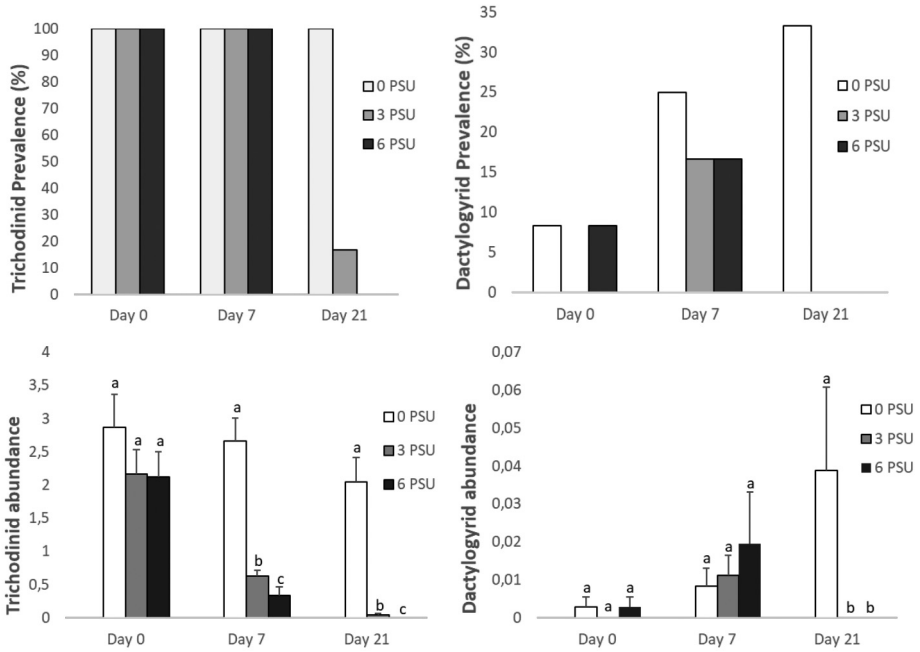

Conclusion

The application of elevated NaCl concentrations (3 PSU and 6 PSU) can reduce the prevalence and abundance of the ectoparasites, Trichodinids and Dactylogyrids. Moreover, there were no ectoparasites found on pikeperch in the 6 PSU treatment after three weeks of the NaCl prolonged application. Though further researches are needed, our findings provide an effective treatment method for controlling both ectoparasites, Trichodinids and Dactylogyrids, on pikeperch maintained in RAS.

References

Bregnballe, J. (2015). A Guide to Recirculation Aquaculture: An introduction to the new environmentally friendly and highly productive closed fish farming systems. FAO and Eurofish Report, 100.

Kirschner, L. B. (2004). The mechanism of sodium chloride uptake in hyperregulating aquatic animals. Journal of Experimental Biology, 207(9), 1439–1452.

Németh, S., Horváth, Z., Felföldi, Z., & Beliczky, G. (2013). The use of permitted ectoparasite disinfection methods on young pike-perch (*Sander lucioperca*) after transition from over-wintering lake to RAS. AACL Bioflux 6(1), 1–11.

Noga, E. J. (2010). Fish Disease: Diagnosis and Treatment, Second Edition (2nd ed.). North Carolina, US: John Wiley & Sons, Inc.

BIOLOGICAL AND GENOMIC CHARACTERIZATION OF A NOVEL JUMBO BACTERIOPHAGE, vB_VhaM_pir03 WITH BROAD HOST LYTIC ACTIVITY AGAINST *Vibrio harveyi*

Gerald N. Misol, Jr[*, 1, 2], Constantina Kokkari [1] and Pantelis Katharios[1]

[1]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, 71500 Heraklion, Crete, Greece
[2]Department of Biology, University of Crete, 71003 Heraklion, Crete, Greece
*Corresponding address: gerald.san89@gmail.com

Introduction
Vibrio harveyi is a Gram-negative marine bacterium that causes major disease outbreaks and economic losses in aquaculture. Phage therapy has been considered as a potential alternative to antibiotics however, candidate bacteriophages require comprehensive characterization for a safe and practical phage therapy.

Materials and methods
Bacteriophages were isolated and purified from Greek water prior to characterization. From the host range tests with the isolates, vB_VhaM_pir03 was selected due to its broad host lytic activity. The life cycle, tolerance, and efficacy of plating of vB_VhaM_pir03 was then determined from further characterization work. The genome of vB_VhaM_pir03 was then extracted and sequenced for genomic analyses. Finally, *in vitro* analysis and *in vivo* trials with *Artemia salina* were then carried out to determine the potential of vB_VhaM_pir03 for phage therapy in aquaculture.

Results
In this work, a lytic novel jumbo bacteriophage, vB_VhaM_pir03 belonging to the *Myoviridae* family was isolated and characterized against *V. harveyi* type strain DSM19623. It had broad host lytic activity against 31 antibiotic-resistant strains of V. *harveyi, V. alginolyticus, V. campbellii* and *V. owensii*. Adsorption time of vB_VhaM_pir03 was determined at 6 min while the latent-phase was at 40 min and burst-size at 75 pfu/mL. vB_VhaM_pir03 was able to lyse several host strains at multiplicity-of-infections (MOI) 0.1 to 10. The genome of vB_VhaM_pir03 consists of 286,284 base pairs with 334 predicted open reading frames (ORFs). No virulence, antibiotic resistance, integrase encoding genes and transducing potential were detected. Phylogenetic and phylogenomic analysis showed that vB_VhaM_pir03 is a novel bacteriophage displaying the highest similarity to another jumbo phage, vB_BONAISHI infecting *Vibrio coralliilyticus*. Experimental phage therapy trial using brine shrimp, *Artemia salina* infected with *V. harveyi* demonstrated that vB_VhaM_pir03 was able to significantly reduce mortality 24h post infection when administered at MOI 0.1 which suggests that it can be an excellent candidate for phage therapy.

PHYSIOLOGICAL EFFECTS OF NACL APPLICATION ON JUVENILE PIKE-PERCH *Sander lucioperca* REARED IN RECIRCULATING AQUACULTURE SYSTEMS – A PILOT STUDY

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

Pike-perch (*Sander lucioperca*) are increasingly produced in recirculating aquaculture systems (RAS). Due to low water exchange rates, RAS allow the economically feasible application of salts to the rearing environment. Salts are known to have beneficial effects on the physiology of percids with regards to somatic growth and welfare. Today, the usage of salt (NaCl) in pike-perch aquaculture can be considered common practice. However, there is only very limited knowledge about the physiological effects of NaCl application on pike-perch reared in RAS.

In a 78-days pilot trial, juvenile pike-perch (31±5g body weight) were exposed to five different salinities (0, 3, 6, 9, 12g NaCl l^{-1}) in five independent RAS, each with a volume of $1m^3$. Daily water exchange rates were set to a maximum of 600 l water per Kg of feed administered. In accordance with animal welfare protocol, the NaCl concentration was increased by a maximum of 3g l^{-1} per day. On day 1, 3, 8, 36 and 78 after reaching the desired NaCl concentration, blood samples were taken by puncture of the caudal vein. Blood samples were first analysed for haematocrit, and subsequently blood plasma for cortisol, glucose, lactate, protein, triglyceride, osmolality and ions (Na^+, Cl^-). Additionally, on day 36 and 78 a group weighing took place in order to evaluate aquaculture performance parameters (body weight increase, specific growth rate, feed efficiency) of the groups.

At the end of the experiment, fish in 3g NaCl l^{-1} had the highest body weight and showed best performance, even though on day 36 of the trial, this group had a significantly lower body weight compared to the control. No negative effects on haematology were observed at 3g NaCl l^{-1}. Fish in 6 and 9g NaCl l^{-1} did not grow throughout the 36 experimental days, but displayed only minor haematological changes. Juvenile pike-perch in 12g NaCl l^{-1} failed to osmoregulate and were intolerant towards this concentration. In this group, high mortalities, body weight losses and significant haematological alterations were observed already after eight days of exposure.

Consequently, when rearing juvenile pike-perch in RAS, the application of 3g NaCl l^{-1} is beneficial with regards to body weight gain and feed efficiency. Based on haematological results, no negative effects on the physiology were observed applying this concentration. However, an adaptation period to the new conditions seems to be necessary if fishes are handled repeatedly. Generally, juvenile pike-perch under RAS conditions should not be exposed to NaCl concentrations ≥ 6g l^{-1} over a prolonged period of time.

DIVERGENT BACTERIAL COMMUNITIES OF EGGS FROM DIFFERENT HATCHERIES OF EUROPEAN SEA BASS AND GILTHEAD SEA BREAM

Babak Najafpour*[1], Patricia I.S. Pinto[1], Katerina Moutou[2], Adelino V. M. Canário[1,3] and Deborah M. Power[1,3]

[1]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal
[2]University of Thessaly, Biopolis, 41500 Larissa, Greece
[3]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China
Email: dpower@ualg.pt

Introduction

European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*) are important aquaculture species in the Mediterranean Sea, with approximately 191,003 and 185,980 tons produced by the sector in 2016, respectively (www.fao.org). Variable egg quality is linked to high mortality rates of early developmental stages and hampers productivity of the farmed sea bass and sea bream sector (Muniesa et al., 2020). The importance for egg quality of bacterial colonization is unclear but the presence of pathogenic bacteria has been linked to high mortality during their incubation period (Olafsen, 2001). Amplicon-based sequencing of marker genes such as 16S rRNA can give a "snapshot" of the global microbiome and provide valuable knowledge about the composition, relative proportion, and potential function of microbial communities. The objective of the present study was to establish the composition and diversity of the microbial community of eggs and broodstock water in European sea bass and Gilthead sea bream hatcheries using 16S rRNA sequencing.

Material and methods

Eggs (before and after disinfection using standard hatchery procedures) and broodstock water samples were collected from three different hatcheries in Greece in January 2020 (Table 1). The extraction of total DNA was performed following the instructions of a DNeasy Blood & Tissue Kit (Qiagen, Germany). Library construction was carried out using an Illumina 16S Metagenomic Sequencing Library protocol with 12.5 ng of DNA extracted from eggs or water samples, using primers targeting the V3 and V4 hypervariable regions of the 16s rRNA gene for amplification (Klindworth et al., 2013)400-1000, ≥1000 bp. QIIME 2 v2020.2 was used for the identification and classification of operational taxonomic units (OTUs) using scikit-learn classifier against the SILVA database (release 132 QIIME), with a cut-off threshold set at 97% similarity.

Table 1. Number of eggs and water samples analysed from different hatcheries.

Hatchery	Egg samples before disinfection	Egg samples after disinfection	Water from Boodstock tanks
Hatchery 1	3 Sa	4 Sa	4 Sa
Hatchery 2	1 Sa, 1 Dl	1 Sa, 1 Dl	2 Dl
Hatchery 3	2 Sa, 2 Dl	2 Sa, 2 Dl	2 Sa, 2 Dl

Sea bass (Dl), gilthead sea bream (Sa). Samples were collected of eggs and the corresponding broodstock tank water

Table 2. The average relative abundance (%) of the top 6 bacterial genera in eggs and broodstock water.

Bacterial genus	Hatchery 1			Hatchery 2			Hatchery 3		
	Water	Egg BD	Egg AD	Water	Egg BD	Egg AD	Water	Egg BD	Egg AD
Vibrio	3.1	11.0	7.0	15.9	4.6	18.0	2.8	2.2	8.1
Glaciecola	11.6	0.9	0.7	16.2	2.4	2.4	10.4	1.0	1.7
Pseudoalteromonas	2.8	7.3	8.4	0.5	2.4	0.3	7.3	2.2	4.9
Colwellia	9.1	6.9	6.7	0.4	1.0	3.0	4.1	2.6	0.7
Psychrobium	0.8	21.4	6.1	0.0	0.0	0.0	0.0	0.0	0.0
Pseudophaeobacter	12.0	1.1	0.9	0.4	1.5	1.1	4.2	1.3	1.4

BD: eggs before disinfection, AD: eggs after disinfection, water: water from broodstock tanks. Sea bass and sea bream egg microbiota were analysed together.

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Results

Bacterial composition and their relative abundance were determined in each sample at the genus level. The relative abundance of bacteria genera was highly divergent between the eggs and water from the different hatchery sites (Table 2). *Vibrio sp*. comprised a high proportion of the microbiota of eggs and water in all hatcheries, *Pseudoalteromonas* and *Colwellia* were the next most abundant genera. *Glaciecola* was more abundant in broodstock water samples compared to the eggs. Some bacterial genera were characteristic of the hatchery site, such as *Psychrobium* in hatchery 1 (Table 2). Egg disinfection differed between hatcheries, but all protocols caused a significant reduction in the total load of bacteria relative to the non-disinfected eggs. The effect of fish species on the microbiota was less important than other factors such as site/hatchery. For example, the average percentage of the top 6 bacteria genera was 2.1 % in sea bass and 2.2 % in sea bream considering the egg microbiota of hatchery 2 and 3 which provided both fish species.

Discussion and conclusion

This study provides metagenomic profiling of the main bacterial communities associated with sea bass and gilthead sea bream eggs and water from broodstock tanks of three commercial hatcheries. The study revealed that geographic location had a high impact on the microbiomes of eggs and water since they diverged between different hatcheries. The identified site/hatchery specific bacterial communities associated with eggs and the broodstock water suggests application of hatchery specific strategies for better management and disease prevention (e.g. different disinfection protocols) may be required. Bacterial genera common between eggs and water samples revealed an obvious contribution of the broodstock tank water to the egg bacterial community, and the high relative abundance on eggs of some potential pathogenic bacterial genera such as *Pseudoalteromonas* (López et al. 2016).

Acknowledgements

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References

Hansen, G.H., Bergh, O., Michaelsen, J., Knappskog, D., 1992. Flexibacter ovolyticus sp. nov., a pathogen of eggs and larvae of atlantic halibut*, Hippoglossus hippoglossus* L. Int. J. Syst. Bacteriol. 42, 451–458.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1.

López, J.R., Lorenzo, L., Marcelino-Pozuelo, C., Marin-Arjona, M.C., Navas, J.I., 2016. Pseudomonas baetica : pathogenicity for marine fish and development of protocols for rapid diagnosis. FEMS Microbiol. Lett. 364, fnw286.

Muniesa, A., Basurco, B., Aguilera, C., Furones, D., Reverté, C., Sanjuan□Vilaplana, A., Jansen, M.D., Brun, E., Tavornpanich, S., 2020. Mapping the knowledge of the main diseases affecting sea bass and sea bream in Mediterranean. Transbound. Emerg. Dis. 67, 1089–1100.

HOW AGRICULTURE CAN HELP AQUACULTURE BECOME GREENER

J. A. Napier

Rothamsted Research, Harpenden, Herts AL5 2JQ, UK
Johnathan.napier@rothamsted.ac.uk

Aquaculture is recognised as a highly efficient system to produce protein for human consumption, and central to feeding the ever-growing growing global population without exceeding planetary boundaries. In contrast, many terrestrial animal production systems are considered to be both inefficient and impacting on land use and climate change. This has led to suggestions that mankind needs to adopt a plant-centric diet, the only exception being fish which also brings important nutrients such as omega-3s. Here, we consider the implications of such a transition, and also the challenges that aquaculture must face to increase productivity within planetary boundaries. In particular, we consider how agriculture, especially crops, can contribute to helping aquaculture become greener. Some of this is via new technologies such as GM crops tailored specifically for use in aquaculture. Examples of these include new plant-based sources of EPA+DHA, ketocarotenoids such as astaxanthin and more tailored protein sources designed to match the dietary requirements of fish. My talk will discuss the potential for these approaches, focusing on our use of GM Camelina to generate novel oils containing omega-3 long chain polyunsaturated fatty acids and their evaluation as a drop-in replacement for fish oils in aquafeed diets.

IN OVO ARGININE SUPPLEMENTATION IMPROVES RESILIENCE AGAINST CHALLENGING CONDITIONS IN ZEBRAFISH LARVAE

C. Navarro-Guillén[1a], I. Jerez-Cepa[2], A. Lopes[1], S. Engrola[1*]

[1] Centre of Marine Sciences (CCMAR), *Campus* de Gambelas, Faro, Portugal
[2] Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), University of Cadiz, *Campus* de Excelencia Internacional del Mar (CEI·MAR), 11519 Puerto Real, Cádiz, Spain
[a]Current affiliation: Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), *Campus* Universitario Rio San Pedro, 11519 Puerto Real, Spain
[*]E-mail: sengrola@ualg.pt

Introduction
Aquaculture industry is facing many evolving sustainability challenges. Improving feeding protocols and fish digestive efficiency will help the sector to attain a higher productivity, concomitant with a lower environmental impact. Therefore, discovering new methods to improve fish larvae gastrointestinal maturation alongside with higher digestive efficiency at early stages without compromising survival and fitness represents a very promising avenue in fish nutrition research. Nutritional programming refers to a nutritional intervention during the early phases of development that will imprint an individual physiological memory resulting in long-term effects on growth and physiological function. Since digestive capacity is key to resilience of fish populations, applying this novel concept to the fish aquaculture production provides numerous possibilities for improving adaptive responses of fish to challenging conditions.

Nutritional research has demonstrated that dietary protein and amino acids (AA) a play fundamental role in the overall fish digestive capacity and consequently growth performance. The objective of this work was to assess *in ovo* AA supplementation as long-term modulator of zebrafish larva intestinal maturation and metabolic capacity at optimum (28ºC) and challenging temperature (32ºC).

Materials and methods
The AA supplementation was performed at zebrafish embryonic stage (3.5 hours post-fertilization), using the sonophoresis technique. The experimental setup was: control (CTRL, no supplementation) and amino acid arginine (ARG) or glutamine (GLN) supplementation. The experiment lasted until 898 growing degree-days (GDD). Growth performance, free amino acids (FAA) profile, methylation index (SAM:SAH ratio) and digestive enzymes activity levels were analysed to evaluate the larval nutrition-induced metabolic plasticity and the effects on fish resilience to challenging conditions.

Results
Results confirmed that fish survival was not affected either by the sonophoresis technique or rearing temperature, with an average survival of 52%. Growth performance was affected by both, temperature, and AA treatment. Overall, 28ºC-fish showed higher final dry weight (DW) than 32ºC-fish. On the other hand, arginine supplementation significantly improved fish weight at 32ºC.

The FAAs profile confirmed the effectiveness of sonophoresis for arginine and glutamine supplementation into zebrafish eggs, with significantly higher levels of arginine and glutamine recorded in ARG and GLN embryos, respectively. In addition, the effects of the early supplementation in the larval FAAs profile were maintained until the end of the experiment. Taurine was the main amino acid clustering the samples, with higher levels in larvae reared at 32ºC, independently of the treatment.

Both, digestive enzymes activity levels and methylation index (SAM:SAH ratio) were higher in 32ºC reared fish. Specifically, for the methylation index, the maximum level was reached in the ARG fish.

Discussion
This study confirms sonophoresis as a good technique to incorporate amino acids in fish eggs. Results shows that *in ovo* arginine supplementation promoted fish performance in challenging condition.

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Taurine is involved in the coping mechanism of several species to thermal stress, mainly by its antioxidants properties and by regulating the expression of heat shock proteins (Nakamura-Kusakabe et al., 2016; Belal et al., 2018; Orhan et al., 2020). Higher taurine levels in 32ºC-fish suggest that this amino sulfonic acid may also have a protective role against thermal stress-induced effects in zebrafish larvae.

Stress events derived from environmental factors, like temperature, can have life-long phenotypic effects on the organisms. These phenotypic differences may indeed occur by epigenetic gene inactivation, such as DNA methylation, rather than changes in gene sequence (Campos et al., 2012). In the present study temperature was found to influence differential methylation index with, 32ºC-fish showing higher values than 28ºC-fish, being the maximum SAM:SAH ratio recorded in ARG larvae. Results suggest a role of DNA methylation in thermal epigenetic regulation of early development in zebrafish, suggesting an improved phenotypic plasticity in the ARG treatment. Results are supported by Campos et al. (2012) who described thermal plasticity of DNA-methyltransferases expression during zebrafish embryonic development.

In conclusion, the present work suggests that *in ovo* arginine supplementation may improve fish larvae resilience and promote a better metabolic adaptation to cope with higher temperatures.

References

Campos C, Valente LMP & Fernandes JMO. (2012) Molecular evolution of zebrafish *dnmt3* genes and thermal plasticity of their expression during embryonic development. Gene 500, 93-100.

Nakamura-Kusakabe I, Nagasaki T, Kinjo A, Sassa M, Koito T, Okamura K, Yamagami S, Yamanaka T, Tsuchida S & Inoue K. (2016) Effect of sulfide, osmotic, and thermal stresses on taurine transporter mRNA levels in the gills of the hydrothermal vent-specific mussel *Bathymodiolus septemdierum*. Comp Biochem Physiol A 191, 74-79

Belal, SA, Kang, DR, Cho, ESR, Park, GH, & Shim, KS. (2018). Taurine reduces heat stress by regulating the expression of heat shock proteins in broilers exposed to chronic heat. Braz J Poult Sci 20, 479-486.

Orhan, C, Kucuk, O, Sahin, N, Tuzcu, M & Sahin, K. (2020). Effects of taurine supplementation on productive performance, nutrient digestibility and gene expression of nutrient transporters in quails reared under heat stress. J Therm Biol 92, 102668.

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DIGESTIVE CAPACITY IN DEVELOPING GREATER AMBERJACK USING MOLECULAR AND BIOCHEMICAL APPROACHES

P. Gamberoni [1,2], M. Yúfera [2], V. de las Heras [3], I. Siguero [3], N. Gilannejad[2], G. Martínez-Rodríguez [2] and C. Navarro-Guillén [1,a]

[1] Centro de Ciências do Mar do Algarve (CCMAR), Universidade do Algarve, *Campus* de Gambelas, 8005-139 Faro, Portugal
[2] Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro, 11519 Puerto Real, Spain
[3] Futuna Blue España S.L. Muelle Comercial S/N, 11500 El Puerto de Santa María, Spain
[a]Current affiliation: Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Rio San Pedro, 11519 Puerto Real, Spain
Email: manuel.yufera@icman.csic.es

Introduction

The greater amberjack, *Seriola dumerili*, is a fast-growing pelagic teleost with great interest for the diversification of farmed marine fish species in the Mediterranean region. As in other fish species that are currently being commercially farmed, a crucial step in the way to industrialisation is to develop suitable spawning and larval rearing methodologies to obtain robust and healthy juveniles. In spite of the advances in the last years, there are still some problems to solve during this stage to prevent mortalities and size dispersion due to pathologies, malnutrition and cannibalism.

Food dependence is still more exigent in a fast-developing species in which the developmental changes may occur faster. Digestive function changes progressively during the larval stage for improving the digestive capacity up to attain the definitive digestion mode of juvenile and adults. These changes are linked to anatomical and physiological changes that appear progressively during the first weeks of larval growth supported by an adequate feeding. Aiming to advance the optimization of feeding during the early stage of this species, this study examined the ontogeny of digestive function using molecular and biochemical approaches during the first weeks of development. Besides, a diurnal cycle was examined in the middle of this larval period.

Materials and methods

Gene expression of digestive enzyme precursors and the enzymatic activity levels has been determined during the first 51 days post hatching (dph) and during the diurnal period of 19 dph larvae reared in semi-intensive conditions. Analysis were performed according to Mata-Sotres et al., 2016 and Navarro-Guillén, C. et al., 2019.

Results

The expression of pancreatic proteases precursors (*try3*, *ctra* and *ctrb1*) increased from first-feeding, while gastric precursors such as gastric chitinase (*chia1*), gastric protease (*pga3*) and proton pump (*atp4a2*) after 10 dph. The expression of pancreatic lipases (*cel1*, *cel2* and *cel3*) peaked between 6 and 10 dph, phospholipase A2 (*pla2g1b*) rose only after 25 pdh, while α-amylase (*amy2a*) increased mainly from 20 dph. The trypsin activity was more evident from first-feeding to 25 dph and chymotrypsin activity from this day onwards. Regarding activity levels, acidic chitinase and pepsin activity appeared, respectively, at 16 and 30 dph. The activity of 7C-like lipase was evident from first-feeding but, as 4C-like lipase, significantly increased from 15 dph, while amylase peaked from 6 to 22 dph. Aminopeptidase and alkaline phosphatase activities started at 20 dph indicating the functional maturation of brush border of the enterocytes.

The daily pattern analysis showed a food anticipatory strategy in the expression proteases related genes. Lipases activity was more evident during the morning hours, followed by amylase, and by alkaline proteases in the afternoon. Results, also, suggested a diurnal alternation in the activity of chymotrypsin and trypsin.

Discussion

There are two recent studies on greater amberjack describing the digestive ontogenetic pattern during larval developmental stages (Navarro-Guillén et al., 2019; Pérez et al., 2020), however, both of them examined only changes in the enzymatic activities. The present study examined for the first time the appearance and functionality profiles of key digestive enzymes at both, molecular and activity levels, in order to complete the previous knowledge on greater amberjack.

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The described patterns in the present work are in agreement with what is expected for a carnivorous and piscivorous fish species, and specifically for fast-growing species, with a trend to decrease its trypsin activity early, due to a precocious stomach development and the consequent transition to adult digestion. Nevertheless, there are some discrepant features that could be due to species-specific characteristics or to the particular experimental conditions and feeding protocol performed in this study.

The ontogenetic pattern of mRNA transcript expression of the examined proenzymes was not linked to that observed for the corresponding activities. In general, the transcript expressions were detected earlier in development than the activity of the corresponding enzymes. The molecular expression and the biochemical activity reflect the produced and used molecules, respectively, and not necessarily may exhibit similar patterns (Mata-Sotres et al., 2016). However, assembling enzymatic activity and gene expression gives insights about regulatory mechanisms underpinning digestive capacity along greater amberjack larval development.

In conclusion, the detailed expression and activity ontogenetic profiles of digestive enzymes reported in this study contributed to advancing in the knowledge of the digestive function in developing larvae of greater amberjack under rearing conditions.

References

Mata-Sotres, J.A. *et al*., 2016. Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream *(Sparus aurata*) during ontogeny. Comparative Biochemistry and Physiology Part A, Molecular & Integrative Physiology 197: 43-51.

Navarro-Guillén, C. *et al.*, 2019. Fast growing greater amberjack post-larvae require a high energy-high protein weaning diet. Aquaculture. 499, 195-202.

Pérez, J.A. *et al*., 2020. The ontogeny of greater amberjack digestive system under different rearing conditions: a histological and enzymatic approach. Aquacult. Nut. 26, 1908– 1925

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MODULATION OF GILTHEAD SEA BREAM GUT MICROBIOTA BY A BIOACTIVE EGG WHITE HYDROLYSATE

F. Naya-Català[1], G.A. Wiggers Peçanha[2], M.C. Piazzon[3], M.I. López-Martínez[4], I. Estensoro[3], J.A. Calduch-Giner[1], M.C. Martínez Cuesta[5], T. Requena[5], A. Sitjà-Bobadilla[3], M. Miguel[4], J. Pérez-Sánchez[1]

[1]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Spain
[2]Cardiovascular Physiology Research Group, Universidade Federal do Pampa, Brazil
[3]Fish Pathology Group, IATS-CSIC, Spain
[4]Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL, CSIC-UAM), Spain
[5]Department of Food Biotechnology and Microbiology, CIAL, CSIC-UAM, Spain
E-mail: fernando.naya@iats.csic.es

Introduction

A bioactive egg white hydrolysate (EWH) treated with pepsin has demonstrated potent *in vitro* and *in vivo* antioxidant and anti-inflammatory properties, improving oxidative stress and inflammation biomarkers on genetically and diet induced obese rats (Requena et al., 2017). However, the effects of protein hydrolysates and bioactive food-derived peptides on gut microbiome remain relatively poorly studied in mammals and fish in particular. Thus, the aim of this study was to unravel the main effects on fish performance, histopathological scoring and mucosal adherent gut microbiota of EWH supplementation in a fish fed a formulation with a high replacement of marine feedstuffs by alternative plant ingredients, using gilthead sea bream as a farmed fish model.

Methods

The feeding trial lasted 8 weeks (May-July) under natural photoperiod and temperature conditions. Juvenile fish (20-24 g initial body weight, 4.8-4.9 kg/m^3) were fed near to visual satiety with control (CTRL) or low fish meal (FM)/fish oil (FO) diets with/without egg white hydrolysate (EWH) supplementation (7.5%). DNA from the adherent bacteria of the anterior intestine 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 with a custom-made pipeline using the RDP database. Alpha diversity was calculated using Phyloseq, and beta diversity using PERMANOVA and partial least-squares discriminant analysis (PLS-DA) models. Metagenome prediction and pathway analysis were performed using Piphillin.

Results

Daily specific growth rates (SGR) varied significantly from 2.16 in CTRL fish to 1.88 in EWH fish as a result of a reduced feed intake. A slight impairment of feed conversion ratio, from 1.03 to 1.10, was also observed. Intermediate values on growth performance parameters were reported with the low FM/FO diet without EWH supplementation. No changes in total plasma antioxidant capacity, and faecal concentrations of lactic acid and short-chain fatty acids were found among dietary groups. The dietary replacement of FM/FO triggered a hyperplasic inflammation of the anterior intestine submucosa that was not alleviated by EWH supplementation. Conversely, alterations on the staining pattern and amount of goblet cells at the level of anterior intestine were reversed in EWH fish, together with a decreased accumulation of lipid vacuoles in the epithelium of posterior intestine, a high abundance of hepatic melanomacrophage centers, and depletion of hepatocyte lipid depots until the restoration of CTRL fish values. Illumina sequencing reads were assigned to 2,117 OTUs and a significantly lower richness was found in the EWH group. Indeed, at the phylum level, *Proteobacteria* reached the highest proportion in CTRL and EWH fish, whereas *Firmicutes* were decreased and *Actinobacteria* increased with the replacement of FM/FO. The proportion of *Actinobacteria* was restored to CTRL values with the dietary EWH supplementation. Additionally, EWH triggered the highest amount of *Bacteroidetes* and *Spirochaetes* phyla. Detailed differences in microbiota composition were analysed with a statistically validated PLS-DA which clearly separated CTRL fish from fish fed low FM/FO diets along x-axis (component 1, 37.4%), whereas component 2 (43.2%) separated the low FM/FO diets with/without EWH along y-axis (Fig. 1). This analysis disclosed 165 OTUs discriminating among diets (VIP ≥ 1), with 46 OTUs representing at least the 1% in one of the groups. For these abundant bacteria, a first type of response was mediated by 17 OTUs that were increasing with the FM/FO replacement and decreasing again in EWH fish. In this group, *Neisseriaceae* family and species of *Ralstonia*, *Lactobacillus*, *Streptococcus*, *Corynebacterium* and *Nocardioides* genera were included. A group of 14 OTUs were present in high proportion in the CTRL group, but decreased in fish fed the two low FM/FO diets. In this case, the dietary plant ingredients drove the decrease of the *Comamonadaceae* family and *Mesorizhobium*, *Brochotrix*, *Bacillus*, *Clostridium sensu stricto* and *Exiguobacterium* genera. The remaining 15 OTUs increased their proportion in fish fed the EWH diet, being in a very low proportion in the other two dietary groups. This response triggered the presence

of *Bacteroidetes* phylum, *Rhodospirilalles* order and *Granucatella*, *Bradyrizhobium*, *Propionibacterium* and *Streptophyta* genera. Inferred metagenome results showed two pathways corresponding to primary bile acid biosynthesis and steroid degradation consistently underrepresented in the microbiota of EWH fish when compared to the other two groups.

Conclusions

These results reinforce the central role of gut microbiota in the regulation of host metabolism and lipid metabolism in particular (Hegyi et al., 2018), supporting a main role of the EWH as an anti-obesity and satiety factor in fish as suggested in rat models of obesity. The potential use of this functional food ingredient in finishing diets, and the role of gut microbiota in tuning fillet fatty acid composition of marketable fish merits further research.

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References

Requena, T. et al. 2017. Pepsin egg white hydrolysate modulates gut microbiota in Zucker obese rats. *Food & function*, 8(1), 437–443.

Hegyi, P. et al. 2018. Guts and gall: bile acids in regulation of intestinal epithelial function in health and disease. *Physiology Review*, 98, 1983-2023.

CHRISTMAS CARP : THE ANIMAL WELFARE AND PUBLIC HEALTH IMPACT OF THE LIVE SALE OF CARP (*Cyprinus carpio var. specularis)* IN POLAND

Máirín-Rua Ní Aodha* [1], Ewa Karuga-Kuzniewska [1,]

[1] Department of Veterinary Epidemiology , Wroclaw University of Life and Environmental Sciences, Wroclaw , Poland
mairinruaniaodha@gmail.com

Introduction
Carp are part of Polish culture and history. Since the 12th century carp, once considered "The fish of the King"have been eaten in the country. Under communism Poland saw the 'Centrala Rybna' programme, a large scale carp breeding programme, designed to provide "Carp on every Christmas table in Poland". Carp is traditionally eaten on Christmas eve.

Societal attitudes towards this tradition are changing as the public's understanding of animal welfare and zoonosis evolves. This project investigated multiple factors affecting the future of this tradition; public awareness, animal welfare impact and public health risks.

Materials and methods
Surveys were conducted with veterinary students and members of the public. There were two surveys, one for veterinary students, the other for consumers purchasing fish.

The next phase of the project was laboratory based. Scale and serum samples were taken from 3 sample groups to measure cortisol levels [1]. The scale and serum samples were evaluated using a Fish Cortisol ELISA kit. Group One consisted of fish slaughtered at the farm by the farmer. Group Two consisted of fish purchased live from street sellers, which were then slaughtered by them. Group Three consisted of fish purchased live, transported to the laboratory live (in a bucket with water) and slaughtered in the laboratory.

Water samples were also taken from the tanks of street sellers and assessed for several bacterial species; Listeria Monocytogenes, Salmonella and Staphylococcus Aureus [2].

Results
Survey results showed that 43% of veterinary students surveyed consume carp at Christmas. 60% of all surveyed see no benefit in purchasing live fish. 4% of students think carp should be slaughtered at home, while 42% think they should be slaughtered at a purpose-built facility. 100% of surveyed students believe carp can experience pain and stress.

Observation by the surveyors stated that carp were transported by consumers in plastic bags or buckets. An alarming finding was that 24% of people buying live carp transported them home in plastic bags without water. Given that 26% of people surveyed didn't know how long they would keep the fish in the transport vessel for, this raises real concern for the welfare of these fish in transport. Furthermore, 66% of people buying live fish were intending to eat them in 4 days time. In the meantime the carp would be kept in a large basin or in the bath in their homes. This places the fish at distinct risk of thermal shock and distress due to variation in water parameters.

The survey also highlighted that the majority (42%) of people slaughter the carp in their home by decapitation, without prior stunning. This method is deemed inhumane by OIE [3].

Statistical Analysis is still being carried out but here are some of our initial findings;

- The highest levels of cortisol were found in Group One, the fish slaughtered by sellers. This is due to poor pre-slaughter stunning techniques and improper slaughter. Five fish purchased and slaughtered by sellers arrived at the laboratory still alive.
- The fish slaughtered at the laboratory consistently had lower serum cortisol levels despite the additional handling transport time to the laboratory. They were stunned percussively and slaughtered by destruction of the brain. This combination proved to me more effective and humane than the stunning and exsanguination by gill cutting carried out by sellers.

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High volumes of Listeria Monocytogenes were identified in one of the tanks by bacterial culture. The species is currently being confirmed by PCR.

Conclusion

The traditional live sale of carp in Poland poses significant risks for animal welfare and public health. Fish experience significant stress due to poor handling and slaughter techniques. Consumers are put at risk by poor qatar quality and presence of zoonotic bacteria.

References

[1] Cortisol and Glucose: Reliable indicators of fish stress? MARCEL MARTÍNEZ-PORCHAS , LUIS RAFAEL MARTÍNEZ-CÓRDOVA & ROGELIO RAMOS-ENRIQUEZ, Pan-American Journal of Aquatic Sciences (2009), 4(2): 158-178

[2] Fish: A potential source of bacterial pathogens for human beings. Novotny, Ladislav & Dvorska, L. & Lorencova, Alena & Beran, Vladimír & Pavlik, Ivo. (2004). Veterinarni Medicina. 49. 343-358. 10.17221/5715-VETMED.

[3] Welfare Aspects of Stunning and Killing of Fish for Human Consumption. OIE 2010 Health (San Francisco) 1-5

Note: All statistical analysis will be completed before the conference occurs, therefore an updated abstract will be submitted in due course.

UPDATED HATCHERY AND CULTIVATION METHODS FOR *Palmaria palmata* CULTIVATION ON LAND AND AT SEA

M. M. Nielsen* & P. S. Schmedes

National Institute of Aquatic Resources, Technical University of Denmark. Øroddevej 80, Nykøbing Mors, Denmark
E-mail: menie@aqua.dtu.dk

Introduction

The red seaweed *Palmaria palmata*, also known as dulse, is a highly demanded food and snack source that shows promising application in multiple products (Mouritsen et al. 2013) why the interest of its cultivation is increasing in Europe. Its high market price of up to 250 euro per dry kilo, justifies cultivation of this cold-water species both on land in controlled environments - but at higher costs - and at sea for large-scale production.

In Denmark, wild beds of dulse are scarce and often not easily accessible. Therefore, for dulse production to develop sustainably there is a need to develop a spore-based approach of production for as well sea-based and land-based cultivation as hatchery methods - despite two decades of cultivation research - still remain inadequate and halt the development of large-scale cultivation.

Here, we present the main results of our recent work with *Palmaria palmata* focusing on innovative methods and new insights into the cultivation steps concerning seed production for both land- and sea-based propagation. This includes methods to increase spore-use efficiency by 1) implementing new seeding strategies, 2) activating female spores and 3) using a multifunctional setup that allows the use of spores for both seeding on substrates and for further propagation in tumbling cultures in a land-based production scenario. Furthermore, we present results on the potential of growing *Palmaria palmata* on land e.g. using process water from a land-based RAS salmon farm.

Findings and conclusions

In a flow-through vertical seeding setup relying on water tumbling to distribute spores uniformly on substrates, we found that 1) nets could be seeded with sufficient densities (>5 seedlings cm^{-1}) using the same sori for three consecutive seedings and 2) that less sori material could be used without any significant difference in seeding density. After the initial seeding, we furthermore demonstrated the potential to double the germination success of seedlings by adding a fertilization step of female gametophytes in the hatchery protocol and thereby increasing spore-use efficiency markedly (Schmedes & Nielsen, 2020).

The seeding setup was accompanied with a filter collector allowing excess spores that did not attach to the substrates in the first place to be collected for later use so no spores were lost from the system. For these excess spores, we have proposed a new seeding method determined **GMA** as it relies on the spores to first **G**erminate, then being **M**acerated and eventually being able to re-attach a seeding substrate under conditions of **A**gitation (Schmedes et al. 2019).

As an alternative to seeding substrates for the purpose of cultivation at sea, excess spores from the abovementioned system were identified as a suitable seed stock for land based production in tumbling conditions allowing for high control of environmental conditions that influence growth of *Palmaria palmata*. In a line of experiments, we have tested the influence of various parameters (light, cleaning, density, nutrients) on the growth performance of *Palmaria palmata* on land in controlled hatchery facilities and found a weekly increase of biomass (wet weight) up to 90% depending on conditions. In a pilot study looking at the growth potential of *Palmaria palmata* in process water from a land-based RAS salmon farm, growth was even higher (up to 110% pr. week), suggesting a hitherto unexploited opportunity to integrate cultivation of the two species. In this perspective, the year-round supply of cold (14-15 degree C) and nutrient rich water from salmon production could act as a mean to lower production costs of *Palmaria palmata* and at the same time expand the normal growing season (Autumn-spring) by avoiding warm summer temperatures.

With these findings showing several means of improving spore-use efficiency for *Palmaria palmata* related to both hatchery production of seeded substrates and possibilities for land-based production, we provide updated methodologies suited for the next steps needed in large-scale and cost-effective production of the highly coveted seaweed, *Palmaria palmata*.

References

Mouritsen OG, Dawczynski C, Duelund L, Jahreis G, Vetter W, Schroeder M (2013) On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr. Journal of Applied Phycology 25: 1777–1791.

Schmedes PS, Nielsen MM & Petersen JK (2019). Improved *Palmaria palmata* hatchery methods for tetraspore release, even settlement and high seedling survival using strong water agitation and macerated propagules. Algal Research. 40, 1014494.

Schmedes PS & Nielsen MM (2020). New hatchery methods for efficient spore use and seedling production of *Palmaria palmata* (dulse). Journal of Applied Phycology. 32: 2183-2193.

AQUAFEED EVALUATION UNDER SPECIFIC FARMING CONDITIONS – A RAS APPLICATION

A. Nobre*[1], F. Soares[1], T. Silva[1], P. Rema, L. Conceição[1]

[1]SPAROS Lda., Olhão (Portugal)

Email: ananobre@sparos.pt

Introduction

Salmonid aquafeeds have evolved over the last half-century to meet the increased industrialization of this sector, with the formulations representing a balance between nutritional requirements, cost, ingredient availability and sustainability standards. Currently, there is a wide variety of commercial aquafeeds designed for different generic farming conditions and fish growth stages. A precise evaluation of the suitability of aquafeeds for the particular conditions of the farm is required to optimize fish feeding and economic feed conversion. This is highly relevant for optimal fish growth and performance, while ensuring optimal water quality, in RAS farms. Optimizing RAS production implies a balance between fish growth, feed efficiency and water quality. Monitoring feeding efficiency indicators is very important not only for the economics of feed conversion but also for planning and managing the biofilter. For instance, decreasing nutrient and solid loads per kg of fish produced enables increasing the fish stocking capacity of the system. In this context, decision supporting tools for monitoring and forecasting the fish nutrient waste, including virtual environments based on mathematical models of fish physiological and metabolic processes, are critical for RAS operation planning.

Herein, we illustrate the application of a nutrient-based model (FEEDNETICS™) to compare two high-energy feeds, including one that is designed for RAS.

Methods

FEEDNETICS™ is a web-application developed by SPAROS 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 highly variable data and is currently available for gilthead seabream, European seabass, rainbow trout and Nile tilapia. The validation charts shown in Figure 1 illustrate the model robustness for this use case.

Both feeds evaluated have a high energy content. However, the RAS feed is denser in nutrients, with higher digestible protein and lipid levels, targeting a reduced FCR thus allowing to increase the farm fish production capacity maintaining the same waste discharges. In terms of DP/DE ratios, the feeds are similar. The feed improvements come at a higher cost (+ 6%).

Results

As expected, the FEEDNETICS™ simulations (Figure 2) indicate that the High Energy RAS feed leads to a better performance: shorter (11%) production cycle, improved FCR by 0.1 units, and a decrease in total N and P wastes of about -12% and -25%, respectively.

For the simulated conditions, the RAS feed leads to a better economic conversion, lower N and P discharges, and with saving estimated around less 48 € of feed per ton of fish produced, despite its higher unit cost. The precise evaluation of aquafeeds will depend on the particular conditions under which fish are reared (e.g. temperature profile). According to the FEEDNETICS™ results, the decrease in the average water temperature affects proportionally more the production performance with the non-RAS feed (Figure 3). For the non-RAS feed, the Total P waste increases about 29% when decreasing the temperature by 2 °C, which is due to a decrease of the P retention.

With the High Energy feed, the production cycle (to grow fish from 50 g to 1 kg) increases by 35 days under the low temperature profile. At 13 °C, the total production time considering the two feeds lags by about 1 month, with the RAS feed representing savings on feed of around 82 € per ton of fish produced when compared with the non-RAS feed.

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Final remarks

The FEEDNETICS™ mechanistic nutrient-based model includes 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. As illustrated in this use case, this type of tools can be used by the aquaculture industry for precision aquafeed evaluation under specific farm conditions. Besides enabling aquafeed evaluation, the FEEDNETICS™ results can help the design and planning of the facilities concerning, for example, biofilter capacity and the cost-benefit assessment of active temperature control. FEEDNETICS™ is available to be used by fish farmers and the aquafeed sector as a web app.

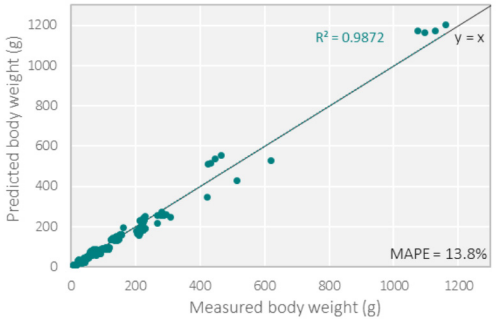

Figure 1 - FEEDNETICSTM validation charts for body weight: measured Vs predicted. Based on a wide range of

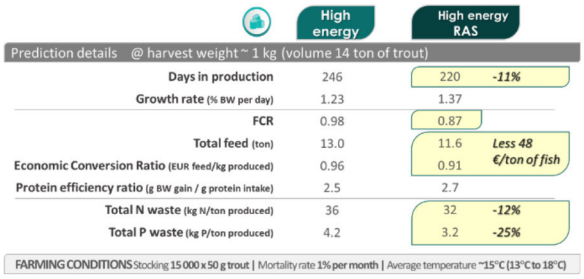

Figure 2 - Results for the comparison of two high-energy feeds under a temperature profile around 15°C.

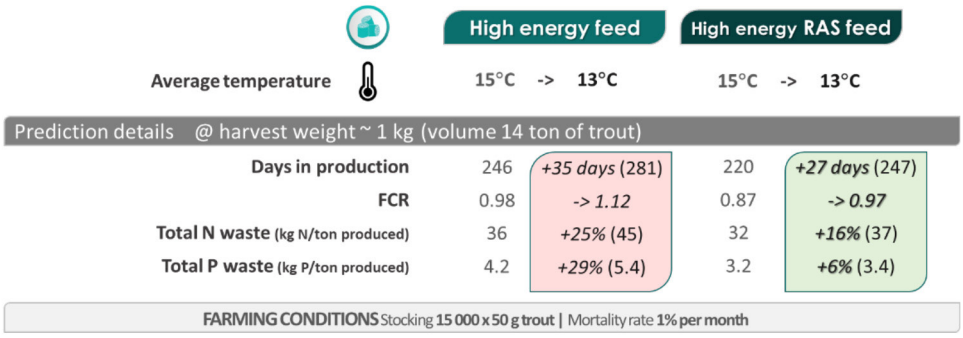

Figure 3 - FEEDNETICSTM model results for the comparison of two high-energy feeds under two temperature profiles around 15°C and around 13°C.

STUDIES OF SOME PREVAILING PARASITIC FISH DISEASES IN THE EXAMINED SOME CULTURED MARINE FISHES AT ISMAILIA PROVINCE, EGYPT

Noor El Deen A I E and Osman H A M

Hydrobiology department, Veterinary Division, National Research Center.
Corresponding author :drahmednoor200254@gmail.com

Objective of this study was assessment of prevailing fish diseases in examined fish in Ismailia province.

Methods of this study was carried out on 1080 premature fish (360 *D. labrax* (225±25 g) and 360 *S aurata* (150±25 g) and 360 *M cephalus* (125±25) collected from assimilatory ponds of studies for examining at the end of treatment.in addition to examined nontreated fish (1080 premature).**Clinical pictures** of the infested fish showed dark colour and respiratory signs.The recorded P.M. lesions were presence of single cymothoid or rarely parasite per fish in the opercular cavity covering gills with congestion or paleness and destruction of gill filaments, in other cases cymothoid parasites were found in buccal cavity in seabass or on the skin of *Mugil Cephalus and* seabream. **Resuts**: The total prevalence of infestation was the total prevalence of parasitic infection of nontreated fishes was 45.83 %. The highest percentage was in *D labrax* 56.94 % followed by*S aurata* 47.22%, the lowest percentage in *M cephalus* 33.33 %., where the prevalence rates.The total prevalence of parasitic infection in premature treated with 2 g algae was 28.79%, followed by 3 g algae was 23.60 %, while the lowest percentage with 5 g algae 20.37 %. respectively. The detected species of parasites were protozoal parasites,*Amyloodinium ocellatum Riboscyphidia* and marine monogenean,*Lamellodiscus dipolicus* crustacean *Lernanthropus kroyeri,Caligus minimus* and *elongatus*,isopoda, *Nerocila orbignyi* isolated from *D Labrex*,*Mugil Cephalus* and*S aurata*. The histopathological alteration was recorded.

SEASONAL PATTERN OF FLESH QUALITY IMPROVEMENT AND SHELF LIFE EXTENSION OF EUROPEAN SEA BASS BY SLURRY ICE COOLING DURING FISH HARVESTING AND TRANSPORTATION

Ntzimani Athina[1], Angelakopoulos Rafael[2], 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

Fish is highly susceptible to spoilage, which can be caused by both intrinsic chemical reactions and microbial growth. The key to fish preservation is the immediate chilling upon catch or harvest to a temperature slightly above the freezing point and maintaining this temperature throughout the cold chain (Kauffeld et al., 2010). The replacement of conventional flake ice with slurry ice as a slaughtering method may result to improved quality stability during subsequent refrigerated storage and shelf-life extension, in terms of microbial growth, flesh quality and sensory degradation of fish (Ntzimani et al., 2021). The objective of the study was the evaluation of the effect of environmental temperature (seasonality) and cooling medium of fish (slurry ice) during harvesting and transportation on flesh quality and key parameters that determine shelf life of European sea bass (*Dicentrarchus labrax*).

Materials and methods

Whole European sea bass (*Dicentrarchus labrax*) was taken from the net cages in Philosofish S.A. farming facilities (Greece) and within 24 h after slaughtering in slurry ice 0, 50 or 100% slurry ice (prepared from seawater), was transported to the laboratory in polystyrene boxes. Four different mixtures of slurry ice and conventional flake ice were tested, coded as C: slaughtered and transported in 100% flake ice, SC: slaughtered in 100% slurry ice and transported in 100% flake ice, S50: slaughtered and transported in 50% slurry ice-50% flake ice, S100: slaughtered and transported in 100% slurry ice. The ratio of ice (slurry or flake) to fish (w/w) was 1:1 and the temperature of the slurry ice was -3.2°C. Sampling was performed in two different periods, i.e. December 2019 and September 2020, in the same fish farm located in Larymna (Fthiotida, Greece). Upon receipt at the laboratory, all fish samples were stored isothermally at 0±0.2°C. Quality evaluation *Brochothrix thermosphacta*, H_2S-producing bacteria yeasts/molds and *Enterobacteriaceae* spp.), colour, texture, lipid oxidation, proteolytic enzymes, and sensory evaluation.

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, 4, 8 and 15 post slaughter, snap-frozen in liquid nitrogen and stored at -80 °C until enzyme extraction. Enzymes were 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 changes. Activity was expressed as fluorescence units (FU) change per minute per mg protein.

Results

At sampling, the average water temperature was 18.5°C and 27°C in December 2019 and September 2020, respectively. The ambient water temperature had no significant effect either on the dominant microflora or on the microbial counts and shelf life of the fish products. *Pseudomonas* spp. and H_2S-producing bacteria were the dominant spoilage microorganisms in all samples tested. However, the use of slurry ice instead of the conventional flake ice led to improved quality and microbial stability during refrigerated storage, as well as to a 2-6 day shelf-life extension of whole sea bass stored at 0 °C. This positive effect did not differentiate with water temperature.

The overall activity of the cytoplasmic calpains and of the collagenases was significantly lower at the low water temperature. No such difference was recorded for the lysosomal cathepsin B and cathepsin L activities. However, the highest cathepsin activities were observed in the C group in the high-water temperature. Significant temporal differences in all enzyme activities were recorded in either water temperature and all methods. A significant correlation between calpain and collagenase activities was observed across slaughter methods and water temperatures. A similar significant correlation between cathepsin B and L activities was observed across methods only at high water temperature.

(Continued on next page)

Discussion and conclusion

The systematic study of the effect of harvesting and transportation conditions on the quality indicators and shelf life during refrigerated storage may provide technological solutions for fish handling with the aim to improve quality and shelf life and reduce food losses during distribution and storage from harvesting up to the consumer level. The use of slurry ice at slaughter appears to lead to improved product quality and extended shelf-life based on microbial growth and intrinsic degrading enzyme activity. It is also evident that water temperature shapes physiological characteristics that are determinant of post-slaughter quality.

Acknowledgment

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References

Barrett, A. J., & Kirschke, H. (1981). [41] Cathepsin B, cathepsin H, and cathepsin L. Methods in Enzymology, 80, 535–561. https://doi.org/10.1016/S0076-6879(81)80043-2

Kauffeld, M., Wang, M.J., Goldstein, V., Kasza, K.E. (2010). Ice slurry applications. International Journal of Refrigeration, 33, 1491-1505.

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), 541–548. https://doi.org/10.1016/j.foodchem.2004.05.015

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 (Accepted, in press).

ASSESSING THE RENEWABLE POWER POTENTIAL FOR OFFSHORE FIN FISH AQUACULTURE

M O'Shea[1], J Fitzgerald[2]

1 University College Cork, 2 Impact 9
Email: Michaeloshea@ucc.ie

Introduction

The development of offshore aquaculture such as the Net9 system has opened up the potential for marine renewable powered integration to supplement or even replace traditional power generation methods on marine based aquaculture facilities. As the aquaculture industry moves further offshore, the potential renewable energy resource increases thus strengthening the case for moving from fossil fuel based generation to a renewable lead power solution. A renewable system potentially offers advantages in reduced fuel consumption, reduced logistical demand and overall reduced operational costs.

Study Description

This study focuses on developing a model to examine the feasibility of a range of renewable energy mix solutions based on the specific energy demands of offshore aquaculture. A power consumption analysis of existing nearshore Atlantic fin fish sites has been undertaken to identify potential similarities and divergences with the proposed offshore installations. Utilising this information, the power demand profiles for a range of typical offshore installations has been synthesized. The development of these power time series, figure 1, enabled an analysis of suitable renewable energy powered generation solutions. Utilising meteo ocean data including wind, wave and solar radiation, an estimate of the available renewable power was calculated. Solutions based on a mix of renewable sources including wind, wave and solar PV, as well as hybrid power solutions including battery banks were then developed and assessed. This work culminated in the application of the model to two case study sites, one in a sheltered Atlantic setting and the other in a deeper exposed location. A renewable hybrid system is sized for both locations and an analysis on the cost implications for reduction in maintenance, diesel and logistics is also presented.

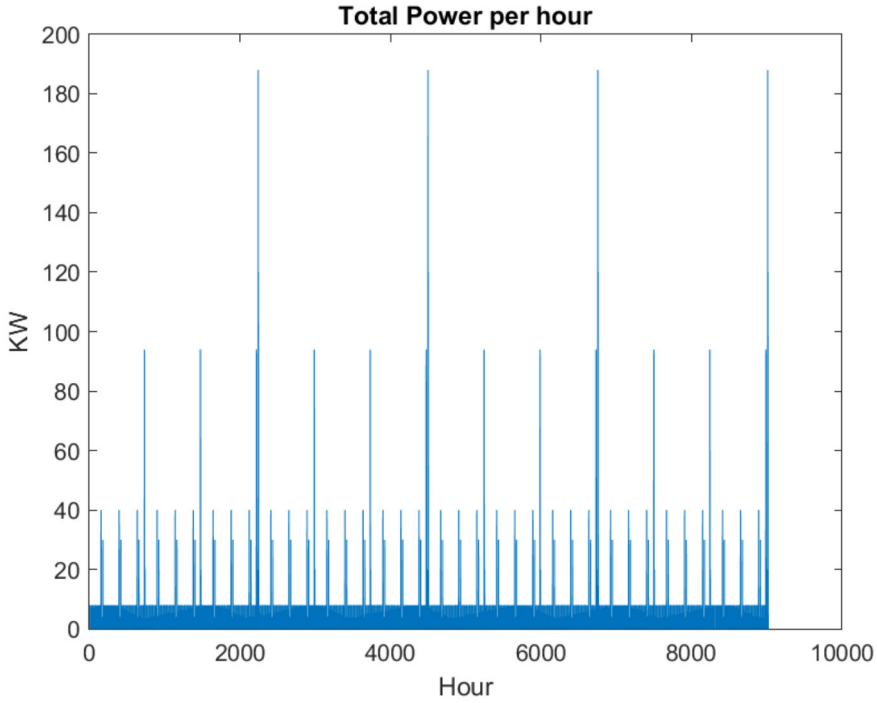

Figure 1 Sample synthesised power consumption of offshore farm

ONTOGENIC DEVELOPMENT OF AFRICAN BONY TONGUE, (*Heterotis niloticus*) (CUVIER, 1829) LARVAE FROM THE YOLK SAC STAGE TO COMPLETE ABSORPTION OF THE YOLK SAC

Ofori-Darkwah, P.*, Adjei-Boateng, D., Edziyie, R.E., Agbo, N.W. and Lund, I.

Department of Fisheries and Watershed Management
Kwame Nkrumah University of Science and Technology, Kumasi Ghana
E-mail:
princedarkwah@yahoo.com
Tel +233246140035.

Introduction

Heterotis niloticus is a potential candidate for commercial aquaculture. However, high larval mortality rates impedes its development for commercial production. Research on the species points to inadequate nutrition of larvae grown in captivity as a possible cause of the high mortality. Thus, a proper understanding of the ontogenic development, and larval behavior will help develop appropriate feeding strategies and proper handling protocols for hatchery management. This study describes the developmental stages of *H. niloticus* larvae observed from hatching of eggs until 10 days after-hatch (DAH) at 28°C.

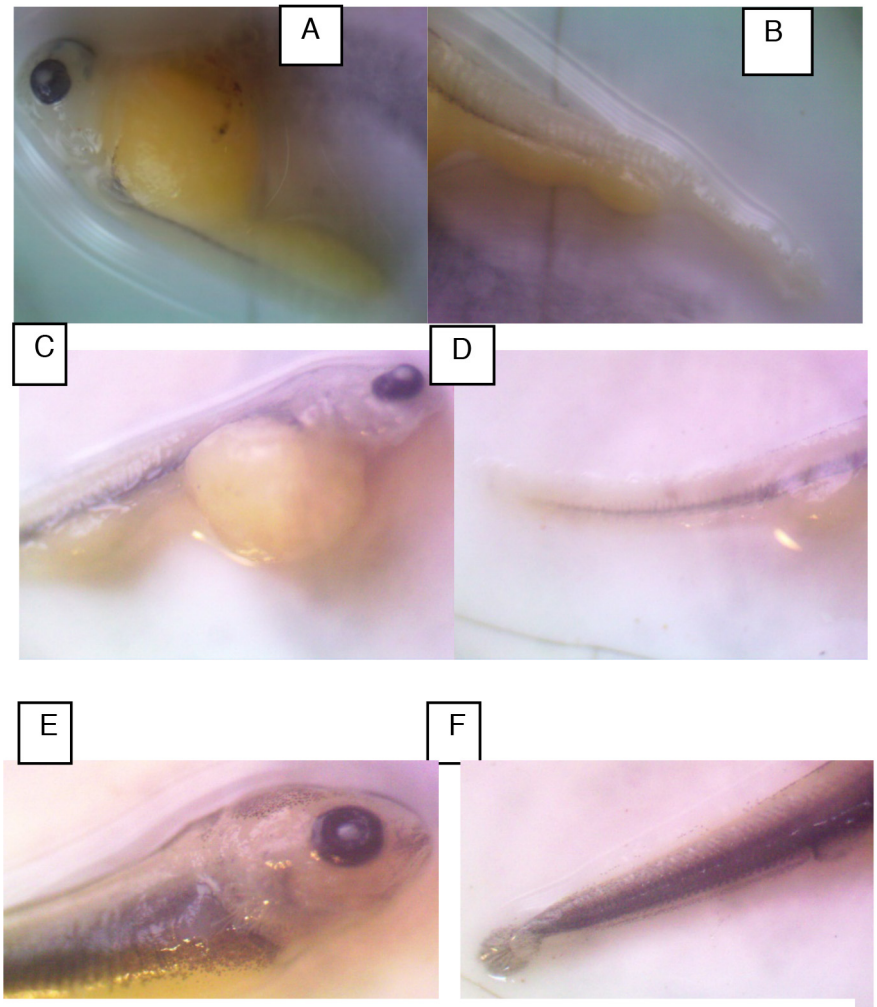

Plate 1. Morphological observations of hatched larvae of *Heterotis niloticus* at different stages of larval development viewed under the microscope: **A and B** larvae at 9 hours after hatch (orange in colour), **C and D**. larvae at 2 DAH, **E and F**. larvae at 4 DAH.

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Material and methods

Fertilized eggs obtained from a natural spawning nest in the Barekese reservoir, Ashanti Region, Ghana, were distributed over three indoor plastic tanks (20L) at a density of about 50 eggsL^{-1}. The survival, growth (weight gain, specific growth rate), and swimming behavior were monitored. Six morphometric characteristics were measured; total length, head length, pre-anal length, trunk length, tail length and yolk sac length. Dead larvae were removed and counted to monitor mortality daily.

Results

At hatching, the larvae had a large yellow vascularized yolk sac filled with yolk platelets, which occupied about 28.5% of the total body length of the abdominal cavity. Yolk platelet reabsorption started 1 DAH (23.5% of the TL) and was fully completed by 5 DAH. At 1 DAH, the mouth and anal parts were closed and no fins were differentiated. Primordial mouth formation and the lower jaw became visible 2 DAH. Between 3–4 DAH, the pelvic fins were visible and the tail fin was pigmented. The larvae started swimming in schools on 5 DAH. Larval length increased from 9.053 ± 0.085 mm at 0 DAH to 14.30 ± 0.081 mm at 10 DAH. The growth curve was defined by the following equation with: $TL = 0.5707x + 9.0545$ ($R^2 = 0.876$), while the increase in total weight was based on the equation $TW = 0.0015x + 0.0085$ ($R^2 = 0.846$), (x = number of DAH). The absolute growth and specific growth rates were 0.525mm day^{-1} (SD, 0.0004) and 4.572% day^{-1} (SD, 0.482) respectively. Larval survival was high above 89.2% up to 8 DAH before mass mortality set in between 9-10 DAH. The morphological and developmental data described constitutes essential baseline information for the culture of *H. niloticus*.

COMPARATIVE ECONOMIC ANALYSES AND DRYING PROFILES OF SMOKE-DRIED CATFISH (*Clarias gariepinus*) USING TRADITIONAL AND ECO-FRIENDLY STANDARDIZED METHODS IN SELECTED FISH MARKETS IN LAGOS STATE, NIGERIA

Ogunbambo*, M. M[1] and Akinleye, S. O.[2]

[1]Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos State, Nigeria
[2]Department of Economics, Faculty of Social Sciences, University of Lagos, Akoka-Yaba, Lagos State, Nigeria
* Corresponding author: mmogunbambo@gmail.com

INTRODUCTION

Fish serves as a healthy source of animal protein, supplying nutrients such as proteins, oils, vitamins and minerals to many people on a global level (Oladimeji, 2017). Freshly harvested fish is however extremely perishable and begins to spoil within a few hours (Pigott, 2015). In order to delay the inevitable spoilage of freshly harvested fish and so prevent spoilage, preservation or processing needs to be carried out (George *et al*., 2014). Over 70 % of freshly harvested fish in Nigeria is processed by smoke-drying (Adeyeye, 2016). This is usually carried out using Traditional Drum Kiln (TDK) and combines heating, drying and application of smoke to the fish, giving it a unique taste that makes it a delicacy. There is however little documented information on costs incurred as well as estimated revenue accrued and the aim of this study was to construct Traditional Drum and standardized Eco Friendly Kilns (EFK), identify market *modus operandi*, assess total costs and estimate revenue generation from smoke-drying catfish (*Clarias gariepinus*) in selected fish markets in Lagos, Nigeria.

MATERIALS AND METHODS

Both Traditional Drum Kiln (TDK) and Eco-Friendly Kiln (EFK) used for this study were constructed according to method of Ogunbambo *et al*., 2018. *Clarias gariepinus* were obtained and prepared according to standard procedures. No form of seasoning was used before arranging the prepared fish samples in both kilns. The smoke-drying process using both kilns was carried out for 24 ± 3 hours at a temperature range of 60 - 85 ^{0}C maintained with the aid of temperature alarm and Visual Light Emitting Diode. The fish were measured on an hourly basis till a fairly constant weight was achieved and the drying profiles of both kilns calculated by plotting the average weights of the smoke-drying catfish in both kilns against time. The economic analyses were carried out by determining the market characteristics, total costs, recurrent costs and revenue generated from the use of traditional drum kiln in eight (8) randomly selected markets covering Lagos State. Personal interviews were also done with the use of validated structured questionnaires and total investment, projected recurrent expenditure, profit per day and payback period over two years were also determined using Eco-Friendly Kiln. All data are presented as means ± standard error (SE). Analysis of variance (ANOVA) was set at 0.05 level of significance and the data collected from structured questionnaire administration was used in determining market characteristics and profit assessment.

RESULTS

Drying Profiles and Weight Loss of *Clarias Gariepinus* Smoke-Dried using Traditional Drum and Eco-Friendly Kiln Fitted with Different Sized Smoke Filters

The initial weights of the fresh catfish prior to smoke-drying using both kilns were above 200 g and most rapid weight loss of an average of 50 % was observed to be in the first five (5) hours using both kilns (Fig. 1). The smoke-drying process however had to be stopped at eighteen hours using TDK as the catfish samples had become very brittle and were starting to disintegrate. The least clogging was found in EFK using combination of two layers of 0.3 cm smoke filters while the highest clogging of the smoke filters was found with EFK using combination of two layers of 0.1 cm smoke filters.

COSTS AND RETURNS

Table 1 shows that the market characteristics and mechanics of fish procurement differ in the investigated fishing villages. It was also found via questionnaire administration that the smoke-during process was predominantly carried out by women with little formal education and also that the processing and distribution activities were profitable, agreeing with Abolagba and Uwagbai, 2011 and Obasohan *et al*., 2012 who did studies in Benin City. This study also showed that profit would be doubled with the use of EFK as against TDK (Table 2).

Conclusion

The use of EFK can ensure better quality smoke-dried catfish as well as improved health conditions of fish processors if introduced to markets across Lagos State. Its lower overall cost, greater turn over, increased profit and longer shelf life would also go a long way in reducing waste and also increase income for fish mongers. Eco-Friendly Kiln can also be easily adopted because of its similar methods of operation to Traditional Drum Kiln and fish processors would not need major training in its usage.

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Table 1: Characteristics of Sampled Markets in Lagos State, Nigeria

Parameters Investigated	Markets							
	Bariga	**Makoko**	**Somolu**	**Ikorodu**	**Epe**	**Itokin**	**Badagry**	**Mainland**
Source of Fresh Catfish	**Open market**	**Badagry lagoon**	**Other Farms**	**Other Farms**	**Contract Basis**	**Epe Lagoon**	**Badagry Lagoon**	**Other Farms**
Number of Drying Trays	Four	Four	Three	Three	Two	Four	One	Two
Freshness Mode	Palm oil + Reheating	Reheating	Reheating	Reheating	None	Reheating	Reheating	Reheating
Marketing Outlet	End Consumers	End Consumers	End Consumers	Retailers and End Consumers	Contract	End Consumers	End Consumers	Retailers and End Consumers

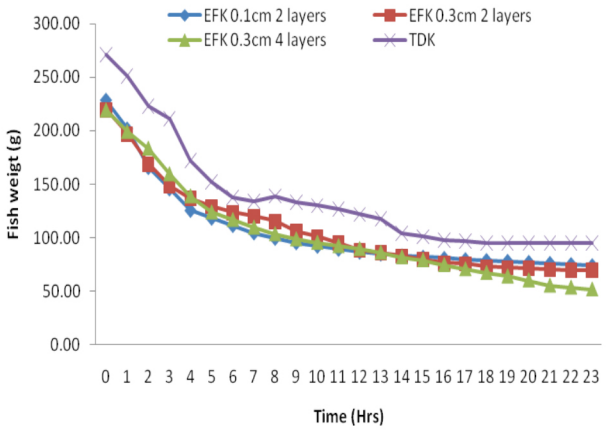

Figure 1: Fish weight of the smoked-dried catfish with Eco-Friendly and Traditional smoking kilns

Table 2: Comparative Cost and Returns for Traditional Drum Kiln and Eco-Friendly Kiln

WEEKLY COSTS (₦)	TRADITIONAL DRUM KILN	ECO FISH KILN
FIXED COST (₦)		
Fixed Cost of Kiln	6,000 (2 months)	100,000 (2 years)
Depreciated Cost of Kiln (Weekly)	750	962
VARIABLE COSTS		
Fish	22,312.50	44,625
Firewood	4,800	4,800
Labour	9,000	18,900
Marketing/Sales Cost	4,200	8,400
Packaging	600	1,200
TOTAL COSTS	41,662.50	78,887
Weekly Revenue	52,500	105,000
Weekly Profit	10,837.50	26,113
Annual Profit	563,550	1,357,876
Profit in Two Years	1,127,100	2,715,752

REFERENCES

Abolagba, O. J., Adekunle, A. T., Dede, A. P. O. and Omoiguil, G. O. (2011). Microbial Assessment of Smoked Fish (*Clarias* spp.) in Benin Metropolis, Edo State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*. 7 (3), 55 - 58.

Adeyeye, S. A. O. (2016). Traditional Fish Processing in Nigeria: A Critical Review. *Nutrition and Food Science*. Vol 46 (3), 321 - 335

George, F. O. A., Ogbolu, A. O. Olaoye, O. J., Obasa, S. O. Idowu, A. A. and Odulate, D. O. (2014). Fish Processing Technologies in Nigeria: A Case Study of Ibeju-lekki Local Government Area, Lagos State. *American Journal of Food Technology*. 9, 302 - 310.

Obasohan, E. E., Imasuen, J. A. and Isidahome, C. E. (2012). Preliminary Studies of the Length-Weight Relationships and Condition Factor of Five Fish Species from Ibiekums Stream, Ekpoma, Edo State, Nigeria. *Journal of Agricultural Research and Development.* 2(3), 061-069.

Ogunbambo, M. M., Osibona, A. O. and Boyo, H. O. (2018). Comparative Drying Profiles and Polycyclic Aromatic Hydrocarbon Content of Catfish Smoke-dried using Traditional Drum and Newly Constructed Eco Fish Kiln. *Unilag Journal of Medicine, Science and Technolgy* 6(2), 17 - 32.

Oladimeji, Y. U. (2017). Trend in Fish Production Parameters in Nigeria and its Total Estimated Demand: Empirical Evidence from Fish Production. *Journal of Animal Production Research Advances*. 29 (1), 410 - 418

Pigott, G. M. (2015). Fish Processing. A Book by Singh, R. P. Encyclopaedia Britania. Last updated 1-2-2015. Retireved 14 January, 2016.

PERMANENTLY RESIDENT ROBOT FOR AUTONOMOUS NET CLEANING AND INSPECTION

Sveinung Johan Ohrem[1]*, Håvard Løvseth Seehuus[2], Martin Føre[2], Eleni Kelasidi[1], Nina Bloecher[1]

[1]SINTEF Ocean, P.O.Box 4762 Torgarden, 7465 Trondheim, Norway
[2]Norwegian University of Science and Technology, NTNU, 7491 Trondheim, Norway
*E-mail: sveinung.ohrem@sintef.no

Introduction

Biofouling, the growth of organisms such as algae, mussels and hydroids on submerged marine structures, is a challenge for finfish farming worldwide. It can lead to decreased oxygen levels in the cages, net deformation, and increased stress on mooring systems due to occlusion of the net. Biofouling can also harbour pathogens that impact fish health. Some fish farmers use antifouling coatings on nets as a preventive measure against biofouling, however, most farmers resort to in-site net cleaning using pressure washers. Net cleaning poses a number of risks to fish health and welfare, as cleaning waste is released into the net pens. Furthermore, it leads to abrasion of the antifouling coatings, reducing their efficacy. As an alternative to pressure washing, brush based grooming robots can be employed. Through regular brushing of the net, these robots aim to prevent the establishment and growth of biofouling communities. [1].

Net inspections and environmental monitoring are also of importance for fish farmers in order to e.g., avoid fish escapes and ensure optimal water conditions (e.g., oxygen concentrations, temperature). With this in mind, a permanently resident autonomous and tetherless subsea robot for cleaning and inspection is currently being developed. This paper addresses the current status of the robots' autonomous functions and the identified requirements that are to be implemented in the future.

Materials and methods

The Netclean 24/7 project (RCN 296392) aims to develop an autonomous net cleaning and inspection robot. The robot will be permanently residing in the fish cage and perform missions on a fixed schedule, or when the operators see fit. An underwater docking station will charge the batteries of the robot and serve as an interface for data transfer to the topside operating station.

To identify the hardware and software requirements for the robot the SEATONOMY design methodology [2] was employed. This method aims to first identify the operations the robot must be capable of performing and then break these operations down into smaller sub-tasks and sub-goals by performing an Autonomous Job Analysis (AJA). Throughout the AJA the designers must answer a set of questions related to e.g., the robots' communication and perception requirements. After the AJA is performed for all operations the designers can collect the requirements in a requirement matrix. Some operations are likely to have the same requirements, and as such, the designers can assign different priority levels to the different requirements.

Results

The SEATONOMY methodology resulted in the identification of the following main operations:
1) Environmental condition monitoring,
2) Net and biofouling inspection,
3) Cleaning and prevention and 4) Docking.

Each operation consists of several sub-operations. Details can be found in [1]. One important sub-operation identified during the AJA for each main operation was that the robot needs to move autonomously on the nets. As such, the robot requires a control system for autonomous operations.

To develop control algorithms and simulate their performance, a mathematical model of the robot was required. This robot, unlike most underwater vehicles, does not float freely in the water. It is attached to a dynamic net which constrains the movement of the robot and affects some of the robots' degrees of freedom. As such, a dynamic model of the robot that considers the effects of the moving net cage and the ocean currents was developed.

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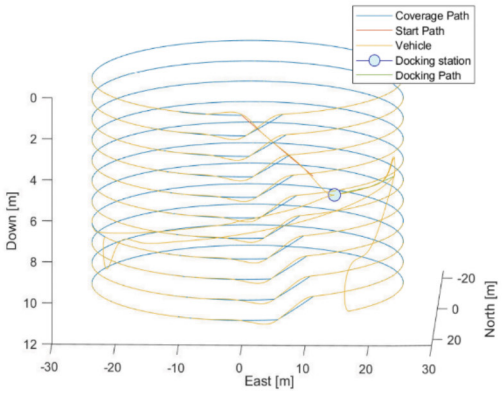

Figure 1: A simulation of the path following with battery charging.

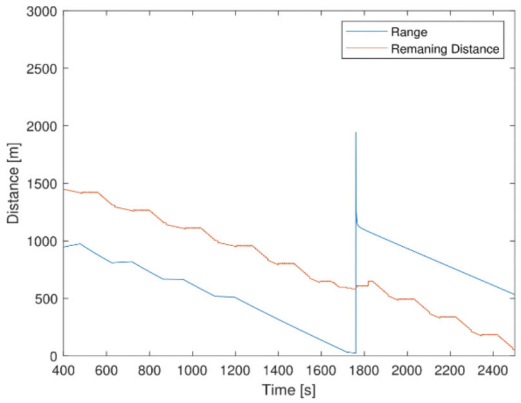

Figure 2: The range and remaining distance. The battery is recharged at t=1800 seconds.

A commonly used path following algorithm is the Line of Sight (LOS) method [3]. LOS guidance provides reference signals to the lower level heading and speed controllers. The operators can create custom paths for the robot to follow, but the robot will autonomously return to the docking station if the battery level is low. When fully charged, the robot can continue the path until completion.

The LOS guidance method was employed on the robot simulation model together with simple PID and PD controllers as low-level heading angle and forward speed controllers. The results of a simulation are shown in Figure 1. Here, the robot starts a cleaning mission and moves around on the net in a circular pattern. When the battery level falls below a threshold, the robot returns to the docking station to recharge the battery. Figure 2 shows the robots' available range and the remaining distance of the chosen path. [4].

Discussion and conclusion

There is need for alternative biofouling mitigation methods in the fish farming industry. An autonomous, tetherless underwater net cleaning robot is currently being developed in the project Netclean 24/7 (RCN 296392). The robot will brush the nets and hence prevent biofouling from forming. The robot will also carry different sensors and cameras and thus provide inspection and measurement possibilities not available today. Currently, the robots' requirements have been defined, a mathematical simulation model has been developed and initial path following capabilities have been demonstrated in simulations. Future work includes developing and implementing adaptive path planners capable of avoiding objects and field testing the autonomous system.

References

[1] S. J. Ohrem, E. Kelasidi, and N. Bloecher, "Analysis of a novel autonomous underwater robot for biofouling prevention and inspection in fish farms," in *2020 28th Mediterranean Conference on Control and Automation (MED)*, 2020: IEEE, pp. 1002-1008.

[2] E. I. Grøtli *et al.*, "SEATONOMY Design, development and validation of marine autonomous systems and operations," 2015.

[3] A. M. Lekkas and T. I. Fossen, "Line-of-sight guidance for path following of marine vehicles," *Advanced in marine robotics,* pp. 63-92, 2013.

[4] H. Løvseth Seehus, "Control Strategies for Autonomous Net Cleaning Operations using Underwater Robotic System," Master of Science, Engineering Cybernetics, Norwegian University of Science and Technology, 2020.

ECOLOGICAL CONCEPTS INTRODUCED TO MANAGE AQUATIC FOOD PRODUCTION

Y. Olsen

Norwegian University of Science and Technology, 7491 Trondheim, Norway
E mail: yngvar.olsen@ntnu.no

Human seafood harvested from the ocean is mainly animal products from more than two trophic levels (TL) higher than food produced in agriculture, which is mainly plant products (Duarte et al 2009). This is a main reason why marine and freshwater food, with a similar initial primary production for oceanic and terrestrial systems, only constitute around 2% of the total human food production. It is difficult to reduce the metabolic losses of two extra trophic transfers in fisheries, but aquaculture open avenues to reduce such losses. The TL of a farmed predator is a function of the TL of the feed ingredients, and a substantial reductions in fish TL has already been achieved by feeding farmed marine fish a feed using more low TL plant ingredients. Cultured marine species like Atlantic salmon has been moved from TL 4.2 for wild salmon to about 2.6 for farmed over the last decades. This is achieved through replacing 80% of fish oil and meal on TL 3.13 (Kautchik and Troell 2010) by first trophic level plant ingredients. This process has already been implemented among others because of limited marine feed resources.

Seafood production are constrained in aquaculture because of limiting available resources with the needed marine lipid quality. Marine primary production is a key ecosystem service supporting human's food production and can be considered as the primary limiting resource for seafood production. The primary production required per unit weight of fish produced can be estimated by back-calculation (Pauli and Christensen, 1995). Fish produced in aquaculture require far less limiting marine primary production than the wild stocks. Wild Atlantic salmon require 105 tons of primary produced C per ton salmon produced whereas cultured salmon on TL 2.4 require around 8.9 tons. The remaining primary production needed for farmed fish is 0.26 tons of terrestrial primary production, lower because these resources are used directly and are fed without going through the amplification of 2.1 trophic transfers like the marine primary production used for producing the fish meal and oil in the feed.

Assuming uniform primary production per ocean area, a sea area of 151 ha ton^{-1} is needed for wild salmon whereas 6.6 ha ton^{-1} is needed for farmed. The changes in feed ingredients and TL has allowed a strong increased production of carnivore fishes, because they are produced on lower TL. It is still a further challenge for sustainability of aquaculture to replace the feed resources by ingredients mainly taken from outside the human food chain. Such replacement is challenging and require time. New marine resources and feed ingredients from microorganisms are perhaps the most promising. The TL concept may serve to help understanding the implication of further changes made and to compare sustainability with other farmed and wild animals.

BIOTECHNOLOGICAL PRODUCTION OF MICROBIAL INGREDIENTS FROM BLUE AND GREEN BIOMASS

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

Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Aas, Norway
E-mail: margareth.overland@nmbu.no

Introduction

Changing climatic conditions and increasing competition for land, water and energy, and fully exploited capture fisheries, emphasize the urgent need for sustainable feed ingredients developed from under-utilized renewable natural resources. While marine ingredients such as fish oil and fishmeal are limited, increased use of some plant proteins as fish feed is questionable from a sustainability standpoint. Reducing competition with human food resources will be key for sustainability, and microbial feed ingredients (MI) can play an important role. MI have rapid growth rate and do not require any agricultural land. Also, they use little fresh water, and can be produced from non-food biomass like trees and seaweeds. Overall, MI do not compete directly with human food. Main categories of microbes are bacteria, yeast, fungi, and microalgae.

Microbial ingredients from natural gas

Gas-based fermentation technology to produce methanotrophic bacteria, such as *Methylococcus capsulatus* grown on natural gas as the energy and carbon source, is advancing. Natural gas is abundant and available at reasonable costs, which suggests that protein production from natural gas could be a realistic large-scale alternative. Bacterial meal contains about 70% crude protein and 10% crude fat and is similar to fish meal in proximate and amino acid composition. Considerable research has been carried out on bacterial meal produced by natural gas fermentation as a protein source for a number of animal species, including Atlantic salmon and rainbow trout (Øverland et al., 2010). Bacterial meal has shown to support high growth performance of salmonids when partially replacing conventional protein sources in nutritionally balanced diets. Bacterial meal also contains a wide range of bioactive components that has shown to have positive effects on gastro-intestinal health in Atlantic salmon (Romarheim et al., 2011; 2013; Skugor et al., 2020 to be submitted).

Other alternative substrates for bacterial meal production are the use of biogas, H_2 and CO_2, but this technology is still in its infancy. Technology to produce microalgae by heterotrophic fermentation or from autotrophic cultivation is also developing and microalgae are increasingly used as a protein and energy source in fish feeds.

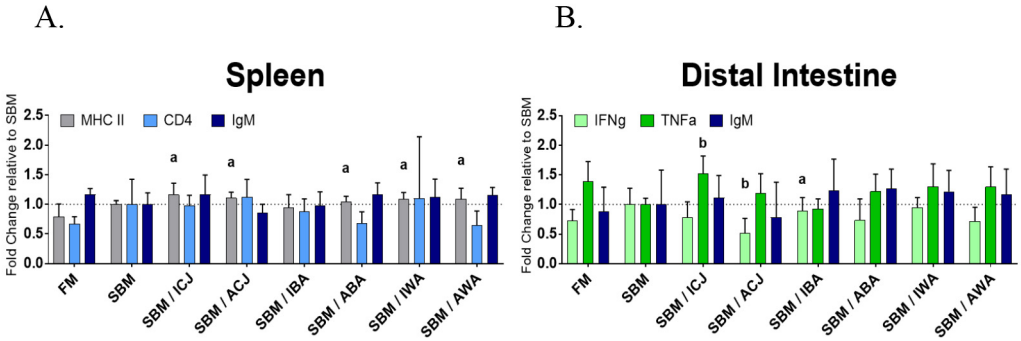

Figure 1. Effect of yeast strain and processing on immune response of salmon. A. Protein expression of markers in Spleen; B. Protein expression of markers in Distal intestine. MHC II: Major Histocompatibility Complex II, CD4: Cluster of differentiation 4, IgM: Immunoglobulin M, IFNg: Interferon gamma, TNFa: Tumor necrosis factor alpha. a: Significant difference vs. FM ($p<0.05$); b: Significant difference vs. SBM ($p<0.05$).

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Yeast from lignocellulosic biomass

Underutilized co-products from agriculture, forestry or aquaculture sectors can provide resources for production of MI. Using lignocellulosic biomass like spruce trees or cultivated seaweeds as substrates for feed production is particularly appealing for a country like Norway, with limited cultivable land area and challenging climatic conditions. Processing of lignocellulosic biomass to produce yeast requires 4 major steps: thermo-chemical pre-treatment; enzymatic hydrolysis of lignocellulosic biomass into sugars; fermentation technology using special yeast strains to convert sugars into microbial biomass; and downstream processing to produce a high-quality yeast-based protein source. The yeast obtained contains about 50-55% crude protein with a favorable amino acid composition and bioactive components like β-glucans and mannoproteins (Lapeña et al., 2020; Øverland & Skrede, 2017). Oleaginous yeast species can also utilize lignocellulosic biomass to produce high-lipid yeast-based feed resources.

Yeast in diets for Atlantic salmon

In general, our studies show that fish perform well when fed yeast-based diets, compared to a high-quality fish meal control as well as plant-based diets (Øverland et al., 2013; Sahlmann & Djordevic et al., 2019). Feeding diets containing moderate levels of yeast (2.5 to 20%) also have positive health effects, including improved gut barrier function and stimulation of the innate immunity (Grammes et al., 2013; Reveco-Urzua & Hofossæter et al., 2019). However, yield, nutritional value and health effects of yeast may vary depending on the species, fermentation process and downstream processing conditions (Lapeña et al., 2020; Hansen et al., 2020 submitted).

Recently, we evaluated three yeast strains (*Cyberlindnera jadinii* (CJ), *Blastobotrys adeninivorans* (BA) and *Wickerhamomyces anomalus* (WA)) in diets for Atlantic salmon fry. The yeast was fermented in a media of sugars from Norwegian spruce, ammonia and enriched nitrogen sources from chicken hydrolysates and then exposed to autolysis. The diets were: a fish meal based control (FM), a challenge diet with 40% soybean meal (SBM), and 6 test diets with 40% SBM and 5% of the following inactivated (I) yeasts: IBA, IWA and ICJ and their autolysates (ABA, AWA and ACJ). Adding yeast to a challenge SBM-based diet did not affect growth performance or morphology in distal intestine (DI), but, yeast strains and autolysis processing induced different immune responses through the modulation of biomarkers associated with innate and adaptive components in spleen and DI (Figure 1). For each strain, the structure and physicochemical properties of the cell wall and the immune response in cell culture were also evaluated.

The use of CJ yeast was also evaluated in a salmon trial during sea water transfer. Adding 25% CJ yeast to a commercial-like diet increased feed intake and growth, reduced secretion of cytokines in distal intestine (DI) (IFNγ, TNF-α, IL-1β and IL-8) on a transcriptional and a protein level, prevented morphological changes, and expression of CD3 cells in DI after seawater transfer (Sahlmann & Djordjevic et al., 2019). Thus, yeast, depending on the species and processing conditions, can be a promising protein source with health-beneficial properties during critical life stages.

Conclusion

Continued research and development in production of MI can make an important contribution to securing the sustainability of the aquaculture industry. As the technology advances and is proven profitable and demand from the feed market exist, industry actors will play a major role in taking this technology further to commercial production.

References

Grammes et al., 2013. PLoS ONE 8(12), e83213 (doi: 10.1371/journal.pone.0083213).
Hansen et al., 2020 submitted to Aquaculture, April 2020.
Lapeña et al., 2020. Microbial Cell Factories 19, 19 (doi: 10.1186/s12934-020-1287-6).
Reveco-Urzua & Hofossæter et al., 2019. PloS ONE 14(12), e0218360 (doi: 10.1371/journal.pone.0218360).
Romarheim et al., 2011. Journal of Nutrition 141, 124–130.
Romarheim et al., 2013. British Journal of Nutrition 109 (6), 1062-1070.
Sahlmann & Djordjevic et al., 2019. Aquaculture, 513, 734396 (doi: 10.1016/j.aquaculture.2019.734396).
Skugor et al., 2020 to be submittet to Plos ONE.
Øverland & Skrede. 2017. Journal of the Science of Food and Agriculture 97, 733-742.
Øverland et al., 2010. Archives of Animal Nutrition 64(3), 171–189.
Øverland et al., 2013. Aquaculture 402–403, 1–7.

BIOTECHNOLOGICAL PRODUCTION OF MICROBIAL INGREDIENTS FROM BLUE AND GREEN BIOMASS

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

Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, P. O. Box NO-1433, Aas (Norway)
E-mail: margareth.overland@nmbu.no

Introduction

Changing climatic conditions and increasing competition for land, water and energy, and fully exploited capture fisheries, emphasize the urgent need for sustainable feed ingredients developed from under-utilized renewable natural resources. While marine ingredients such as fish oil and fishmeal are limited, increased use of some plant proteins as fish feed is questionable from a sustainability standpoint. Reducing competition with human food resources will be key for sustainability, and microbial feed ingredients (MI) like yeast, can play an important role. MI have rapid growth rate and do not require any agricultural land, they use little fresh water, and can be produced from non-food biomass or underutilized by-products from agriculture, aquaculture, forest and seaweeds. Overall, MI do not compete directly with human food.

Yeast species can also use local available waste streams such as food and agricultural waste to produce high-lipid or protein-based microbial feed resources. Using lignocellulosic biomass like spruce trees or cultivated seaweeds as substrates for feed production is particularly appealing for a country like Norway, with limited cultivable land area and challenging climatic conditions. Processing of lignocellulosic biomass to produce yeast requires four major steps: thermo-chemical pre-treatment; enzymatic hydrolysis of lignocellulosic biomass into sugars; fermentation technology using special yeast strains to convert sugars into microbial biomass; and downstream processing to produce a high-quality yeast-based protein source. The yeast obtained contains about 50-55% crude protein with a favorable amino acid composition and bioactive components like β-glucans and mannoproteins (Lapeña et al., 2020; Øverland & Skrede, 2017; Agboola et al., 2020).

Results

In general, our studies with Atlantic salmon showed that fish perform well when fed yeast-based diets, compared to a high-quality fish meal control as well as plant-based diets. Feeding diets containing moderate levels of yeast also have positive health effects, including improved gut barrier function and stimulation of the immune system, especially during sensitive period such as seawater transfer of salmon smolt where the aquaculture industry can experience high losses. However, yield, nutritional value and health effects of yeast may vary depending on species, fermentation and downstream processing conditions (Lapeña et al., 2020; Hansen et al., 2020).

Recently, we evaluated the structure and physicochemical properties of the cell wall of three yeast strains (*Cyberlindnera jadinii* (CJ), *Blastobotrys adeninivorans* (BA) and *Wickerhamomyces anomalus* (WA)). For this, each yeast was fermented in a media of sugars from Norwegian spruce trees, ammonia and enriched nitrogen sources from chicken hydrolysates and then exposed to autolysis. Then, the yeasts were evaluated in diets for Atlantic salmon fry. The diets were: a fish meal based control (FM), a challenge diet with 40% soybean meal (SBM), and 6 test diets with 40% SBM and 5% of the following inactivated (I) yeasts: IBA, IWA and ICJ and their autolysates (ABA, AWA and ACJ). Adding yeast to a challenge SBM-based diet did not affect growth performance or morphology in distal intestine (DI). Yeast strains and autolysis processing, however, were able to differentially modulate biomarkers associated with the immune response in the spleen and DI (Figure 1).

The use of CJ yeast was also evaluated in a salmon trial during sea water transfer. Feeding a commercial-like diet containing 25% CJ yeast for 4 weeks in fresh water and 4 weeks in sea water increased feed intake and growth rate, reduced secretion of cytokines in distal intestine (DI) (IFNγ, TNF-α, IL-1β and IL-8) on transcriptional and protein levels, prevented morphological changes, and modulates CD3 cell expression in DI after seawater transfer (Sahlmann & Djordjevic et al., 2019). The results suggest that yeast has beneficial health properties in diets for smoltifying Atlantic salmon. Thus, yeast, depending on the species and processing conditions, can be a promising protein source with health-beneficial properties during critical life stages.

Conclusion

Continued research and development in production of microbial ingredients can make an important contribution to securing the sustainability of the aquaculture industry. As the technology advances and is proven profitable and demand from the feed market exist, industry actors will play a major role in taking this technology further to commercial production.

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References

Agboola, J., et al., 2020. Reviews in Aquaculture, 1-22. /doi.org/10.1111/raq.12507
Grammes et al., 2013. PLoS ONE, 8(12), e83213.
Hansen, J.Ø., et al., 2019. Aquaculture, 511, 734239.
Hansen, J.Ø., et al., 2021. Aquaculture, 530, 735707.
Lapeña, D., et al. 2020. Microbial Cell Factories, 19(1), 19.
Sahlmann, C., Djordevic, B., 2019. Aquaculture, 513, 73439.
Øverland, M. & Skrede, A. 2017. J. of the Sci, of Food and Agric, 97, 733-742.
Øverland, M., et al., 2013. Aquaculture, 402–403, 1–7.

FIELD EVALUATION OF ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY POINT-OF-CARE DIAGNOSTIC DEVICE FOR PRESENCE OF SAV (*Salmonid alphavirus)* IN FARMED ATLANTIC SALMON (*Salmo salar*) IN NORWAY

Katarzyna Pala*, Michalina Alicka, Karolina Lechowicz, Ivanna Zlobenko

Fish Farm Solutions Ltd, Bierutowska Street 57-59, 51-317 Wrocław, Poland

karolina.lechowicz@etongroup.eu

Introduction

Salmonid alphavirus (SAV) is a single-stranded RNA virus affecting Atlantic salmon (*Salmo salar)*. It is known to be responsible for pancreas disease (PD) and sleeping disease (SD) which are increasing problems, causing high fish mortality and economic losses in the European aquaculture industry.

Point-of-care diagnostic kit (PD-Sensor Kit) by Fish Farm Solutions company is based on electrochemical impedance spectroscopy. The diagnostic set consists of single-use 8-channel electrodes (microsensors) covered with bioreceptor bonding specifically SAV capsid protein, allowing detection of SAV infection in fish.

Real Time RT-PCR and results obtained using PD-Sensor Kit were compared using samples of heart tissue taken from two different farmed Atlantic salmon populations with different infection status - one population regarded negative and one in a late stage of infection.

Materials and methods

The presence of salmonid pathogens was determined using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR). Heart tissue from each single fish sample was individually isolated and placed into tubes containing 1 ml of Fenozol reagent (A&A Biotechnology, Gdańsk, Poland). Briefly, tissues were disrupted and homogenized using manual microhomogenizator (Scientific Specialties, Inc. Lodi, CA USA). Extraction of total RNA was performed using the Total RNA Mini Kit (A&A Biotechnology, Gdańsk, Poland) according to the manufacturers instructions.

In order to evaluate the presence of salmonid pathogens, cDNA was synthesized using SensiFAST cDNA Synthesis Kit (Bioline, London, UK). The procedures were performed, following the protocol provided by the producer. qRT-PCR was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The PCRs were performed in 10µL reaction volume with 1 µL of cDNA, 500 nM of specific primers, using the AMPLIFYME SG Universal Mix (AM02) (Blirt, Gdańsk, Poland). The amplification was carried out with initial activation of the enzyme at 95°C for 30 s followed by 40 cycles of the following: 94°C for 15 s, annealing at 60°C for 10 s, and 72°C for 55 s for SAV, IPNV, PMCV and PRV, whereas the optimal annealing temperature for primers specific to ISAV and VHSV was 55°C.

Sample preparation and measurements using Fish Farm Solutions technology were conducted according to PD-Sensor Kit Instructions for Use. Firstly, 10 mg piece of frozen before in -80°C salmon heart tissue was chopped finely using scalpel (Bayha®). Secondly, tissue was placed in 1ml of lysis buffer in 2ml test-tube and subjected to the process of disruption and homogenization using manual microhomogenizator (Scientific Specialties, Inc.

Lodi, CA USA). Sample was left for 15 minutes in temperature -10°C for the lysis process to finish.

Afterwards, to separate heart tissue from the lysate, the sample was filtered through the cell strainer (pluriStrainer Mini 20µm, pluriSelect Life Science UG & Co. KG) directly into the test-tube filled with 1ml of measurement buffer.

According to established procedure, 250µl of measurement buffer was applied on the microsensor (electrode). After 5 measuring cycles (calibration), 50µL of prepared earlier sample was added on the surface of the electrode and measured up to 10th cycle.

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Results and conclusions

A significant difference between impedance changes due to SAV positive and SAV negative sample addition was observed. In case of SAV negative fishes, level of impedance after addition of the sample did not change, curves representative for following cycles were on the same level as during calibration. On the contrary, addition of SAV positive samples showed significant reaction represented by an increase of impedances. Additionally, no cross-reactions with VHSV (*Viral hemorrhagic septicemia virus*) were observed. According to RT-qPCR assay, all of the fishes with negative SAV results were infected with VHSV (Cq values: 30.780 ± 0.663). Despite the presence of VHSV pathogen in the samples, tests conducted using PD-Sensor Kit presented no reactions when VHSV infected samples were tested on SAV detecting microsensors.

Acknowledgements

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AQUAPONICS IN MECKLENBURG-WESTERN POMERANIA, NORTHERN GERMANY: INTEGRATION OF AQUACULTURE PRODUCTION INTO REGULAR FARMING PRACTICES SUPPORTS RESOURCE EFFICIENCY AND THE CIRCULAR ECONOMY

Harry W. Palm[1*]

[1]Aquaculture and Sea-Ranching, Faculty of Agricultural and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany
[*]Mail: harry.palm@uni-rostock.de

Introduction

Aquaponics combines the production of aquatic organisms with different plants that are cultivated with the help of feed-derived nutrients converted through bacteria. It is "a production system where the majority (> 50%) of the nutrients sustaining the optimal plant growth derives from waste originating from feeding the aquatic organisms" (Palm et al., 2018). This definition includes the production of algae in bioreactors and the soilless cultivation of plants in hydroponics (aquaponics s.s.) as well as horticulture of plants in substrate and agriculture on farmland (aquaponics s.l.).

Beside insects, the protein production through aquatic organisms is most resource efficient compared with other farmed animals. Due to its high feed efficiency, bony fish are a more sustainable source of protein in human nutrition. For instance, to produce one kilo of fish about 0.8-1.5 kg of fish feed is needed, whereas for the same amount of meat in poultry 2-3 kg, in pigs 3-4 kg and in cattle production about 7-8 kg concentrated feed is used (Tidwell 2007). Although fish are kept in their natural habitat, their water consumption is significantly lower. In state-of-the-art recirculation aquaculture systems (RAS), the production of one kilogram of fish consumes about 0.03-0.1 m^3 of water per kilogram of feed (Martins et al., 2010), thus 0.6-1.5 kg of fresh biomass produced. Water consumption is significantly higher for terrestrial farm animals, for poultry meat at 1.5-2.5 m^3/kg, for pork at 3.7-4.0 m^3/kg and for beef at 3.9-10.3 m^3/kg (Mekonnen and Hoekstra 2012).

The CO_2 balance of, for example, salmon from Norwegian aquaculture with approximately 2.5 kg CO_2/kg of fish is significantly lower than that of pigs (2.4-16 kg CO_2/kg) and cattle (8-33 kg CO_2/kg) (Müller-Lindenlauf et al., 2013). As a result, the carbon footprint of aquaponic production processes is even better. Consequently, aquaponics further increases resource efficiency and can be considered one of the most sustainable agricultural system for the future.

Because of dwindling soil for agriculture, increasing desertification and increasing human population, the future generations must move towards a circular economy. Aquaponics (*s.s.*/soilless; *s.l.*/with soil) increases resource efficient protein production and also allows a better reuse of aquaculture waste products. Because of the nearly unlimited possibilities for system designs, local adaptations and a wide range of produce, these systems can be adapted to many locations and conditions. This is exemplified through first farms in Mecklenburg-Western Pomerania that integrate aquaculture and regular farming practices. Constraints and potential of these production methods are discussed.

References

Palm, H.W., U. Knaus, S. Appelbaum, S. Goddek, S.M. Strauch, T. Vermeulen, M.H. Jijakli, and B. Kotzen. 2018. Towards commercial aquaponics: A review of systems, designs, scales and nomenclature. Aquaculture International 26(3): 813-842.

Martins, C. I. M., E. H. Eding, M. C. Verdegem, L. T. Heinsbroek, O. Schneider, J. P. Blancheton, ... & J. A. J. Verreth. 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquacultural Engineering, 43(3): 83-93.

Mekonnen, M.M., A.Y. Hoekstra. 2012. A Global Assessment of the Water Footprint of Farm Animal Products. Ecosystems 15: 401–415

Müller-Lindenlauf, M., Zipfel, G., Münch, J., Gärtner, S., Rettenmaier, N., Paulsch, D., Reinhardt, G. (2013). CO2-Fußabdruck und Umweltbilanz von Fleisch aus Baden-Württemberg. 66 p.

Tidwell, J. 2007. Aquaculture Production Systems, Whiley-Blackwell, p.60

ECO-INTENSIFICATION OF COMMON CARP FARMING IN EUROPE, FIRST LESSONS LEARNT FROM THE GAIN PROJECT

Panicz R.[1], Eljasik P.[1], Troszok A.[2], Sobczak M.[1], Lisiecki S.[1], Nędzarek A.[3], Sadowski J.[3]

[1] Department of Meat Science, Faculty of Food Science and Fisheries, West Pomeranian University of Technology, Szczecin, Poland
[2] Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences, Chybie, Poland,
[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

Pond aquaculture is an important component of freshwater aquaculture in Europe and globally. The main target species cultured in the European pond culture is common carp (*Cyprinus carpio* L.) contributing to 1.8% (0.17 Mt) of the total inland aquaculture production (9.42 Mt) during 2015–2016 (FAO FishStat, 2017). This biased farming mode, with very short market season concentrated around Christmas, constantly keeps farmers on the brink of profitability. Moreover, carp farms have limited options of specialisation, vertical integration, diversification or upscaling, and domestic markets are being eroded by the changing consumer preferences, price competition and imbalances at the value chain (Lasner et al., 2020; Raftowicz et al., 2020). Therefore, carp farmers urgently seek cost-effective and sustainable methods to intensify common carp production and additionally use available on-site by-streams (morts, suspended solids, farm vegetation) to increase profitability and circularity. The aim of this study was to assess influence of wintering in RAS on the performance, welfare and health indices of common carp. The second aim was to develop three (Basic, Hydrated and Commercial) silaging methods of common carp by-streams to develop a straightforward and highly flexible silaging method of production of soil fertiliser.

Material and methods

The study was conducted during 2019/2020 wintering period (October – May) in RAS system (6 tanks, 3 m^3 each) under the tent (ambient conditions). Each tank was stocked with 100 fish (average starting weight of 47 ± 2 g). The feeding trial was performed for 203 days during which fish were automatically fed with two feed blends of different level of protein and fat, i.e., AL (42/12) and AG (30/9). Growth performance and fish weight were assessed every month and feeds dose was calculated based on the current water temperature and fish weight. At the end of the trial, n = 6 fish from each variant were slaughtered and zootechnical data (SGR, WG, TG, FCR, VSI, HSI) collected. Subsequently, liver and intestine samples were dissected for gene expression analysis [*cholesterol 7 alpha-hydroxylase* (*cyp7a1*), *sterol regulatory element-binding protein 1* (*srebp-1*), *lipoprotein lipase* (*lpl*), *fatty acid synthase* (*fas*), *carnitine palmitoyltransferase I* (*cpt-1*), *zonula occludens-1* (*zo-1*), *occludin*, *claudin-3c*, *claudin-11*, γ-glutamyl transpeptidase (*ggt*), *alkaline phosphatase* (*akp*)] and histological assessment. The RNA extraction, cDNA synthesis, qPCR, bioinformatic analysis as well as processing of histological samples were carried out according to previous study (Eljasik et al. 2020). To reach the second aim of the study, by-streams (morts induced by CyHV 3, *Cyprinus herpesvirus* 3) were chopped, minced and mixed with starter bacteria culture and wheat bran (Basic), water and wheat bran starter (Hydrated) or Bokashi commercial mix (Commercial), and placed in a bioreactors (in quadruplicates). The CyHV 3 reactivation potential was assessed by cell cultures (CCB and KF1) and expression of the virus genes classified into three temporal kinetic classes (*orf134*, *orf78*, *orf3*). The feasibility of the silaging method and selection of the best method assessed by pH monitoring, characterisation of the odour profile, elemental analysis and calculating the cost of the in-farm silaging.

Results and Conclusions

In the winter fish trail, no significant mortalities (0.28%) were observed and even in low water temperature AL and AG feeds were consumed by carps. The final body weight, specific growth rate, weigh gain and total growth in the AL group were higher ($P = 0.01$) than in the AG. Feed conversion ratio for AL carps (1.98 ± 0.09) was lower compared to AG fish (2.72 ± 0.07). Gene expression analysis showed that activity of the hepatic *fas* was significantly higher for the fish fed AL feed comparing to AG fish. Whereas, in intestine samples *occludin* and *ggt* in AG fish had lower activity than in AL. The microscopy analysis of carp liver and intestine revealed no pathological indicators (e.g., vacuolisation, nuclei displacement, necrosis, pyknosis) in AL and AG fed fish.

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In the experiment related to management of carp mortalities, cell cultures and the subsequent gene expression analyses showed that the virus was successfully inactivated in the Basic, Hydrated and Commercial silages, confirming their safety. Among the three silaging methods, the Hydrated was the cost-effective one; however, concerning the other features (odour profile, feasibility and final pH level), the Basic was selected as the most promising for implementation. Additionally, elemental analysis showed that the level of nutrients in the Basic silage was higher than in commonly used natural fertilisers, while the content of heavy metals (Pb, Cd, Zn) met the official recommendations for organic fertilisers. The study provides fish farmers with an effective method of silaging mortalities that offers pathogen inactivation (via the combination of decreased pH and microbial activity), turns common by-stream into a valuable product and increases profitability of the farm in a sustainable and cost-effective way.

Concluding, overwintering of common carp in RAS is an alternative to earthen ponds in which high losses of body weight and fast depletion of energetic reserves, fish losses were commonly reported (Hurst, 2007). However, as shown in our study in order to produce market size carp in a hybrid 2-year cycle (pond-RAS-pond) an optimal feed is essential, both to maintain fish growth and to improve overall condition before transferring the carp to the ongrowing pond. Additionally, our study showed that silaging method is straightforward and highly adaptable, as unexpected mortality events of various size and frequency can be easily managed, while the produced organic fertiliser is also safe in terms of pathogens, e.g., CyHV 3. Eco-intensification of common carp farming through sustainable management of morts and reduction of framing cycle is an opportunity to increase the profitability and sustainability of freshwater aquaculture.

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References

FAO FishStat, 2017. Fisheries and Aquaculture Software. FishStatPlus – Universal Software for Fishery Statistical Time Series. FAO Fisheries and Aquaculture Department (online), Rome. http://www.fao.org/fishery/ (Accessed 31 October 2020).

Lasner, T., Mytlewski, A., Nourry, M., Rakowski, M., Oberle, M., 2020. Carp land: Economics of fish farms and the impact of region-marketing in the Aischgrund (DEU) and Barycz Valley (POL). Aquaculture, 519, 734731.

Raftowicz, M., Kalisiak-Mędelska, M., Struś, M., 2020. Redefining the Supply Chain Model on the Milicz Carp Market. Sustainability, 12(7), 2934.

Eljasik, P., Panicz, R., Sobczak, M., Sadowski, J., Barbosa, V., Marques, A., & Dias, J. (2020). Plasma biochemistry, gene expression and liver histomorphology in common carp (*Cyprinus carpio*) fed with different dietary fat sources. Food and Chemical Toxicology, 111300.

Hurst, T.P., 2007. Causes and consequences of winter mortality in fishes. Journal of Fish Biology, 71: 315–345.

MOLECULAR INBREEDING AND GENETIC LOAD FOR FEMALE REPRODUCTION TRAITS IN A RAINBOW TROUT SELECTED LINE

Paul, K.[1]*, D'Ambrosio, J.[1,2], Phocas, F.[1]

1. Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France
2. SYSAAF, LPGP-INRAE, Campus de Beaulieu, 35042 Rennes, France
E-mail : katy.paul@inrae.fr

Introduction

Selection for production traits in closed and small broodstock populations of rainbow trout (*Oncorhynchus mykiss*) over the last 30 years have induced significant levels of inbreeding [1]. Molecular inbreeding can be derived from the identification of homozygous genomic segments, named ROH for run of homozygozity [2]. ROH allow to identify the regions responsible for inbreeding depression along the genome [3]. The aim of the study was to identify through ROHs the main chromosomes that contribute to inbreeding depression for female reproduction traits in a rainbow trout selected line.

Materials and Methods

To answer this question, we analyzed the performance of 1,366 females under linear animal models including the fixed effects of the cohort and spawn week within cohort and the animal additive and dominance genetic effects. A first model fitted a single covariate accounting for the individual inbreeding coefficient (F) derived at the all genome scale. A second model fitted 30 covariates accounting for the distinct inbreeding effects (Fi) of the 30 chromosomes. The traits studied were the female post-spawning weight (PW), the female fork length (FL), the spawning date (SD), the spawn weight (SW), the cœlomic fluid weight (CF), the egg number (EN) and the egg average weight (EW).

Results

The dominance variance explained about 4 to almost 19% of the phenotypic variance of each trait under the second model, the largest effect being estimated for SW and the lowest ones for CF and FL. At the genome scale, a significant effect of inbreeding was only observed for SD and EW, with +10% in F level leading to variations of +12.3% and -3.8% of SD and EW performance, respectively (Figure 1). However, at the chromosome scale, we observed some positive or negative significant effects (ranging from -3.9 to +4.6%) of the Fi for all the traits studied (Figure 2). For example, we estimated positive effects of inbreeding on Omy5 for SD, EW and SW, but negative effects on Omy23 for the three traits. On Omy10, we saw a positive effect of inbreeding for SD while effects were negative for EW and SW.

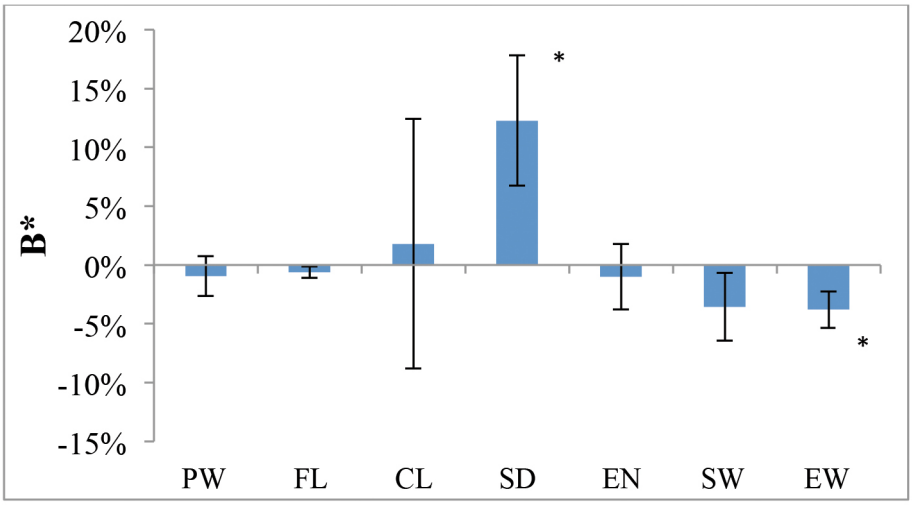

Figure 1. Performance variations expressed in proportion of trait mean for an increase of 10% of total inbreeding coefficient F (e.g. standardized genetic load B*)
*Significant effect of inbreeding at the all genome scale.

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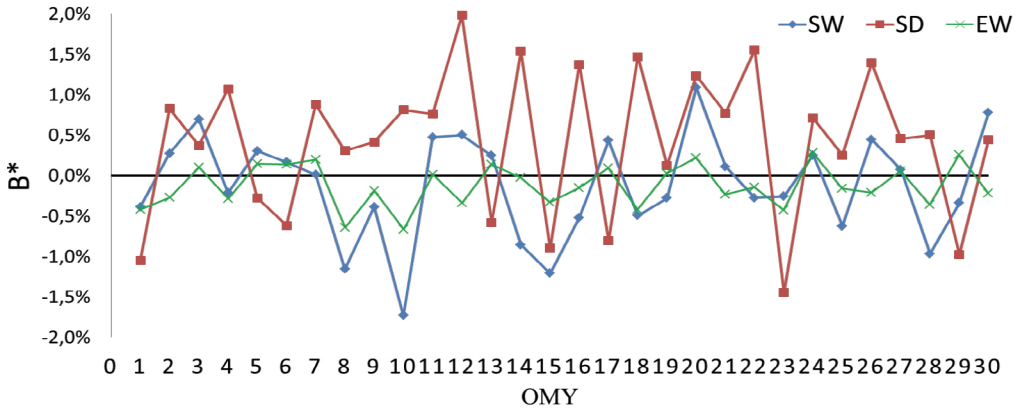

Figure 2. Representation of the standardized genetic load for EW (egg average weight), SW (spawn weight) and SD (spawning date) traits along the chromosomes Omy1 to Omy30.

Discussion and conclusion

We estimated higher dominance ratios for reproduction traits (particularly for SW and EN) in comparison to size traits. It may correspond to a higher number of loci with overdominant alleles playing a role on female fecundity. As expected, we estimated rather unfavorable effects of genome-wide inbreeding on female size and reproduction traits. However, we observed very variable inbreeding effects along the genome, with some favorable or unfavorable effects of local inbreeding. These results suggest that local inbreeding can strongly impact the performance despite the fact that no effect is associated to the total inbreeding cofficient F. To conclude, this work helps identify genomic areas whose genetic diversity is essential for good female reproduction performance and provide tools for a better management of genetic diversity in breeding programs.

Acknowledgments

Material was provided by the breeding company Viviers de Sarrance and funded by the European Maritime and Fisheries Fund and FranceAgrimer (SG-Truite project, n° RFEA47 0016 FA 1000016). Ana Acin Perez and R. Morvezen' implication in data collection is heartfully acknowledged.

References

[1] D'Ambrosio et al. 2019, Genetics Selection Evolution, 51:26.
[2] McQuillan et al. 2008, The American Journal of Human Genetics, 83:359.

DEVELOPMENT OF DROPLET DIGITAL PCR ASSAY FOR MONITORING OF *Saprolegnia parasitica*, AND DEMONSTRATION OF ITS APPLICABILITY IN AQUACULTURE AND NATURAL FRESHWATER ENVIRONMENTS

Dora Pavić*[,1], Anđela Miljanović[1], Uršula Prosenc Zmrzljak[2], Rok Košir[2], Dorotea Grbin[1], Javier Diéguez-Uribeondo[3], Ana Bielen[1]

[1]Faculty of Food Technology and Biotechnology, Department for Biochemical Engineering, University of Zagreb, Zagreb, Croatia
dpavic@pbf.hr
[2] Labena d.o.o., BIA Separations CRO – Molecular Biology Laboratory, Ljubljana, Slovenia
[3]Real Jardin Botánico-CSIC (RJB-CSIC), Department of Mycology, Madrid, Spain

Introduction

Oomycetes are fungal-like microorganisms parasitic towards a large number of plant and animal species. Genera from order Saprolegniales, such as *Saprolegnia* and *Aphanomyces*, cause devastating infections in freshwater ecosystems and aquaculture facilities. *Saprolegnia parasitica* is a widely distributed oomycete pathogen that causes saprolegniosis, disease responsible for significant economic losses in aquaculture, as well as declines of natural populations of fish and other freshwater organisms. Despite its negative impact, no monitoring protocol for *S. parasitica* has been established to date. Thus, we aimed to develop droplet digital PCR (ddPCR) assay for detection and quantification of *S. parasitica* DNA in environmental samples.

Material and methods

Saprolegnia parasitica-specific primers were designed to target internal transcribed spacer region 2 (ITS 2), based on the alignment of ITS sequences of *S. parasitica* and a range of *Saprolegnia* spp., as well as other oomycetes. The limit of detection (LOD) of the assay was established by using serial dilutions of the *S. parasitica* genomic DNA. Specificity of the designed primer pair was tested using genomic DNA of *S. parasitica* (as positive control) and DNA of non–*S. parasitica* oomycete isolates, as well as trout/crayfish DNA. Assay performance was further assessed with water and swab samples from aquaculture (trout farms) and natural environments. Water samples were collected from 21 different locations in Croatia, while swab samples were collected from *S. parasitica* host/carrier species: (i) skin (30 samples) and eggs (15) of rainbow (*Oncorhynchus mykiss* Walbaum, 1792) and brown trout (*Salmo trutta* Linnaeus, 1758), and (ii) cuticle (20) of signal (*Pacifastacus leniusculus* Dana, 1852) and narrow clawed crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823). Samples were classified into agent levels A0 to A6, depending of the number *of S. parasitica* ITS copies per ng of total DNA.

Results

Designed primers specifically amplified a segment of the ITS region of oomycete pathogen *S. parasitica*, while no amplification (i.e. no positive droplets) was obtained for closely related *Saprolegnia* spp. (like *Saprolegnia* sp. 1 and *S. ferax*) and other more distantly related oomycetes. Sensitivity of the assay was high: LOD was 15 fg of pathogen's genomic DNA per μL of reaction mixture. The assay performance was further assessed using environmental DNA samples (water and swab samples). *Saprolegnia parasitica* was detected in 16 out of 21 water samples (76 %) and the range of pathogen's ITS copies in positive samples was between 0.02 and 14 copies/ng of total DNA (agent levels A1 to A3). Furthermore, *S. parasitica* was detected in swab samples collected from the host surface (i.e. adult trout, trout eggs and crayfish). *Saprolegnia parasitica* load was significantly higher in diseased trout samples then in those with healthy appearance: 9375 vs 3.28 *S. parasitica* copies/ng of total swab DNA (median 8, agent level A6 vs. A2, respectively). Despite the fact that none of the sampled crayfish had sings of infection, the pathogen was detected in all tested cuticle swabs. Swabs of *P. leniusculus*, a known *S. parasitica* host, had significantly higher *S. parasitica* load then swabs of *P. leptodactylus*, *S. parasitica* carrier: 390 vs 83 *S. parasitica* copies/ng (median 63 agent level A5 vs. A4, respectively).

Conclusion

Our results demonstrate the applicability of the newly developed ddPCR assay in monitoring and early detection of *S. parasitica* in aquaculture facilities and natural freshwater environment. This would help in better understanding of *S. parasitica* ecology and its effects on the host populations.

DE NOVO TRANSCRIPTOME ASSEMBLY AND ANALYSIS OF TRANSCRIPTOMIC PROFILES DURING LARVAL DEVELOPMENT OF THE MANILA CLAM, *Ruditapes philippinarum* (ADAMS AND REEVE, 1850)

Antonio J. Pazos[1]*, M. Luz Pérez-Parallé[1], Susana Nóvoa[2], Justa Ojea[2], Dorotea Martínez-Patiño[2] and José Luís Sánchez[1]

[1] Dpto. de Bioquímica y Biología Molecular. Instituto de Acuicultura. Universidade de Santiago de Compostela. Campus Vida. 15782-Santiago de Compostela. Spain antonioj.pazos@usc.es
[2] CIMA. Consellería do Mar. Xunta de Galicia. 27700 Ribadeo, Lugo, Spain

Introduction

Settlement and metamorphosis are key events during development of bivalve larvae that allow the transition to an adult life stage. In bivalve molluscs when competence (an appropriate developmental stage) is reached the larvae are receptive to external chemical cues or inducers (Degnan & Morse, 1995; Joyce and Vogeler, 2018). Competent molluscan larvae can be induced to settle and metamorphose using several chemical inducers (Coon et al. 1985; Mesías-Gansbiller et al., 2013; Joyce and Vogeler, 2018), but the molecular mechanisms that allow larvae to become competent are poorly understood. We hypothesize that the expression of adequate levels of membrane receptors for the putative inducer molecules is necessary to reach competence. In the present research, the transcriptome of Manila clam (*Ruditapes philippinarum*) larvae was sequenced and the expression of genes coding for plasma membrane receptors putatively involved in the settlement and metamorphosis was analysed over the course of the larval development.

Materials and methods

Between September and November 2019 *Ruditapes phillipinarum* larvae in four different stages of development (D, umbonate, pediveliger and pre-metamorphic) were collected. After mRNA extraction and cDNA synthesis the cDNA libraries were sequenced by paired-end sequencing (2x100 bp) on Illumina NovaSeq 6000 sequencer. The *de novo* assembly and the quantification of the expression of the transcripts were performed using Trinity (Grabherr et al., 2011) and Kallisto (Bray et al., 2016) respectively. For the analysis of the differential expression, the statistical program Sleuth (Pimentel et al., 2017) was used. The genes with an absolute fold change (FC) > 2, and an adjusted p-value < 0.05 were considered differentially expressed. The completeness of the *de novo* assembly was evaluated with BUSCO (Seppey et al., 2019), using the metazoa database. The transcripts were annotated with OmicsBox software (BioBam Bioinformatics, Valencia, Spain) (Conesa et al., 2005; Götz et al., 2008), using local Blastx 2.10.0+, (E-value threshold of 10^{-3}) against a database of *Mizuhopecten yessoensis*, *Crassostrea gig*as and Swiss-Prot proteins obtained from NCBI and Uniprot.

Table 1. Assembly Summary Metrics

Step	Transcripts	GC (%)	Contig N50	Mean Length	Median Length	No. bases
1st Assembly	923,698	34.23	1,032	657.90	357	607,705,055
Post-filtering	579,027	34.04	1,183	837.13	506	484,720,928

Table 2. Summary of Differentially Expressed Transcripts.

	Down-regulated	Up-regulated
Pre-metamorphic vs D-shape larvae	21807	11105
Pre-metamorphic vs umbonate larvae	18406	12420
Pre-metamorphic vs pediveliger larvae	10424	3884

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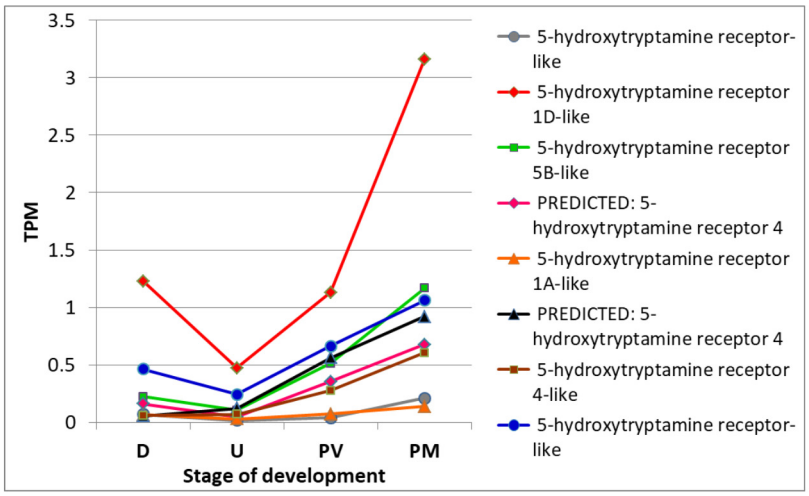

Figure 1. Normalized expression level (TPM) of several transcripts that code for 5-hydroxytryptamine receptors in four larval stages of development (D: D-shape; U: umbonate; PV: pediveliger; PM: pre-metamorphic) in *R. phillipinarum*.

Results and discussion

The summary metrics of the *de novo* assembly is displayed in Table 1, while Table 2 shows the number of differentially expressed transcripts. BUSCO completeness analysis identified 97.6% complete orthologues and 1.8% fragmented orthologues. The score, very close to 100%, indicates the high quality of the assembly.

In the transcriptome we have searched for transcripts that code for membrane receptors for hormones and neurotransmitters. Several authors showed that serotonin induced metamorphosis in *R. phillipinarum* larvae (Urrutia et al., 2004; Perez-Parallé et al., 2021). We found 27 transcripts that code for putative 5-hydroxytryptamine (serotonin) receptors. Several of these transcripts (Figure 1) showed an increased expression level in the pre-metamorphic stage when compared with the two anterior stages of development, and might play an important role in the settlement and metamorphosis of clam larvae.

Acknowledgement

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References

Bray, N.L., Pimentel, H., Melsted, P. and Pachter, L. 2016. Nature biotechnology. 34(5):525–7.
Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M. and Robles, M. 2005. Bioinformatics 21, 3674–3676.
Coon, S.L., Bonar D.B. and Weiner R.M. 1985. J. Exp. Mar. Biol. Ecol. 94, 211-221.
Degnan, B.M. and Morse, D.E. 1995. American Zoology 35, 391-398.
Götz, S., García-Gómez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talón, M., Dopazo, J. and Conesa, A. 2008. Nucleic Acids Res. 36, 3420–3435.
Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., et al. 2011. Nature biotech. 29(7):644.
Joyce, A. and Vogeler, S., 2018. Aquaculture 487, 64–82.
Mesías-Gansbiller, C., Silva, A., Maneiro, V., Pazos, A., Sánchez, J.L., and Pérez-Parallé, M.L. 2013. Aquaculture 376–379, 85–89.
Pérez-Parallé, M.L., Sánchez, J.L., Martínez-Patiño, D., Nóvoa, S., Ojea, J. and Pazos, A.J. 2021. Aquaculture Europe 2020.
Pimentel, H., Bray, N.L., Puente, S., Melsted, P. and Pachter, L. 2017. Nature methods.;14(7):687.
Seppey, M., Manni, M. and Zdobnov, E.M. 2019. In: Gene prediction. Springer;. pp. 227–45.
Urrutia, P.M., Okamoto, K. and Fusetani, N. 2004. J. Shellfish Res. 23: 93-100.

EDDIETARY TRYPTOPHAN SUPPLEMENTATION MODULATES THE IMMUNE RESPONSE TO ACUTE INFLAMMATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES UNDER STRESSFUL CONDITIONS

D. Peixoto[1,2], M. Machado[1], P. Santos[1,3], A. Ricardo[1,2], I. Duarte[1,2], 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: bcostas@ciimar.up.pt

Introduction

Amino acids (AA) play several regulatory functions on key metabolic pathways that may be important, among other functions, to immune and neuro-endocrine responses (Hoseini et al., 2019). Tryptophan is an essential AA with important roles other than protein synthesis such as being precursor of several compounds with a wide range of effects in the modulation of stress response, antioxidant system, behavioural response and immune system. Most studies in fish focused on tryptophan effects regarding welfare issues (Hoseini et al., 2019), whereas few studies explored the role of tryptophan during the fish response to infection under stressful conditions. Therefore, this study aimed to contribute to this endeavour by assessing the immunological profile, inflammatory response and disease resistance of European seabass (*Dicentrarchus labrax*) fed dietary tryptophan supplementation under acute stressful conditions followed by a exposure to a bacterial challenge.

Materials and methods

European seabass juveniles (12.02 ± 2.77 g) were randomly distributed in 16 tanks with a density of 5 g L^{-1} and maintained in two recirculated seawater systems (temperature 20 ± 0.5 °C; salinity 32; photoperiod 10h:14h D:L). By lowering the water level in one of the systems (i.e. 8 tanks with a density of 10 g L^{-1}), fish were kept at higher density and consequently, under stressful conditions. In a complete randomized design, two dietary treatments were evaluated in quadruplicate 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 15 days twice a day with a daily average ration of 2 %. Sampling at 15 days (0h, n=10) allowed the collection of data regarding haematological profile, plasma cortisol and immune parameters (i.e. peroxidase and bactericidal activities) and the gut catalase activity. The remaining fish were intraperitoneally-injected with 100 μL of *Photobacterium damselae piscicida* (Phdp, 5×10^7 cfu mL^{-1}) (temperature 24 ± 0.5 °C; salinity 32; photoperiod 10h:14h D:L) and sampled after 4, 24, 48 and 72 h. For all five sampling times fish were euthanized by anaesthetic overdose with 2-phenoxyethanol.

Results

Plasma cortisol levels tended to increase in unstressed fish fed TRP until 24 h post-infection and were significantly lower at 48 and 72 h post-infection (Fig. 1A). Differently, unstressed and stressed fish fed CTRL presented higher cortisol levels at 4 h post-infection compared to those recorded at 72 h post-infection. No significant differences were observed in cortisol levels from 0 to 72 h post-infection in stressed fish fed TRP. Still, cortisol levels decreased in stressed fish fed TRP at 4 h post-infection compared to their counterparts fed CTRL at the same time post-infection. Neutrophilia and monocytosis were observed during the first hours post-infection regardless diet and stress condition. In contrast, lymphocytes in stressed fish fed TRP increased from 4 to 72 h post-infection and the same occurred in stressed fish fed CTRL from 4 to 48 h post-infection (Fig. 1B). No significant differences were observed in catalase, peroxidase and bactericidal activities as well as peripheral thrombocytes and in total white and red blood cells.

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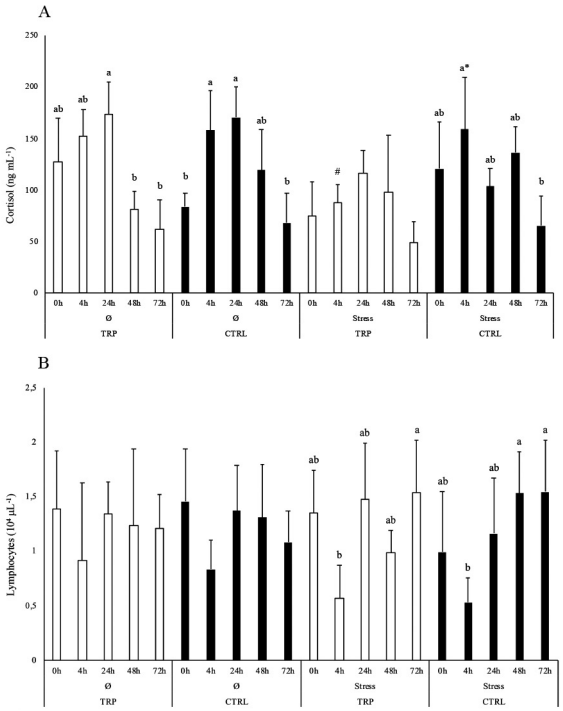

Figure 1. Plasma cortisol levels (A) and lymphocytes concentration (B) of European seabass juveniles fed dietary treatments for 15 days and intraperitoneally injected with Phdp. Values are expressed as means ± SD (n=10). Lower case letters indicate differences between time within the same diet. Symbols indicate differences between dietary treatments within the same stress condition.

Discussion and conclusion

Most peripheral immune parameters (both cellular and humoral) and catalase activity in the gut were not significantly modulated by tryptophan dietary supplementation after 15 days of feeding. However, cortisol levels dropped in stressed fish fed TRP compared to their counterparts fed CTRL following the inflammatory insult, suggesting that this AA might counteract stress-induced cortisol production, thereby potentially reverting cortisol-mediated immunosuppressive effects. Preliminary data from the present study point to dietary tryptophan supplementation as a nutritional strategy to modulate fish endocrine responses during acute inflammation and stressful conditions. More parameters related to molecular endocrine and immune responses as well as oxidative stress are being analysed and will assist to better understand the interactive effects of tryptophan nutrition, inflammation and stressful conditions.

Acknowledgements

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References

Hoseini SM, Pérez-Jiménez A, Costas B, Azeredo R, Gesto M (2019). Physiological roles of tryptophan in teleosts: current knowledge and perspectives for future studies.

DIFFERENT FISH MEAL AND FISH OIL DIET LEVELS EFFECTS ON WELFARE AND PERFORMANCE OF EUROPEAN SEABASS *Dicentrarchus labrax* SUBJECTED TO ACUTE CONFINEMENT STRESS

N. F. Pelusio[1]*, L. Parma[1], S. Busti[1], A. De Marco[1], A. Marchi[1], L. Mariani[1], L. Morsiani[1], F. Dondi[1], E. Brini[1], E. Gisbert[2], K. B. Andree[2], M. A. Esteban[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]IRTA, Centre de Sant Carles de la Ràpita, Programa d'Aqüicultura, Crta. del Poble Nou Km 5.5, 43540 Sant Carles de la Rápita, Spain
[3]Department of Cell Biology and Histology, Faculty of Biology, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain
[4]Unit of Microbial Ecology, Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
E-mail: nicole.pelusio2@unibo.it

Introduction
To maximize productivity, sustainability, and fish welfare of the Mediterranean aquaculture, research needs to focus on the interaction between feeding and rearing conditions. To provide practical feeding management guidelines for fish farmers, the aim of this study was to evaluate the effect of different fishmeal (FM) and fish oil (FO) dietary levels on growth, immune parameters on skin mucus, plasma biochemistry, and gene expression of stress-related markers in European sea bass exposed to short fasting, and acute confinement.

Materials and methods
Three isonitrogenous (46 % protein), and isolipidic (18 % lipid) diets were formulated to contain high, medium and low FM and FO dietary levels (FM30/FO15, FM20/FO7 and FM10/FO3; 30 % FM, 15 % FO, 20 % FM and 7 % FO, and 10 % FM and 3 % FO, respectively). Fish (initial body weight: 76.9g) were fed to satiation twice a day (10% overfeeding) over 60 days.

At the end of the trial, growth performances, feed utilization and carcass composition were estimated. Then, after 36 hours of fasting (T0), 2 hours long crowding stress (80 kg m^{-3} biomass) exposure was achieved by lowering the water level in tanks (T1) mimicking animal confinement during common husbandry operations like size sorting or fish harvest, followed by a recovery lasting 24 hours (T2). At the end of this time frame, the water level was brought back to the initial one, letting animals recover from the stressful event for 24 hours. Before (time 0, T0), at the end of the 2 hours of crowding stress (time 1, T1) and 24 hours after confinement (time 2, T2) at high density, 8 fish per tank were sacrificed with MS222 (300 mg L^{-1}) and sampled each time to explore the capacity of animals to cope stress attributed to the feed formulation administered during the trial. Skin mucus (8 fish per tank), plasma biochemistry (5 fish per tank), and stress-related gene expression (3 fish per tank) were analysed according to Parma et al. (2020) and Mateus et al., (2017) respectively. All gained data were analysed by Two-way ANOVA followed by a Tukey's multiple comparison test. Differences among treatments were considered significant when the P value was lower than 0.05.

Results
Significant differences were found on FBW, WG and SGR, with the lowest values in animals fed FM10/FO7 diet ($p < .05$). No significant diet effect was found neither on carcasses proximate composition nor on nutritional utilization. Regarding humoral immune parameters in skin mucus, no significant difference was found in protease activity ($p > 0.05$), while significant variations occurred in peroxidase, antiprotease, lysozyme, esterase, and alkaline phosphatase activities ($p < 0.05$). Furthermore, significant changes were also found in skin mucus IgM levels, and bactericidal activity against *Vibrio anguillarum* and *V. harveyi* ($p < 0.05$). Most of the blood parameters met significant differences among different time points rather than experimental diets ($p < 0.05$).

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Discussion and conclusion

Among diets, FM10/FO7 was found to exert a lower SGR thus leading to the negative outcomes on FBW. Once those sea bass specimens were exposed to fasting, stress confinement and recovery, most of their innate humoral immune components analyzed in skin mucus aspecific humoral immunity components went towards some modifications that tend not to completely cope by 24 hours. At the end of recovery time glucose, cortisol, calcium, potassium, sodium, magnesium, and lactate managed to come back to basal levels in blood-stream. Contrarywise, plasma triglycerides, creatine kinase, lactate dehydrogenase and phosphorus levels did not cope after stress-induced modifications, while total proteins were not consistently altered. In the light of this study, at each considered FM and FO dietary level, sea bass is able to cope with fasting and stress confinement without compromising its health status.

Acknowledgements

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References

Parma, L., Pelusio, N.F., Gisbert, E., Esteban, M.A., D'Amico, F., Soverini, M., Candela, M., Dondi, F., Gatta, P.P., Bonaldo, A. 2020. Effects of rearing density on growth, digestive conditions, welfare indicators and gut bacterial community of gilthead sea bream (*Sparus aurata*, L. 1758) fed different fishmeal and fish oil dietary levels. Aquaculture, 518, 734854.

Mateus, A. P., Costa, R. A., Cardoso, J. C. R., Andree, K. B., Estevez, A., Gisbert, E., Power, D. M. 2017. Thermal imprinting modifies adult stress and innate immune responsiveness in the teleost sea bream. *J Endocrinol*, *233*, 381-94.

METHODOLOGY FOR THE ANALYSIS OF SILVER NANOPARTICLES IN CULTIVATED MUSSELS (*Mytilus Galloprovincialis*)

E. Peña-Vázquez*, J.J. López-Mayán, M.C. Barciela-Alonso, P. Bermejo-Barrera, A. Moreda-Piñeiro

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
E-mail: elenamaria.pena@usc.es

Introduction

The aquaculture industry is a very important sector for Western Europe. It is growing exponentially due to needing to feed an ever-increasing population. In Spain, mussel is one of the most cultivated aquaculture species. Spain stands out for its production of these filtering organisms worldwide, and it is necessary to ensure their quality avoiding water contamination.

Nowadays, silver nanoparticles (Ag NPs) have a widespread use in industry due to their strong antimicrobial activity and their particular physicochemical properties. Ag NPs can be emitted into the wastewater and the environment, where they can react and release ions. Thus, the different silver compounds can be toxic to the aquatic organisms[1]their interaction with the environment and toxicity in live terrestrial or aquatic organisms is still a matter of intense debate. More detailed knowledge is still required about the toxicity of AgNPs, their possible uptake mechanisms and their adverse effects in live organisms. Several studies have reported the interactions and potential negative effects of AgNPs in different organisms. In this review, we report and discuss the current state of the art and perspectives for the impact of AgNPs on different organisms present in the environment. Recent progress in interpreting uptake, translocation and accumulation mechanisms in different organisms and/or living animals are discussed, as well as the toxicity of AgNPs and possible tolerance mechanisms in live organisms to cope with their deleterious effects. Finally, we discuss the challenges of accurate physicochemical characterization of AgNPs and their ecotoxicity in environmentally realistic conditions such as soil and water media.”,”author”:[{“dropping-particle”:””,”family”:”Tortella”,”given”:”G. R.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Rubilar”,”given”:”O.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Durán”,”given”:”N.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Diez”,”given”:”M. C.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Martínez”,”given”:”M.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Parada”,”given”:”J.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Seabra”,”given”:”A. B.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”container-title”:”Journal of Hazardous Materials”,”id”:”ITEM-1”,”issue”:”November 2019”,”issued”:{“date-parts”:[[“2020”]]},”page”:”121974”,”publisher”:”Elsevier”,”title”:”Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment”,”type”:”article-journal”,”volume”:”390”},”uris”:[“http://www.mendeley.com/documents/?uuid=1214f844-7ce7-4bd1-85e6-e34729ac2d3e”]}],”mendeley”:{“formattedCitation”:”¹”,”plainTextFormattedCitation”:”1”,”previouslyFormattedCitation”:”¹”},”properties”:{“noteIndex”:0},”schema”:”https://github.com/citation-style-language/schema/raw/master/csl-citation.json”}. On the other hand, AgNPs are also getting a lot of attention in aquaculture because of their possible applications as a disinfectants of tanks and as a replacement of antibiotics.

Methods and results

This study describes the methology developed in our research group to determine the total concentration of silver in mussels, and to isolate AgNPs from the complex matrix and perform their subsequent analysis using spectroscopic techniques.

For the measurement of total Ag content in mussels, a microwave acid digestion using HNO_3 (69% v/v) and H_2O_2 (33% v/v) is necessary previous to the ionic metal determination by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

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Afterwards, mussel samples were submitted to an enzymatic hydrolysis extraction with the objective of separating AgNPs and ensuring their integrity. The enzymatic extraction is performed overnight using a pancreatine:lipase mixture solution (0.3 g L^{-1}/0.3 g L^{-1}, pH 7.4) under continuous stirring (150 rpm) at physiological pH and temperature[2]. After centrifugation, the extracts are diluted with 1% (v/v) glycerol and analyzed by single-particle ICP-MS (SP-ICP-MS). SP-ICP-MS allows the visualization of individual AgNPs and obtaining information about their concentration in the extracts and the distribution of their particle sizes. The developed methodology was applied to mussel samples from the Atlantic area, some of them exposed to nanoparticles.

References

1. Tortella, G. R. , Rubilar, O., Durán, N., Diez, M.C., Martínez, M., Parada, J., Seabra, A.M., Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment. *J. Hazard. Mater.* **390**, 121974 (2020).
2. Taboada-López, M. V., Alonso-Seijo, N., Herbello-Hermelo, P., Bermejo-Barrera, P., Moreda-Piñeiro, A. Determination and characterization of silver nanoparticles in bivalve molluscs by ultrasound assisted enzymatic hydrolysis and sp-ICP-MS. *Microchem. J.* **148**, 652–660 (2019).

Acknowledgments

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INSIGHTS INTO CHITIN REGULATION AND SHELL FORMATION IN THE MANTLE OF BIVALVES IMPORTANT FOR AQUACULTURE

Maoxao Peng*[1,2], João CR Cardoso[1] and Deborah M Power[1,2]

[1]Comparative Endocrinology and Integrative Biology, Centre of Marine Science (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[2]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China Shanghai Ocean University DMP
dpower@ualg.pt

Introduction

Shellfish aquaculture is one of the fastest growing food production sectors in the world (FAO 2018). There is concern about the impact on its productivity of changing ocean conditions caused by climate change. In Mollusca, chitin, a widespread natural amino polysaccharide, is an important organic structural polymer of the shell since it provides the framework for mineralization of calcium carbonate (Falini et al., 1996). The regulation of chitin turnover (synthesis/breakdown) and how it affects shell growth is poorly understood. Furthermore, it is unclear if the effects of climate change on shell growth and mineralization are mediated by modifications in its organic framework. With a view to answering these questions the chitin-synthases (CHS), a group of enzymes that are members of the glycosyltransferase family 2 (Zakrzewski et al., 2014), and add UDP-GlcNac units to the oligosaccharide chain during chitin synthesis, were investigated. The evolution, gene family expansion and potential functional diversity of CHS in the mantle of bivalve species was investigated.

Material and methods

Orthologues of CHS in molluscs were procured (Blastp) by searching with the vertebrate and arthropod proteins against, i) Mollusca datasets at NCBI (https://www.ncbi.nlm.nih.gov, taxid:6447), ii) ENSEMBL GENOMES (http://ensemblgenomes.org/) databases and iii) in mantle edge transcriptomes available "in house" for two Mytilids (*Mytilys galloprovincialis, Mga* and *Mytilis coruscus, Mco*) (Bjärnmark et al., 2016). Deduced mollusc CHS and homologues were aligned with MUSCLE and the main protein motifs were identified with Pfam. Phylogenetic trees were constructed using the Maximum-Likelihood (ML) method with branch analysis in the CIPRES Science Gateway v3.3. Bivalve mantle transcriptomes were explored to assess if CHS expression changed under acidified sea water conditions.

Results

The predicted mollusc CHS were confirmed by sequence similarity and their clustering in the phylogenetic tree (Figure 1A). In the genome of the pacific oyster (*Crassostrea gigas*) 10 genes were found and in the mantle transcriptomes of *M. galloprovincialis* and *M. coruscus*, 5 and 8 gene transcripts were retrieved, respectively. The phylogenetic tree topology suggests that two distinct CHS clusters exist in bivalves (CHS-type I and CHS-type II), in other invertebrates and in the vertebrates. Within the CHS-type II cluster four main branches exist and the genes within them were designated A-, B-, C- and D-subtypes (Figure 1). The multiple CHS genes in bivalves all had well conserved functional domains apart from modifications in the donor saccharide binding site. In acidified sea water, mussel CHS transcripts responded differently in the mantle suggesting their function is different (Figure 1).

Discussion and conclusion

The number of CHS members in bivalves and other Mollusca was species-specific. In the two Mytilid species examined many different genes were expressed in the mantle edge, an important region for shell production. The phylogenetic analysis revealed that a diversity of CHS-types exist in bivalves. Homologues of vertebrate CHS-type II were identified and CHS-type I genes, previously only described in the early metazoans, cnidarians and Porifera, were also identified in bivalves. Overall, the data suggest that chitin synthesis in bivalves is more complex than previously thought. The differing CHS-gene complement in different species of bivalves and their varying response to acidified sea water suggests that the vulnerability of bivalve species to changes in the marine environment will not be uniform.

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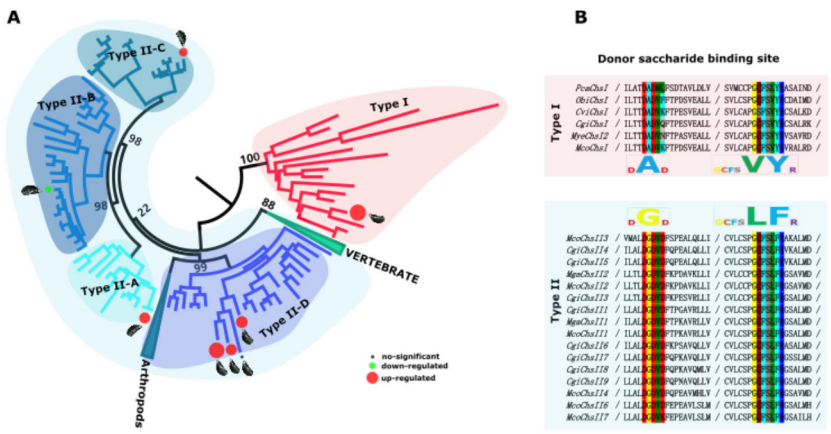

Figure 1. A) ML tree of CHS from Mollusca and other species rooted with the Fungi CHS (P30588.2). Bootstrap values for major branches are shown. Circles indicate CHS gene transcripts in the mantle that respond to decreased pH (pH= 7.4, Zhao et al., 2020); the size of the circles is indicative of the fold change. B) Multiple sequence alignment of the DSBS domain. Residues that differ between the CHS types are indicated.

Acknowledgements

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References

FAO. (2018). The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. Food and Agriculture Organization of the United Nations, Rome.

Bjärnmark, N.A., Yarra, T., Churcher, A.M. et al. (2016). Transcriptomics provides insight into *Mytilus galloprovincialis* (Mollusca: Bivalvia) mantle function and its role in biomineralisation. Marine genomics, 27: 37-45.

Falini, G., Albeck, S., Weiner, S. et al. (1996). Control of aragonite or calcite polymorphism by mollusk shell macromolecules. Science, 271, 67–69.

Zakrzewski, A.C., Weigert, A., Helm, C., et al. (2014). Early divergence, broad distribution, and high diversity of animal chitin synthases. Genome biology and evolution, 6(2): 316-325.

Zhao, X., Han, Y., Chen, B., et al. (2020). CO2-driven ocean acidification weakens mussel shell defense capacity and induces global molecular compensatory responses. Chemosphere, 243: 125415.

EFFECT OF ANTIOXIDANT AND B-GLUCAN SUPPLEMENTED DIETS ON IMMUNOLOGICAL AND OXIDATIVE STRESS RESPONSES IN JUVENILES OF SENEGALESE SOLE (*Solea senegalensis*)

S. Pereira[1,3*], P. Santos[1], W. Pinto[2], I. Blanquet[4], L. Conceição[2], B. Costas[1,3]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental, University of 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, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto (Portugal)
[4]Safiestela SA (SEA8), Lugar Do Rio Alto, Estela, Póvoa de Varzim (Portugal)
e-mail: susana.pereira@ciimar.up.pt

Introduction

Evidence that dietary supplementation with antioxidants and β-glucans can modulate the ability of different fish species to stimulate the immune system and stress-resistance is fairly established [1, 2]. This work examined the effects of dietary antioxidant and β-glucan supplementation on the immune responses and oxidative stress biomarkers from juveniles of Senegalese sole subjected to acute stress.

Materials & Methods

Juveniles of Senegalese sole (5g) were fed with four diets. A Commercial diet used as positive control; and 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. Each diet was administered to 3 random replicates (n= 80/tank), divided in 8 daily meals and fed to apparent satiety. Animals were fed with the experimental diets for 7 days and submitted to an acute stress by a standardized 30 min transport. Thereafter, fish were returned to the experimental tanks and continued to be fed dietary treatments for 7 days. After this period, all fish were fed the Commercial diet for 7 more days. All 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). Plasma (pooled) and liver samples were collected from 5 animals per tank to assess immune parameters and oxidative stress biomarkers.

Results

No changes in growth performance were observed among dietary treatments. Regarding plasma immune parameters, nitric oxide levels decreased 7 days after transport in juveniles fed with Commercial diet compared to those fed CTRL, CTRLox and CTRLas, whereas anti-proteases activity dropped in those fed with CTRL compared to their counterparts fed with Commercial diet at day 7.

Hepatic thiobarbituric acid reactive substances (TBARS) levels decreased in fish fed CTRLox and CTRLas compared to those fed the Commercial diet after 21 days (Fig. 2a). In contrast, hepatic total glutathione (GSH) levels increased in soles fed CTRLas compared to control diets after 7, 14 and 21 days (Fig. 2b). Moreover, catalase (CAT) activity increased in fish fed CTRLox and CTRLas compared to those fed Commercial and CTRL after 21 days. Glutathione-S-transferase (GST) activity augmented in fish fed CTRLox compared to those fed Commercial at 14 days and 21 days.

Discussion

Nitric oxide levels decreased in the plasma of fish fed Commercial, contrasting with CTRL, CTRLox and CTRLas fed fish where nitric oxide levels kept stable. This trend was also observed in fish fed with diets supplemented with antioxidants, such as curcumin [3], and β-glucans [4]. TBARS levels lowered in both CTRLox and CTRLas, preventing the peroxidation of lipids, which is stated in fish supplemented with low doses of antioxidants [2]. The increase of TGSH levels and GST activity in CTRLox and CTRLas, respectively, suggest boosting of GSH synthesis influenced by the antioxidants [2] and β-glucans [1]. Catalase activity also increased in CTRLox and CTRLas, hinting that the presence antioxidants [2] and β-glucans [1] up-regulated the production of CAT. In conclusion, fortified diets supplemented with natural antioxidants and β-glucans seem to have an overall beneficial influence in both immune and oxidative stress responses. Still, further gene expression analysis is being proceeded to have a more detailed picture of the eventual health-related benefits of these diets in juveniles of Senegalese sole.

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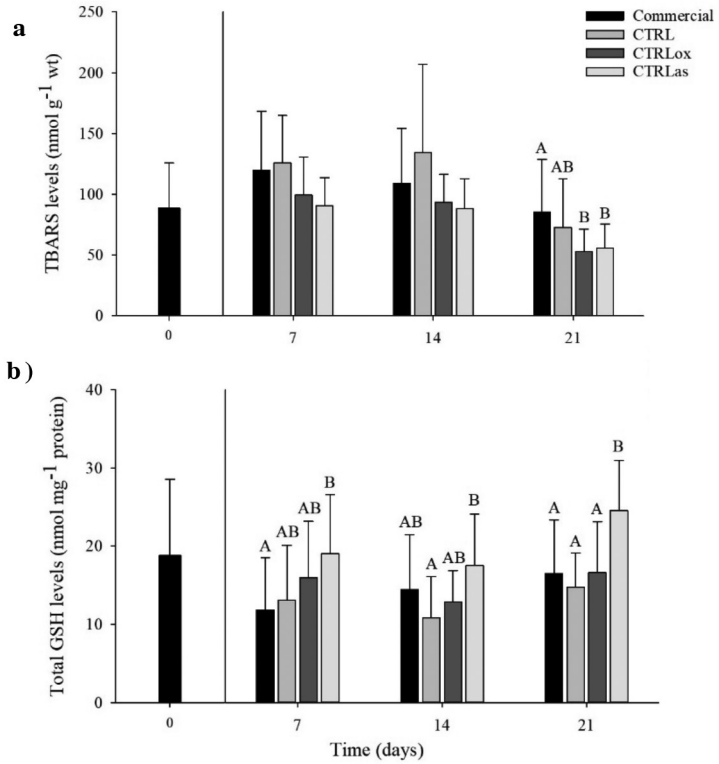

Fig. 1 a) TBARS and b) Total GSH levels after 0, 7, 14, and 21 days in the liver of juveniles of Senegalese sole fed with four diets (n = 15). Letters show significant differences among diets in the same day (One-way ANOVA; Tukey test, $p \leq 0.05$).

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.

References

1. Cao, H., et al., Aquaculture, 2019. **508**: p. 106-112.
2. Mahmoud, H.K., et al., Aquaculture, 2017. **475**: p. 16-23.
3. Abd El-Hakim, Y.M., et al., Aquat Toxicol, 2020. **220**: p. 105406.
4. Pietretti, D., et al., Fish Shellfish Immunol, 2013. **35**(3): p. 847-57.

EFFECT OF AMINO ACID, ANTIOXIDANT AND VITAMIN SUPPLEMENTED DIETS ON IMMUNOLOGICAL AND OXIDATIVE STRESS RESPONSES IN JUVENILES OF SENEGALESE SOLE (*Solea senegalensis*)

S. Pereira[1,3*], P. Santos[1], W. Pinto[2], I. Blanquet[4], L. Conceição[2], B. Costas[1,3]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental, University of 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, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto (Portugal)
[4]Safiestela SA (SEA8), Lugar Do Rio Alto, Estela, Póvoa de Varzim (Portugal)
e-mail: susana.pereira@ciimar.up.pt

Introduction

Plant-based supplements as antioxidants (e.g., curcumin) and vitamins (e.g., vitamin E) to fish diets can be immune-modulatory and improve stress-resistance for several fish species [1-4]. This work aimed to study the effects of amino acid, antioxidant and vitamin supplemented diets on immune responses and oxidative stress biomarkers from Senegalese sole (*Solea senegalensis*) juveniles subjected to acute stress.

Materials & Methods

Senegalese sole juveniles (7.8 ± 1.3 g) were fed with four diets. Two commercial diets (COMM 1 and COMM 2) were used as controls; COMM 2 and two experimental diets were formulated and manufactured by SPAROS with premium marine and plant-based ingredients: NSUP, negative control of SUP; and SUP, diet supplemented with amino acids and natural antioxidants (i.e., vitamins and curcumin). Each diet was administered to 3 random replicates (n= 120/tank), divided in 8 daily meals and fed to apparent satiety. Animals were fed with the experimental diets for 7 days and submitted to an acute stress by simulating a standardized 30 min transport. Thereafter, fish were returned to the experimental tanks and continued to be fed dietary treatments for 14 days. All fish were sampled at the beginning (time 0), prior transport (time 7) and at the end of the feeding trial (time 21). Plasma (pooled) and liver samples were collected from 5 animals per tank to assess immune parameters and oxidative stress biomarkers.

Results

No changes in growth performance were observed among dietary treatments, or in oxidative stress biomarkers before the acute stress. Regarding plasma immune parameters, lysozyme activity increased at day 21 (i.e. two weeks after acute stress) in juveniles fed with NSUP and SUP compared to those fed COMM1 diet (Fig. 1a), whereas proteases activity boosted in those fed with NSUP and SUP compared to their counterparts fed with both controls at day 21 (Fig. 1b).

Hepatic thiobarbituric acid reactive substances (TBARS) levels augmented in fish fed SUP compared to those fed COMM 1 after 21 days. In contrast, total glutathione (total GSH) levels in the liver decreased in Senegalese sole fed COMM 2 compared to COMM 1 diet after 21 days, but kept stable in both NSUP and SUP diets.

Discussion

Lysozyme and proteases activity increased in the plasma of fish fed NSUP and SUP. This trend was also observed in fish fed with diets supplemented with vitamin C [2] and antioxidants, such as curcumin [1, 4]. However, TBARS levels also increased in SUP, a trend that was previously observed in fish supplemented with high doses of vitamin C and E [3]. These findings may be indicative of oxidative stress and lipid peroxidation.

In conclusion, diets non-supplemented and supplemented with amino acids, vitamins and natural antioxidants (i.e. NSUP and SUP) had an overall beneficial influence in the immune responses. Results of oxidative stress biomarkers are inconclusive, thus gene expression is currently being analysed to achieve a better understanding of the health-related effects of these diets in juveniles of Senegalese sole. A bacterial challenge assay could be an additional tool to understand if these diets would help to prevent disease outbreaks following acute stress by transport.

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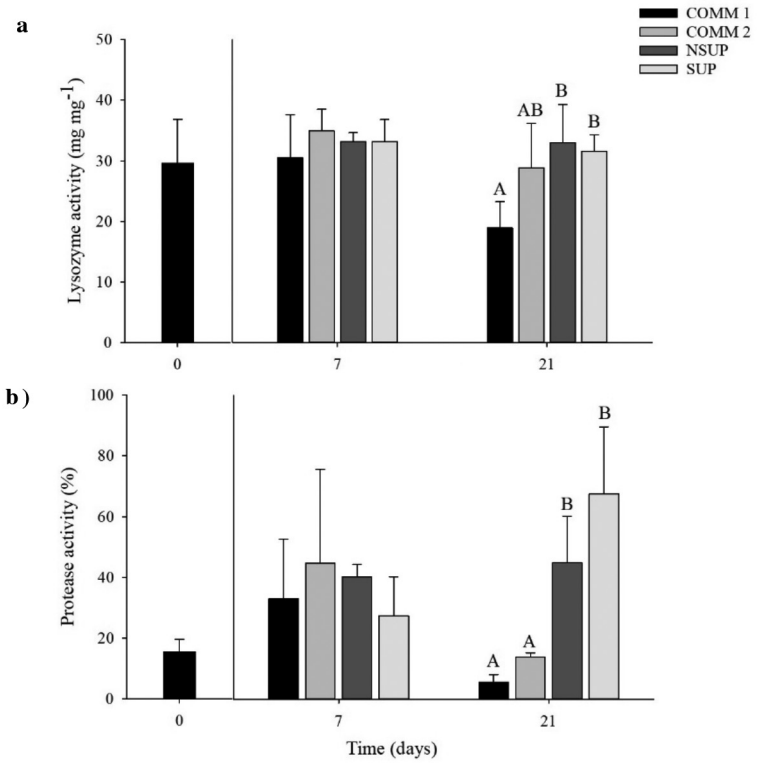

Fig. 1 a) Lysozyme and b) protease activity in the plasma of juveniles of Senegalese sole after 0, 7, and 21 days fed with four diets (n = 3). Letters show significant differences among diets in the same day (One-way ANOVA; Tukey test, $p \leq 0.05$).

Acknowledgments

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References

1. Mahmoud, H.K., et al., Aquaculture, 2017. **475**: p. 16-23.
2. Ibrahem, M.D., et al., Fish Shellfish Immunol, 2010. **29**(2): p. 241-6.
3. Gao, J., et al., Aquaculture, 2014. **422-423**: p. 84-90.
4. Giri, S.S., et al., Fish Shellfish Immunol, 2019. **92**: p. 612-620.

DEVELOPMENT OF MULTIPLEX PCRs OF MICROSATELLITES FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) AND THEIR APPLICATION IN THE CHARACTERIZATION OF BROODSTOCK

L.C. Pachón-Meza[1], C. Pérez-García[1]*, A. Lorenzo-Felipe[1], S. Leon-Bernabeu[2], J.M. Afonso[1], H.S. Shin[1], J.J. Sánchez[3], M.J. Zamorano[1]

[1]Aquaculture Research Group (GIA), Institute of Sustainable Aquaculture and Marine Ecosystems (ECOAQUA), Universidad de las Palmas de Gran Canaria (ULPGC), Carretera de Taliarte, s/n, 35214 Telde, Spain
[2]Aquanaria S.L., Calle Bentejui, s/n, 35107 San Bartolomé de Tirajana, Spain
[3]Instituto Nacional de Toxicología y Ciencias Forenses (INT), La Cuesta, La Laguna, Sta. Cruz de Tenerife, 38320, Spain

Presenting author: C. Pérez-García (cathaysa.perez108@alu.ulpgc.es)

Introduction

The European seabass (Dicentrarchus labrax) is one of the most important commercial species cultivated in Mediterranean areas (FAO Fisheries Division, 2020). In this species, different microsatellites markers have been developed by García de León et al. (1995) and Tsigenopoulos et al. (2003) in order to be applied in genetic selection programs; the SSR are useful to understand the flow of genes between populations, highlighting their availability in marker-assisted selection.

The aim of this study was to design the most robust Multiplex PCR may be proposed as a panel of markers for genetic identification necessary in seabass breeding programmes.

Materials and methods

DNA was extracted from 188 samples by using the *DNA BLOOD Kit of QIAgen* by BIOSPRINT96 robot. Five multiplexes of PCR were designed from a total of 50 markers described previously for European seabass. Initially, four samples and negative control were used to do simple and multiplex PCR, to confirm the presence of amplification product. Secondly, amplification patterns (peak`s morphology), variability genetics, and allelic ranges, were estimated.

PCR conditions: PCR volume was 12.5 μL, containing 1.25 μL of 10X PCR Buffer II; 1.5 μL of $MgCl_2$ solution (25 mM); 0.25 μL of dNTPs solution, for a final concentration of 10 mM of each ones; AmpliTaq's 0.04 U/μL Gold DNA polymerase; 0.5 μL of bovine serum albumin (BSA); 4.9 μL of water PCR grade and 2 μL of DNA from the sample.

Initial denaturation at 95ºC for 10 minutes; 28 cycles of 94ºC for 30 seconds, 60ºC for 1 minute and 65ºC for 1 minute, were carried out, with a final extension at 65ºC for 60 minutes, and stabilization at 12ºC.

Data were analysed in *GeneMapper™* , and *Cervus* 3.0 software.

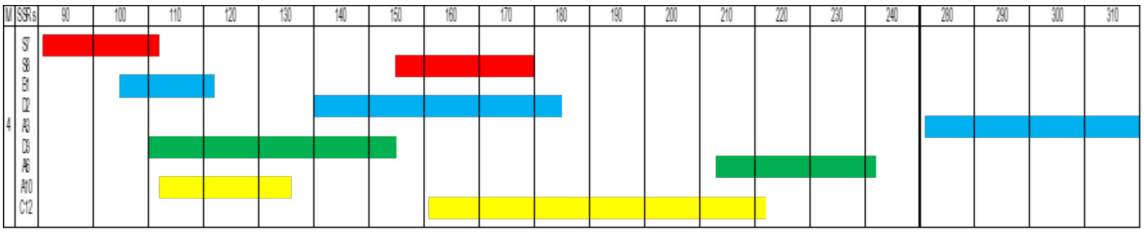

Figure 1. lour and allelic range (bp) for the nine SSRs makers of Eubass4 Multiplex PCR.

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Results

A selection of microsatellite markers (27) of *D. labrax,* with redesigned primer sets,has been considered as highly informative (PIC), amplified under the same PCR conditions, and integrable in different PCR multiplex reactions.

Following the formulation of five different multiplex and the analyses of their PIC, exclusion probability, Ho and He; one of them was selected as a reference panel to parental assignment in European seabass (named Eubass4). It is easy to read and high reproducibility, and proposed as a reference panel for parental assignment in this species. An additional panel of 27 markers were also selected to form a final list of backup markers, all of them with high PIC, that permits to the user customize the identification panel accord to the laboratory necessities.

To conclude a total of 9 highly polymorphic markers, easy to read and high reproducibility, is proposed as a reference panel for parental assignment in this species.

References

FAO Fisheries Division, 2020. Fisheries and aquaculture software [WWW Document]. FishStatJ - Softw. Fish. Aquac. Stat. Time Ser. URL http://www.fao.org/fishery/culturedspecies/*Dicentrarchus_labrax*/ en (accedido 7.20.20).

García de León, F., Dallas, J., Chatain, B., Canonne, M., Versini, J.J., Bonhomme, F., 1995. Development and use of microsatellite markers in sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (Perciformes: Serrandidade). Mol. Mar. Biol. Biotechnol. 4, 62-68.

Garcia de Leon, F.J., Canonne, M., Quillet, E., Bonhomme, F., Chatain, B., 1998. The application of microsatellite markers to breeding programmes in the sea bass, *Dicentrarchus labrax*. Aquaculture 159, 303-316. https://doi.org/10.1016/S0044-8486(97)00188-9

Tsigenopoulos, C.S., Hellemans, B., Chistiakov, D.A., Libertini, A., Kotoulas, G., Volckaert, F., 2003. Eleven new microsatellites of the sea bass (*Dicentrarchus labrax L.*). Mol. Ecol. Notes 3, 352-354. https://doi.org/10.1046/j.1471-8286.2003.00447.x.

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SEROTONIN AND ACETYLCHOLINE INDUCE METAMORPHOSIS IN THE LARVAE OF THE MANILA CLAM, *Ruditapes philippinarum* (ADAMS AND REEVE, 1850)

M. Luz Pérez-Parallé[1]*, José Luís Sánchez[1], Dorotea Martínez-Patiño[2], Susana Nóvoa[2], Justa Ojea[2] and Antonio J. Pazos[1]

[1] Dpto.de Bioquímica y Biología Molecular. Instituto de Acuicultura. Universidade de Santiago de Compostela. Campus Vida. 15782-Santiago de Compostela. Spain
luz.perez-paralle@usc.es
[2] CIMA. Consellería do Mar. Xunta de Galicia. 27700 Ribadeo, Lugo, Spain

Introduction

Metamorphosis is a bottleneck in the bivalve aquaculture. Bivalve larvae must achieve the developmental stage of competence to be able to recognize appropriate exogenous morphogenetic cues to settle and metamorphose (Degnan & Morse, 1995). The molecular mechanisms underlying the metamorphosis process are not well known although several chemical inducers have been identified (Boettcher and Targett 1998; Pires et al. 2000, Joyce & Vogeler, 2018). Competent molluscan larvae from different species have been induced to metamorphose using analogues of natural chemical cues (Coon et al. 1985; García-Lavandeira et al. 2005; Alfaro et al. 2011; Mesías-Gansbiller et al. 2008, 2013). Neurotransmitters such as serotonin and acetylcholine could be involved in metamorphosis through several neuroendocrine pathways. In this research we investigate the potential effect of serotonin and acetylcholine as inducers on the larval metamorphosis of the clam *Ruditapes philippinarum*.

Materials and methods

Competent larvae of Manila clamp were provided by the CIMA of Ribadeo. Up to five experiments of induction of the metamorphosis have been done according to the protocols described in García-Lavandeira et al. (2005) and Mesías-Gansbiller et al. (2013). The larvae were treated with the neurotransmitters: serotonin and acetylcholine. Three different concentrations of the inductor were used: 10^{-4}M, 10^{-5}M and 10^{-6}M. Experiments were performed in triplicate in 90mm-glass Petri dishes for each one of the concentrations in a total volume of 20 mL. A control with filtered seawater and without potential inductor was set in each experiment. Larval density was 4 larvae/ml. Metamorphosis was monitored with a Nikon SMZ-2T microscope at 72h. The percentage metamorphosis was calculated as total number of larvae metamorphosed/total number larvae multiplied by 100. Larvae have undergone metamorphosis when they lose their velum and have ability to crawl. Percentages of metamorphosis were analysed by means of SPSS 20.0 (ANOVA). The results were considered to be significantly different when $p \leq 0.05$.

Results and discussion

The highest percentages of metamorphosis were induced by 10^{-4} M and 10^{-5} M serotonin and 10^{-4} M acetylcholine ranging from 90.54% to 94.4% ($p \leq 0.5$). After 72h, the exposure to 10^{-6} M serotonin also induced significant levels of metamorphosis in *R. philippinarum* (87.52 ± 5.66%) In contrast, a concentration of 10^{-6} M acetylcholine failed to induce significant levels of metamorphosis in the larvae comparing to the control. The proportion of larvae that succeeded metamorphosis in the control experiments, that is, in the absence of inducers, was 67.9 ± 7.73%. Acetylcholine and serotonin were effective inducers of the metamorphosis in Manilla clam larvae, increasing the percentage of metamorphosis to values close to 95%.

These inducers did not affect the mortality rates of *R. philippinarum* larvae since the different concentrations of inducers used in this study were not toxic for the larvae.

Significant larval metamorphosis rates of *R. philippinarum* were also found by Urrutia et al. (2004) using acetylcholine and serotonin however metamorphosis rates were very low comparing to those obtained in our study, especially the control larvae where the percentage of metamorphosis was less than 20%. In our study, the effects of serotonin and acetylcholine are evident even with a 68% of metamorphosis in the control. All these results support the existence of serotonergic and cholinergic pathways associated to settlement and metamorphosis processes.

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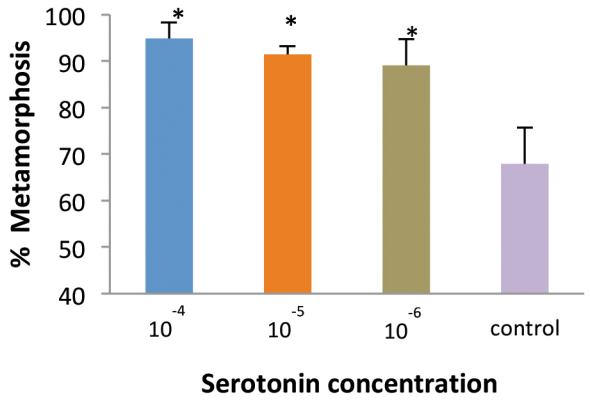

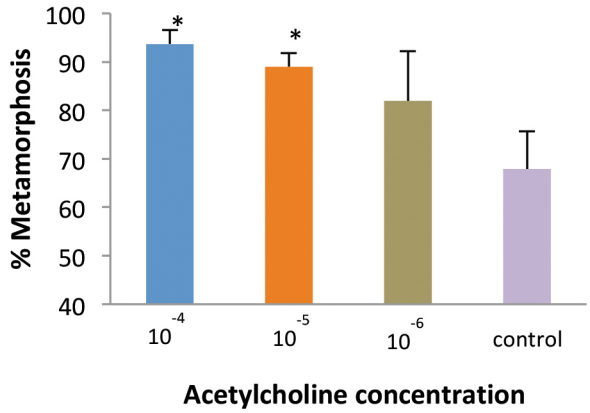

References

Alfaro, A.C., T. Young and A.M. Ganesan. 2011. Aquaculture 322-323, 158-168.
Boettcher, A.A. and N.M. Targett. 1998. Biological Bulletin 194, 132-142.
Coon, S.L., D.B. Bonar and R.M. Weiner. 1985. J. Exp. Mar. Biol. Ecol. 94, 211-221.
Degnan, B.M. and D.E. Morse. 1995. American Zoology 35, 391-398.
García-Lavandeira, M., A. Silva, M. Abad, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2005. J. Exp. Mar. Biol. Ecol. 316, 149-156.
Joyce, A. and Vogeler, S. 2018. Aquaculture 487, 64-82.
Mesías-Gansbiller, C., M.E.A. Bendimerad, G. Román, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2008. J. Shellfish Res. 27, 261-264.
Mesías-Gansbiller, C., A. Silva, V. Maneiro, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2013. Aquaculture 376-379: 85-89.
Pires, A., Croll, R.P., Hadfield, M.G., 2000. Biol. Bull. 198, 319-331.
Urrutia, P.M., Okamoto, K. and Fusetani, N. 2004. J. Shellfish Res. 23: 93-100.

DIVERSITY OF VIBRIO SPECIES IN CROATIAN BIVALVE AQUACULTURE

L. Perić[1]*, S. Kazazić[1], D. Kapetanović[1], I. Vardić-Smrzlić[1], J. Legrou[2], T. Buha[3], A. Kolda[1], J. Bolotin[4], V. Kožul[4], V. Nerlović[5], S. Bobanović-Ćolić[4], F. Barac[1]

[1]Ruđer Bošković Institute, 10000 Zagreb, Croatia
[2]Hogeschool Gent, University College Ghent, 3000 Ghent, Belgium
[3]Faculty of Science, University of Zagreb, 10000 Zagreb, Croatia
[4]Institute for Marine and Coastal Research, University of Dubrovnik, 20000 Dubrovnik, Croatia
[5]University Department of Marine Studies, University of Split, 21000 Split, Croatia
*Lorena.Peric@irb.hr

Introduction:
Vibrios are Gram-negative bacteria ubiquitous in marine and brackish environment. Since this genus includes several bivalve pathogens, monitoring of *Vibrio* sp. occurrence is crucial for seafood products safety. The aim of this work was to investigate the composition and diversity of vibrios in Croatian bivalve aquaculture by means of MALDI TOF MS profiling. This technique has been applied as a "first-tier" screening tool of bacterial isolates for identification of *Vibrio* strains at genus and sub-genus level (Bauer et al, 2018; Culot et al, 2021).

Materials and methods:
Sampling was carried out in November 2020 and January 2021 at two Croatian bivalve farms: Lim Bay (Northern Adriatic) and Mali Ston Bay (Southern Adriatic). Vibrios from Mediterranean mussel *Mytilus galloprovincialis* and European flat oyster *Ostrea edulis* tissues, sediment and seawater were isolated on selective medium Difco™ Thiosulphate Citrate Bile Salt Sucrose (BD) agar. Colonies of different morphologies were plated on tryptic soy agar with 2% NaCl for analysis by matrix-assisted laser desorption ionization time of-flight mass spectrometry (MALDI-TOF-MS) and subsequent comparison of peptide mass fingerprints with Bruker Biotyper database (Bruker Daltonics, Germany). Results were interpreted in accordance to Maldi Score as follows: highly probable species identification (2.3-3.0), secure genus identification (2.0-2.299), probable genus identification (1.7-1.99) and unreliable identification (< 1.699). For *Vibrio* identity confirmation, sequence analyses of marker genes 16S rRNA, gyrB, mreB and rpoD were performed on selected isolates (Table 1). The sequences were submitted to BLASTn search program with the NCBI GeneBank database, applying the criterion of ≥99% sequence identity for reliable species identification.

Sample Origin	Code	Closest species Biotyper	*Maldi Score (1st hit)	*Vibrio* sub-genus	Marker gene	Reference	Best match BLASTn	Sequence similarity
LB oyster	4LK7	*V. aestuarianus*	2.24	*Anguillarum*	gyrB	Pascual et al (2010)	*V. aestuarianus*	≥99%
LB oyster	4LK8	*V. aestuarianus*	2.26	*Anguillarum*	gyrB		*V. aestuarianus*	≥99%
LB mussel	4LK50	*V. aestuarianus*	2.31	*Anguillarum*	gyrB		*V. aestuarianus*	≥99%
LB mussel	4LK51	*V. aestuarianus*	2.18	*Anguillarum*	gyrB		*V. aestuarianus*	≥99%
LB seawater	4LK85	*V. anguillarum*	2.10	*Anguillarum*	gyrB		*V. anguillarum*	≥99%
LB seawater	4LK88	*V. anguillarum*	1.93	*Anguillarum*	gyrB		*V. anguillarum*	≥99%
LB mussel	2LK28	*V. alginolyticus*	1.81	*Harveyi*	16S rRNA	Mougin et al (2020)	*V. alginolyticus*	≥99%
LB mussel	2LK100	*V. alginolyticus*	2.01	*Harveyi*	16S rRNA		*V. alginolyticus*	≥99%
LB mussel	2LK101	*V. alginolyticus*	2.11	*Harveyi*	16S rRNA		*V. natriegens*	≥99%
LB oyster	4LK24	*V. harveyi*	2.18	*Harveyi*	rpoD	Pascual et al (2010)	*V. jasicida*	≥99%
LB oyster	4LK25	*V. harveyi*	1.91	*Harveyi*	rpoD		*V. jasicida*	≥99%
LB oyster	4LK26	*V. harveyi*	2.37	*Harveyi*	rpoD		*V. jasicida*	≥99%
LB seawater	4LK123	*V. tasmaniensis*	2.02	*Splendidus*	mreB	Sawabe et al (2007)	*V. splendidus*	≥99%
LB seawater	4LK125	*V. tasmaniensis*	1.81	*Splendidus*	mreB		*V. splendidus*	≥99%
MSB mussel	4MS112	*V. tasmaniensis*	1.93	*Splendidus*	mreB		*V. splendidus*	98.73%
MSB mussel	4MS98	*V. gigantis*	1.54	*Splendidus*	mreB		*V. splendidus*	98.21%
MSB mussel	4MS103	*V. gigantis*	1.99	*Splendidus*	mreB		*V. crassostreae*	97.97%
MSB mussel	4MS94	*V. pomeroyi*	2.05	*Splendidus*	mreB		*V. celticus*	98.43%
MSB seawater	3MS95	*V. mediterranei*	1.79	*Mediterranei*	16S rRNA	Mougin et al (2020)	*V. mediterranei*	≥99%
MSB oyster	3MS262	*V. mediterranei*	2.05	*Mediterranei*	16S rRNA		*V. mediterranei*	≥99%
MSB oyster	3MS263	*V. mediterranei*	1.71	*Mediterranei*	16S rRNA		*V. mediterranei*	≥99%
LB mussel	3LK96	*V. mediterranei*	1.79	*Mediterranei*	16S rRNA		*V. mediterranei*	≥99%
MSB oyster	3MS12	*V. orientalis*	2.04	*Orientalis*	16S rRNA		*V. hyugaensis*	≥99%
LB sediment	3LK120	*V. orientalis*	1.94	*Orientalis*	16S rRNA		*V. orientalis*	≥99%

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Results and Discussion:
MALDI-TOF MS screening of bacterial isolates (N=431) resulted in around 75% spectra profiles that matched those of the members of genus *Vibrio*. High probability of species identification (N=9), secure genus identification (N=136) and probable genus identification (N=153) was found for *Splendidus, Harveyi, Orientalis, Anguillarum* and *Mediterranei* sub-genus members. At both sites, nearly 70% of vibrios (Maldi 1.70-2.37) accounted for *Splendidus* sub-genus, owing to high abundance of *V. pomeroyi* and *V. gigantis* and moderate occurrence of *V. tasmaniensis, V. chagasii* and *V. fortis*. The sub-genus *Harveyi* (~10% of total) was represented by *V. harveyi, V. alginolyticus, V. rotiferianus* and *V. parahaemolyticus*. Vibrios from *Orientalis* sub-genus (*V. orientalis* and *V. brasiliensis*) were also found at both sites, whereas *V. aestuarianus* and *V. anguillarum* (*Anguillarum* sub-genus) were found only at Lim Bay (bivalve tissue). Identification was confirmed by marker genes for *V. mediterranei*, *V. aestuarianus*, *V. anguillarum* and two *V. alginolyticus* isolates (Table 1). Analyses based on MLSA phylogeny are underway in order to better characterise and classify *Vibrio* strains identified by MALDI TOF MS.

Acknowledgements:
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References:
Bauer et al, 2018. J Fish Dis, 41 (12), 1877-1897.
Culot et al, 2021. J Microbiol Methods, 180, art. no. 106106, .
Mougin et al, 2020. Front Microbiol, 11, art. no. 586536, .
Oden et al, 2016. Aquaculture, 464, 420-427.
Pascual et al, 2010. Int J Syst Evol Microbiol, 60 (1), 154-165.

COMPARISON OF CONCEPTS FOR REMOTE MONITORING IN AN OFFSHORE MULTI-USE PROJECT

Eva Strothotte*, Julian Pforth

R&D Centre Kiel University of Applied Sciences GmbH, Schwentinestr. 24, D-24149 Kiel
Email: eva.strothotte@fh-kiel-gmbh.de

Introduction

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 is co-funded by the Horizon 2020 EU programme. The project aims to examine the technical, regulatory, economic, social and environmental viability of ocean multi-use and reduce associated risks through the development of research pilots to test integrated bio-technological approaches under real environmental conditions. Thus, five demonstration pilots are set up in the North, Baltic and Mediterranean Seas, to assess the benefits of integrating various offshore industries such as wind farms, aquaculture and tourism. Three of these five demonstration pilots (German Pilot; Dutch Pilot; Greek Pilot) have developed different concepts to remotely monitor environmental and infrastructure conditions at their pilot sites which are adapted to meet the different challenges and different needs at each of the locations, under their specific multi-use concept.

a. German Pilot – North Sea – 80km of the coast (extreme exposed offshore site) Multi-use: blue mussel and seaweed cultivation & offshore wind research
b. Dutch Pilot – North Sea – 12km of the coast (offshore site) Multi-use: seaweed cultivation & floating solar energy
c. Greek Pilot – Mediterranean Sea – within 1km of the coast (nearshore site) Multi-use: fish aquaculture & tourism

Materials and Methods

To monitor the operational conditions and performance of the "pilots", various sensors will be installed to continuously record hydrographical, biological and infrastructure data, the analysis of which will allow to draw conclusions about the interactions between the environmental conditions with those of the multi-use activities. Therefore different concepts serving the existing specifications at each of the locations and the specifications of the multi-use concepts have been designed. These concepts have been compared regarding to the challenges and anticipated and target benefits at each location.

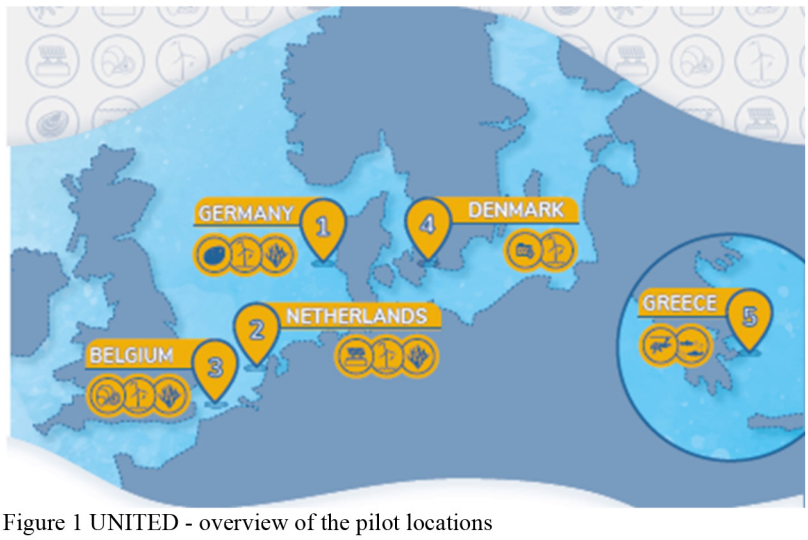

Figure 1 UNITED - overview of the pilot locations

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Results

The three developed monitoring systems will have the following overall characteristics:

a. German Pilot: This pilot will primarily focus on the integration of shellfish and seaweed farming with other stakeholder (offshore wind research) activities. Subsea-lander will be installed in between the mussel and seaweed system and connected via sea cable to the FINO3 platform for power supply and data communication. A data-buoy with a LoRaWAN connection to the FINO3 platform will be attached to the mussel system. The FINO3 platform provides a satellite connection to permit instant remote access to the lander and the data-buoy from the main land.
b. Dutch Pilot: This pilot will focus on integration of seaweed farming with other stakeholder (offshore solar energy) activities. Two data buoys are installed at the test site. They are connected to each other via LoRaWAN and utilize solar panels for independent power supply. The main buoy is directly connected via a cellular network (4G) to the main land for data transfer and remote access. The main buoy is installed independent while the second buoy is installed directly on one of the seaweed systems.
c. Greek Pilot: This pilot will primarily focus on the integration of finfish farming with other stakeholder (tourism) activities. Sensors and cameras are attached to the fish aquaculture cages. From there they are connected to a cellular network antenna (4G) which provides data transfer and remote access from the main land.

Conclusion

The challenges when dealing with an extremely exposed offshore site while attempting to integrate multi-uses at each location requires over-arching safety concepts and operational considerations in order to achieve the intended advantages such as using jointly power supplies and communication networks of the research platform FINO3 (German Pilot) nearby via an existing sea cable (no need for concerns on on-site energy storage, (e.g. battery lifetimes)) thereby allowing to measure more frequently key parameters and online data processing (important for early warning systems to managers). This possibility also exists for future commercial multi-use projects in wind farms if the supply of energy can be obtained onsite from the cooperating wind operator. Sites that are located closer to the shoreline have the advantage of using the cellular network to receive the data from the sensors directly while having the opportunity to reach the monitoring system for maintenance more frequently at reasonable/acceptable costs (Dutch and Greek Pilot). Thus, for the further offshore site (German Pilot) where regular site visits must be more spaced (for economic reasons) the priority on long-term, highly reliable monitoring systems is imperative.

GENETIC SELECTION FOR GROWTH DRIVES DIFFERENCES IN INTESTINAL MICROBIOTA COMPOSITION AND PARASITE DISEASE RESISTANCE IN GILTHEAD SEA BREAM

M.C. Piazzon*[1], F. Naya-Català[2], E. Perera[1,2], O. Palenzuela[1], 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
The key effects of intestinal microbiota in animal health led to an increasing interest in manipulating these bacterial populations to improve animal welfare. The aquaculture sector is no exception, and in the later years many studies have described these populations in different fish species. However, this is not an easy task, as intestinal microbiota is composed of very dynamic populations that are influenced by different factors, such as diet, environment, host age and genetics.

In gilthead sea bream (*Sparus aurata*) aquaculture, genetic selection has been applied to improve growth rates, feed conversion, mortality rates, skeletal deformities, disease resistance and quality (García-Celdrán *et al*., 2015). Selection for growth has been associated with a more continuous growth across seasons and high intestinal plasticity to maximize nutrient absorption when fed plant-based diets, allowing adaptation to dietary changes with no impact on growth and health (Perera *et al*., 2019). In the current study, we aimed to determine whether genetic selection in gilthead sea bream influences the intestinal microbial composition, how these bacterial populations are modulated by dietary changes, and the effect on disease resistance.

Methods
Three different groups of families of gilthead sea bream that were selected for growth (fast: e5e6, intermediate: c2c7 and slow: c4e4) (Perera *et al*., 2019) were kept together in the same open-flow tanks, and fed a control or a well-balanced plant-based diet during nine months. Twelve animals per group were sacrificed, and the adherent bacteria from the anterior intestinal portion were collected and immediately used for DNA extraction. 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. In parallel, 30 fish of the fast- and slow-growth groups were experimentally infected with the intestinal parasite *Enteromyxum leei* and the disease signs, prevalence, intensity and parasite abundance were evaluated.

Results
No differences were detected in alpha diversity indexes among families. The composition at the level of phylum was also not different, indicating that all families harbour the typical intestinal core microbiota of gilthead sea bream, with *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* as the most abundant phyla.

PERMANOVA and PLS-DA analyses showed significant differences in the microbial composition among families and dietary groups. The plant-based diet significantly changed the microbiota in the intermediate- and slow-growth families, with a much lower effect on the fast-growing group, where only a small percentage of the microbiota was changing (Fig. 1). However, when performing pathway analysis from the inferred metagenomes, the small changes detected in the fast-growing families potentially accounted for more changes at the metabolic level when compared to the other families. This was reflected in the 17, 11 and 4 pathways significantly changing (padj < 0.05, log2 fold change > |1|) in the fast-, intermediate- and slow-growing families, respectively, when fed plant-based diets. Upon parasitic infection, the fast-growing group showed significantly lower disease signs and parasite intensity and abundance than the slow-growing animals.

Conclusions
The genetic background, microbiota composition, and physiological plasticity are intricately linked, yielding selection for fast heritable growth more robust individuals. They adapt better to dietary changes, reshape their intestines and organosomatic indexes for an efficient nutrient digestion and absorption, and cope more efficiently with intestinal pathogens. These animals also harbour a plastic microbiota, which effectively adapts to the metabolic challenges induced by dietary changes.

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References

García-Celdrán M., Ramis G., Manchado M., Estévez A., Navarro A., Armero E. 2015. Estimates of heritabilities and genetic correlations of raw flesh quality traits in a reared gilthead sea bream (*Sparus aurata* L.) population sourced from broodstocks along the Spanish coasts. *Aquaculture*, 446:181–186.

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.

ARE HIGH LIPID/ENERGY DIETS BENEFICIAL FOR MARINE FISH LARVAE?

Wilson Pinto[a*], Maria Morais[a], Sara Castanho[b], Ana Candeias-Mendes[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: wilsonpinto@sparos.pt

Introduction
In fish larvae the main metabolic fate of dietary lipids is towards the generation of energy. As energy production also depends on protein, a true dietary lipid requirement is extremely difficult to establish. For this purpose, diet formulations typically include an optimal range of lipids that ensures levels of specific essential fatty acids are satisfied. Growing much faster than juveniles, fish larvae may have a higher requirement for energy and long chain polyunsaturated fatty acids (e.g. docosahexaenoic acid - DHA), which are necessary for organogenesis and formation of cellular membranes. Yet, the dietary requirement for essential fatty acids changes within species and even during ontogeny (Tocher, 2010). In Senegalese sole post-larvae, growth was promoted by diets with both higher DHA and lipid levels (Pinto et al., 2016). While a high dietary lipid level may be beneficial as an additional source of energy and essential fatty acids, excessive lipid levels may negatively affect liver and intestinal function (Morais et al., 2007). To this end, this study aimed at reviewing optimal ranges for dietary lipids levels in early life-stages of traditional (gilthead seabream and European seabass) and emerging fish species (greater amberjack, meagre and Senegalese sole) in Europe.

Materials and methods
Growth trials were conducted during the first weeks of development of target species: gilthead seabream (22-58 days after hatching; DAH), meagre (20-46 DAH), European seabass (23-64 DAH), greater amberjack (33-78 DAH) and Senegalese sole (31-65 DAH). In these trials, larvae were reared in triplicate tanks under standard zootechnical conditions and fed *ad libitum* on diets containing different lipid and energy profiles, all using premium practical ingredients (e.g., squid meal, krill meal, fish meal, wheat fish oil, DHA-rich algae products, soy lecithin) according to the composition in Table 1. Growth performance (weight, total length and relative growth rate), survival, skeletal malformations, liver and gut histology and feed conversion ratio (FCR) were monitored.

Results
At the end of the trials, a significantly higher weight was found in larvae fed HFAT versus MFAT diets in larvae of seabream, meagre, amberjack and seabass (Figure 1). In Senegalese sole, larvae from MFAT treatment had a significantly higher weight at the end of the trial and a lower FCR than larvae from the HFAT treatment.

No significant differences were obtained in skeletal malformations and histological observation of the liver and intestine between treatments of seabream and meagre.

Discussion
Results showed that the capacity to deal with high dietary lipid/energy levels varied among species. Beneficial effects for high lipid / energy diets were observed in species where higher activity patterns and growth rates are also found, such as greater amberjack (Navarro-Guillén et al., 2019) and meagre. Yet, these were also found in seabream and seabass, which are also active but display lower growth rates. In seabass, a diet rich in protein with moderate lipid levels (COMM) also resulted in increased larval growth performance, indicating that the extra supply of protein may help larvae covering energetic requirements. Moreover, dietary DHA supplementation did not bring additional benefits to those observed in larvae fed high dietary lipid levels, suggesting that DHA levels in the HFAT diet fulfilled DHA requirements in seabass. Conversely, Senegalese sole was the only species amongst those studied where no beneficial effect was observed in larvae fed a diet containing higher lipid levels, as a higher growth was observed when dietary lipid levels were reduced. Interestingly, sole is the least active species amongst those studied. Overall, results suggest that high dietary lipid levels may be more adequate for active species with higher growth rates. A high energy input derived from dietary lipids may spare dietary proteins from being allocated for energetic purposes, instead being directed towards protein deposition and growth, as observed in juveniles of several fish species. In general, results support that optimal dietary lipid levels should be determined for the early-life stages of each species, and particularly, that diets should be tailored according to each species nutritional requirements.

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Table 1. Proximal composition of microdiets tested in experimental trials.

	Seabream and Meagre			Amberjack		Seabass				Sole	
	COMM	MFAT	HFAT	COMM	HFAT	COMM	MFATDHA	HFAT	HFATDHA	MFAT	HFAT
Prot %	62	64	61	57	64	67	65	62	64	64	62
Lipid %	17	16	22	15	18	14	16	20	19	17	18
DHA	-	-	-	-	-	-	+	-	+	-	-
Energy (MJ/Kg)	20.9	21.0	22.6	19.0	21.7	20.9	21.4	22.2	22.0	21.4	21.3

*values expressed in diet wet weight basis; COMM: commercial diet; MFAT and HFAT: diets containing moderate and high fat levels, respectively; Prot: crude protein; lipid: crude lipid; DHA: unsupplemented (-) or supplemented (+).

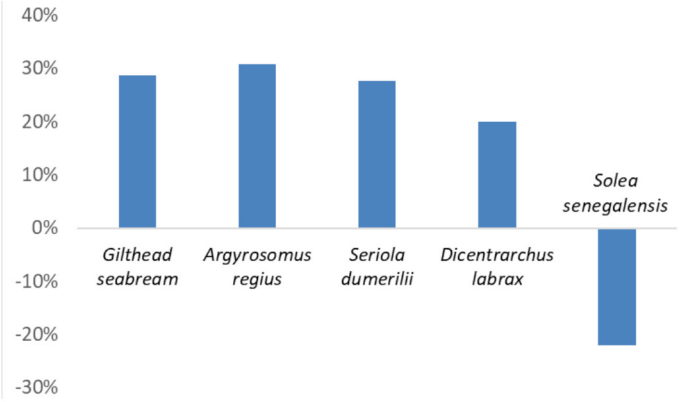

Figure 1. Variation in final weight in larvae fed HFAT versus MFAT diets.

References

Morais, S., Conceicao, L.E.C., Ronnestad, I., Koven, W., Cahu, C., Infante, J.L.Z., Dinis, M.T., 2007. Dietary neutral lipid level and source in marine fish larvae: Effects on digestive physiology and food intake. Aquaculture 268, 106-122.

Navarro-Guillén, C., Conceição, L.E.C., Pinto, W., Siguero, I., Urrutia, P., Moyano, F.J., Yúfera, M., 2019. Fast growing greater amberjack post-larvae require a high energy-high protein weaning diet. Aquaculture 499, 195-202.

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.

Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac Res 41, 717-732.

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LESIONS IN PORTUGUESE OYSTERS ASSOCIATED WITH THE PRESENCE OF *VIBRIO SP.*

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 Lisbon
*dspires@fmv.ulisboa.pt

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

Introduction

Oysters are a rich and healthy seafood, however they can contain pathogens harmful to Human, such as certain Vibrio, like *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which are naturally present in the aquatic marine environment (Baker-Austin *et al*., 2013; Froelich & Noble, 2016). 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).

A complex interaction of factors including high temperature and pathogens like Ostreid herpesvirus or bacteria, like *Vibrio sp*. are suggested to be involved in mortality events affecting oysters. For example, Lacoste *et al*., (2001) attributed the cause of summer mortality of *Crassostrea gigas* in Bay of Morlaix (France) to an infection by *Vibrio splendidus*. For *Vibrio sp*. it is known that virulence depends on environmental parameters, such as temperature or salinity (Martin *et al*., 2002). However, further work is needed to better understand how environmental conditions may influence interactions of host-pathogen in these animals.

The Portuguese oyster, *Crassostrea angulate,* is a resource with relevant importance in Portugal, due to its socioeconomic value and also to its historically and genetic values. Understanding the impact of pathogens that affect other important species of oysters, like *C. gigas*, in the Portuguese species, is a priority to the management and for the sustainability of stocks.

The Vibrio genus encompasses Gram-negative bacteria species indigenous of marine and estuarine waters. To date, one hundred and thirty species of vibrios have been described and twelve were classified as human pathogens implicated mostly in food- or water-borne diseases (Thompson et al. 2006).

The Vibrio genus encompasses Gram-negative bacteria species indigenous of marine and estuarine waters. To date, one hundred and thirty species of vibrios have been described and twelve were classified as human pathogens implicated mostly in food- or water-borne diseases (Thompson et al. 2006).

Materials and Methods

Two populations of Portuguese oysters from Sado estuary (n=30) and Mira estuary (n=30) were surveyed. Anatomohistopathological and bacteriological studies were performed. The tissue samples for histopathology were fixed in Davidson's fixative for 48h, dehydrated, embedded in paraffin and cut with a microtome in sections less than 5 μm thick, then stained with Hematoxylin-Eosin (H&E).

For the bacteriology study, the hemolymph was collected from pericardial cavity and dilutions to the 1/100 and 1/10,000 were performed in Artificial Sterilized Marine Water (ASMW) and fifty (50) μl of those dilutions were plated on TCBS (Thiosulfate-Citrate-Bile Salts Sucrose (Difco)) agar in Petri plates and incubated for 48 to 96h at 20°C. Colonies observed (in non-confluent plates) with a similar macroscopic aspect are counted.

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Results

The histopathological exam revealed in both populations the presence of *Trichodina sp*. ciliates in gills and mantle epithelium and *Ancistrocoma sp*. in the digestive gland tubules and in the connective tissue, both with a moderate prevalence. The copepod *Mytilicola sp*. was also observed within the intestine of oysters in both populations. In both populations, it was were observed the presence of hemocitosis, mainly in connective tissue, tissues necrosis, edema and metaplasia in digestive gland. In the population from Mira estuary the prevalence were slightly higher, with the exception of individuals with metaplasia.

The analysis of bacteriological exam revealed significant differences between both populations. In the population, from Mira Estuary it was observed 20 individuals positive to *Vibrio sp*. contrasting with just 1 positive case in the Sado population.

Discussion and conclusion

In the present study, two *C. angulata* populations were sampled with the objective to understand the prevalence of *Vibrio sp*. in the production of oysters in Portugal.

The destruction observed in the epithelium of the diverticula of the digestive gland, the hemocytic infiltration and necrosis are usually associated with an inflammatory process, can be related with the presence of the virus and *Vibrio sp*., such as *V. splendidus* and *V. aestuarianus*.

The histopathological examination of infected oysters with *Vibrio sp*. showed the presence of necrosis and hemocitosis, mainly in the connective tissue and digestive gland. *Vibrio sp*. invades all tissues, especially the connective tissue and is usually dispersed in the individual infected (Garnier et al., 2007). Hemocyte phagocytosis capacities were also reduced (Labreuche et al., 2006).

Vibriosis is the most commonly encountered disease associated with intensive bivalve culture in hatcheries and nurseries (Bower *et al*., 1994).

Further studies will be performed to identify the prevalence of Vibrio sp during the year and in farmed and wild populations.

References

Baker-Austin, C., Trinanes, J., Taylor, N., Hartnell, R., Siito-nen, A., Martinez-Urtaza, J., 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. *Nature Climate Change*, 3, 73–77.

Bower, S., McGladdery, S., Price, I., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review of Fish Diseases* 4: 1-199.

Cheney, D., MacDonald, B., Elston R., 2000. Summer mortality of Pacific oysters, Crassostrea gigas (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *J. Shellfish Res*. 19, 353– 359.

Froelich, B. and Noble, R., 2016. *Vibrio* bacteria in raw oysters: managing risks to human health.

Garnier, M., Labreuche, Y., Garcia, C., Robert, M., Nicolas, J., 2007. Evidence for the involvement of pathogenic bacteria in summer mortalities of the pacific oyster *Crassostrea gigas*. *Microb Ecol*. 53:187-96.

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*. *Appl Environ Microbiol*. 67(5): 2304–2309.

Martin, Y., Bonnefont, J., Chancerelle, L., 2002. Gorgonians mass mortality during the 1999 late summer in French Mediterranean coastal waters: the bacterial hypothesis. *Water Res*. 36, 779 – 782.

Wendling, C., Batista, F., Wegner, K., 2014. Persistence, seasonal dynamics and pathogenic potential of Vibrio communities from Pacific oyster hemolymph.

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ANTIBACTERIAL AND IMMUNOSTIMULANT ACTIVITIES OF CHITOSAN ULVAN NANOPARTICLES AGAINST *Photobacterium damselae* SUBSP *piscicida*

M. Ponce*, E. Zuasti, V. Anguís and C. Fernández-Díaz

[a]IFAPA Centro El Toruño. Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
E-mail: marian.ponce@andaluciajunta.es

Introduction

Infectious diseases are one of the main causes of the social and economical losses in world aquaculture. In particular, Senegalese sole (*Solea senegalensis*) is an important species for aquaculture in southern Europe, whose production is affected by the appearance of bacterial diseases such as photobacteriosis, a septicaemia caused by *Photobacterium damselae* subsp. *piscicida* (*Phdp*). In this context, the identification of bioactive compounds in order to limit the use of antibiotics and be able to conduct preventive strategies has been one of the main lines of research in aquaculture in the last years. In this sense, ulvan is a marine derived polysaccharide from green seaweed of the genus *Ulva* that possess immunomodulating, antioxidant, anticoagulant, anticancer, antiviral and antihyperlipidemic activities (reviewed in (Kidgell et al., 2019). Recently, the role of ulvan from *U. ohnoi* as bioactive compound with immunomodulatory activity as well as with possible use as vaccine adjuvant against *Phdp* in Senegalese sole has been reported (Ponce et al., 2020). As drug delivery vector for fish vaccination and protection against diseases, the use of chitosan (CS) in the fish farming industry has great potential due to its biodegradability, biocompatibility, bioadhesion and immunomodulatory properties (Wu et al., 2020). The aim of this study was to evaluate the antibacterial and immunostimulant activities of chitosan ulvan NPs (CS-UL-TPP NPs). For this reason, *in vitro* antibacterial activity assay against *Phdp* was performed. CS-TPP NPs and CS-UL-TPP NPs were administered orally to Senegalese sole juveniles and the expression profile of a set of genes related to the immune system was evaluated in spleen at 30 days post administration. Juveniles were challenged with *Phdp* and the mortality rate was determined.

Material and methods

Ulvan extraction and determination of its molecular weight (MW) were performed as described by (Fernández-Díaz et al., 2017). In the same way, CS-TPP NPs and CS-UL-TPP NPs were prepared as described previously by (Fernández-Díaz et al., 2017). Size and polydispersity index (PDI) of CS-TPP and CS-UL-TPP NPs were calculated by dynamic light scattering on a ZetaSizer Nano-ZS90 (Malvern, UK) and Z-potential was calculated using the same device by Laser Doppler Micro-Electrophoresis. NPs were dissolve to 0.6 mg mL^{-1} and serial two-fold dilutions were made for the antibacterial activity assay against *Phdp* (strain Lg41/01). Bacterial suspension containing 10^5 cfu mL^{-1} was added to each well. Absorbance was measured at 0 and 24 h at 600 nm using a microplate reader. For oral administration of NPs, Senegalese sole juveniles (average weight 12.8 ± 1.7 gr) were provided by Cultivos Piscícolas Marinos S.A. (CUPIMAR) (Cádiz, Spain) and they were acclimated for 7 days (three replicates per treatment). The treatment groups were as follows: i) control groups; ii) CS-TPP NPs and iii) CS-UL-TPP NPs. Administration of each treatment (50 μL) was performed by oral intubation using a semi rigid 1 mm diameter veterinary cat catheter (SMI). At 30 days post treatment, spleen was sampled and total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and treated twice with DNase I using the RNase-Free DNase kit (Qiagen). Total RNA was reverse-transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). Expression analyses of *il1b*, *il6*, *c3a*, *hamp1* and *tf* were performed by RT-qPCR using specific primers. *S. senegalensis* juveniles were challenged with the strain Lg41/01 of *Phdp* (10^4 cfu gr^{-1}) and mortality was monitored daily.

Results and discussion

Ulvan was obtained from extraction and purification process of U. ohnoi and the MW was 132 ± 23.5 KDa. The mean size and zeta potential of CS-TPP NPs were 137 ± 5.44 nm and 39.7 ± 0.21 mV, respectively. Regarding CS-UL-TPP NPs, the mean size and zeta potential were 325.5 ± 4.95 nm and 35 ± 0.1 mV, respectively. These data indicate that the NPs obtained in this work are suitable for delivery into host tissues. *In vitro* antibacterial activity was observed for both, CS-TPP NPS and CS-UL-TPP NPs at all concentrations assayed, being 100% inhibition at 0.3 and 0.15 mg mL^{-1}. It is noteworthy that at 0.03 and 0.01 mg mL^{-1}, the percentage inhibition was significantly higher in the case of CS-UL-TPP NPs than in the case of CS-TPP NPs (1.2 fold at both concentrations). Regarding gene expression analyses, *il1b*, *il6*, *c3a*, *hamp1* and *tf* mRNA levels were significantly higher in CS-UL-TPP NPs treated groups than in CS-TPP NPs and control groups in spleen at 30 days after oral administration. Moreover, mortality in CS-TPP NPs treated groups was 25% higher than in sole juveniles from CS-UL-TPP NPs treated groups. These results suggest that ulvan boost the antibacterial and immunostimulant effects of chitosan nanoparticles against *Phdp* in Senegalese sole juveniles.

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References

Fernández-Díaz, C., Coste, O., & Malta, E. jan. (2017). Polymer chitosan nanoparticles functionalized with Ulva ohnoi extracts boost in vitro ulvan immunostimulant effect in Solea senegalensis macrophages. *Algal Research*, *26*, 135–142. https://doi.org/10.1016/j.algal.2017.07.008

Kidgell, J. T., Magnusson, M., de Nys, R., & Glasson, C. R. K. (2019). Ulvan: A systematic review of extraction, composition and function. In *Algal Research* (Vol. 39, p. 101422). Elsevier B.V. https://doi.org/10.1016/j.algal.2019.101422

Ponce, M., Zuasti, E., Anguís, V., & Fernández-Díaz, C. (2020). Effects of the sulfated polysaccharide ulvan from Ulva ohnoi on the modulation of the immune response in Senegalese sole (Solea senegalensis). *Fish and Shellfish Immunology*, *100*, 27–40. https://doi.org/10.1016/j.fsi.2020.02.054

Wu, Y., Rashidpour, A., Almajano, M. P., & Metón, I. (2020). Chitosan-Based drug delivery system: Applications in fish biotechnology. In *Polymers* (Vol. 12, Issue 5). MDPI AG. https://doi.org/10.3390/POLYM12051177

EVALUATION OF AN ORAL DNA NANOVACCINE AGAINST PHOTOBACTERIOSIS IN *Solea senegalensis*

M. Ponce*[,a], E. Zuasti[a], E. Reales[b], V. Anguís[a] and C. Fernández-Díaz[a]

[a]IFAPA Centro El Toruño. Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
[b]Department of Organic Chemistry, School of Sciences, University of Cadiz, and Biomedical Research and Innovation Institute of Cadiz (INiBICA), Cádiz, Spain
E-mail: marian.ponce@andaluciajunta.es

Introduction

Photobacteriosis is a septicemia caused by *Photobacterium damselae* subsp. *piscicida* (*Phdp*) that causes severe economical losses in world aquaculture of marine fish due to its capacity for inducing massive mortality, ubiquitous distribution and widespread antibiotic resistance (Romalde, 2002). Photobacteriosis is one of the main bacterial diseases that affects the culture of *Solea senegalensis*, an important species for aquaculture in southern Europe. Vaccination is one of the most appropriate methods of disease control and thereby limits the use of antibiotics. However, the only commercially available vaccine for photobacteriosis is an ECP-enriched bacterin preparation that gave unreliable results (reviewed in (Andreoni & Magnani, 2014)). In this sense, DNA vaccines offer a number of advantages over conventional vaccines being the most important benefit that they can promote humoral and cell-mediated immune response (Dadar et al., 2017). Nevertheless, naked pDNA is susceptible to degradation by DNases and lysosomes and rather unable to transpose cellular barriers. Encapsulating pDNA within degradable delivery vehicles such as chitosan nanoparticles (CS-NPs) provides an effective way to protect the DNA. The aim of this study was to obtain a DNA nanovaccine and to evaluate its efficacy against *Phdp* after oral vaccination in *S. senegalensis* juveniles. For this purpose, the inosine-5´-monophophate dehydrogenase (*impdh*) gene from *Phdp* was used to obtain a DNA vaccine named as pPDPimpdh. Correct transcription and protein expression were verified at 48 h post transfection in HEK293 cells. pPDPimpdh was conjugated with CS-TPP NPs giving as a result a DNA nanovaccine prototype referred as CS-TPP+pPDPimpdh NPs that was administered orally to *S. senegalensis* juveniles. The expression profile of a set of genes related to the immune system was evaluated in the posterior gut and spleen as well as the mortality rate after challenge with *Phdp*.

Material and methods

The gene encoding for *impdh* was amplified with a specific primer set and cloned into the expression vector pcDNA™6.2/C-EmGFP-GW/TOPO® (Invitrogen). Plasmid was referred as pPDPimpdh. Transfection in HEK293 cells was performed using GenJet™ In Vitro DNA Transfection Reagent (SignaGen® Laboratories) and RT-PCR and western blotting assays were performed at 48 h post transfection. For RT-PCR assay, total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and reverse-transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). For the western blotting assay, 20 μg of total protein were loaded into 10% separating gel SDS-PAGE and transferred to a 0.45 μm Pure Nitrocellulose Membrane (Bio-Rad). Membranes were probe with a monoclonal antibody GFP and an anti-rabbit IgG HRP conjugated secondary antibody. CS-TPP NPs were prepared as described previously by (Fernández-Díaz et al., 2017). Elaboration of CS-TPP+pPDPimpdh NPs was carried out by a complex coacervation method. Size and polydispersity index (PDI) were calculated by dynamic light scattering on a ZetaSizer Nano-ZS90 (Malvern, UK) and Z-potential was calculated using the same device by Laser Doppler Micro-Electrophoresis. For oral vaccination, sole juveniles (average weight 19.5 ± 5.3 gr) were provided by CUPIMAR S.A. (Cádiz, Spain) and they were acclimated for 7 days. Each treatment (50 μL) was administered by oral intubation using a semi rigid 1 mm diameter veterinary cat catheter. At 30 dpv, juveniles were challenged with *Phdp* (10^4 cfu gr^{-1}). At 7 and 30 days post vaccination (dpv) and 4 days post challenge (dpc) the posterior gut and spleen were sampled.

Results and discussion

A transcription analysis was performed by RT-PCR assay at 48 h post transfection in HEK293 cells. The amplified product was observed at the size of ~1,470 bp corresponding to the expected size of the *impdh* gene of *Phdp*. Furthermore, to confirm that pPDPimpdh was able to express the protein, a western blotting assay was performed and a specific band of approximately 75 KDa was observed corresponding to the expected molecular weight of the IMPDH protein (~50 KDa) plus the molecular weight of EmGFP protein (~27 KDa). Once the correct transcription and expression of IMPDH was

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confirmed, the DNA vaccine pPDPimpdh was conjugated with CS-TPP NPs. Some features of NPs, such as size and zeta potential, can affect the uptake of the complexes by the cells. The mean size of CS-TPP and CS-TPP+pPDPimpdh NPs obtained in this work was 208.30 ± 1.13 and 369. 60 ± 6.36 nm, respectively, which is in the size range considered as suitable in the area of drug delivery systems (between 100 to 500 nm). On the other hand, in order to ensure the stability of the dispersion and avoid the aggregation of the particles, it is desirable to obtain high zeta potential values, whether positive or negative. In this study, Z-potential of CS-TPP NPs and CS-TPP+pPDPimpdh NPs was 20.50 ± 2.33 and -56.20 ± 4.03 mV, respectively. Taken together, all these results indicate that CS-TPP+pPDPimpdh NPs prepared in this study are suitable for oral delivery. After oral vaccination, *cd8a* and *igm* mRNA levels increased significantly in CS-TPP+impdh NPs treated groups at 7 and 30 dpv whereas *cd4* expression levels were significantly higher at 4 dpc in the posterior gut. In spleen, expression levels of *igm*, *igt* and *cd4* were significantly higher in CS-TPP+pPDPimpdh NPs treated groups at 4 dpc. At 4 dpc, mortality began first in pPDPimpdh treated groups and the RPS was 6.25 %. The RPS of CS-TPP+pPDPimpdh NPs groups was 40%. All these results indicate that the DNA nanovaccine prepared in this work and delivery orally to sole juveniles could induce the specific cellular and humoral immune response.

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References

Andreoni, F., & Magnani, M. (2014). Photobacteriosis: prevention and diagnosis. *J Immunol Res*, *2014*, 2014. https://doi.org/10.1155/2014/793817

Dadar, M., Dhama, K., Vakharia, V. N., Hoseinifar, S. H., Karthik, K., Tiwari, R., Khandia, R., Munjal, A., Salgado-Miranda, C., & Joshi, S. K. (2017). Advances in Aquaculture Vaccines Against Fish Pathogens: Global Status and Current Trends. In *Reviews in Fisheries Science and Aquaculture* (Vol. 25, Issue 3, pp. 184–217). Taylor and Francis Inc. https://doi.org/10.1080/23308249.2016.1261277

Fernández-Díaz, C., Coste, O., & Malta, E. jan. (2017). Polymer chitosan nanoparticles functionalized with *Ulva ohnoi* extracts boost in vitro ulvan immunostimulant effect in *Solea senegalensis* macrophages. *Algal Research*, *26*, 135–142. https://doi.org/10.1016/j.algal.2017.07.008

Romalde, J. L. (2002). *Photobacterium damselae* subsp. piscicida: An integrated view of a bacterial fish pathogen. In *International Microbiology* (Vol. 5, Issue 1, pp. 3–9). Sociedad Espanola de Microbiologia. https://doi.org/10.1007/s10123-002-0051-6

OYSTER PRIMARY CELL CULTURE

Robert Potts*, Alejandro Gutierrez, 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) (Degremont et al. 2019. Understanding of OsHV pathogenesis is limited, and experimental replication of the virus requires serial passage through live oysters. This is partly due to the lack of a relevant immortalised cell line. Establishing an immortalised pacific oyster cell line is a key goal for several research fields including ecotoxicology, virology, immunology, and genetic resistance to disease (Yoshino et al. 2013). The aim of the current study was to establish primary cell cultures from multiple pacific oyster tissues using an optimised culture method, with a view to future cell culture and live oyster disease challenge experiments to better understand host response and resistance to OsHV.

Materials and methods

Live pacific oysters were obtained from a UK oyster farm. Tissues were dissected and sterilized using antibiotic and antifungal treatments under sterile conditions. Tissue explants between 2 mm and 10 mm across were transferred to cell culture well plates pre-coated with matrigel. Sterile filtered media for all cultures was prepared using Leibovitz's L15 media and artificial seawater with antifungal and antibiotic treatments. Plates were sealed and incubated at 22 ºC, and media was refreshed regularly to promote cell proliferation.

Results

Using this optimised method, axenic primary cell cultures from heart, mantle, gill, adductor muscle, gonad and hemolymph have been successfully established (e.g. Figure 1). The cell cultures demonstrate highly confluent and varied cell assemblages demonstrating that the culture technique can be successfully applied to all these tissues without modification. Successful culture of oyster muscle cells has been achieved, which has not been reported previously.

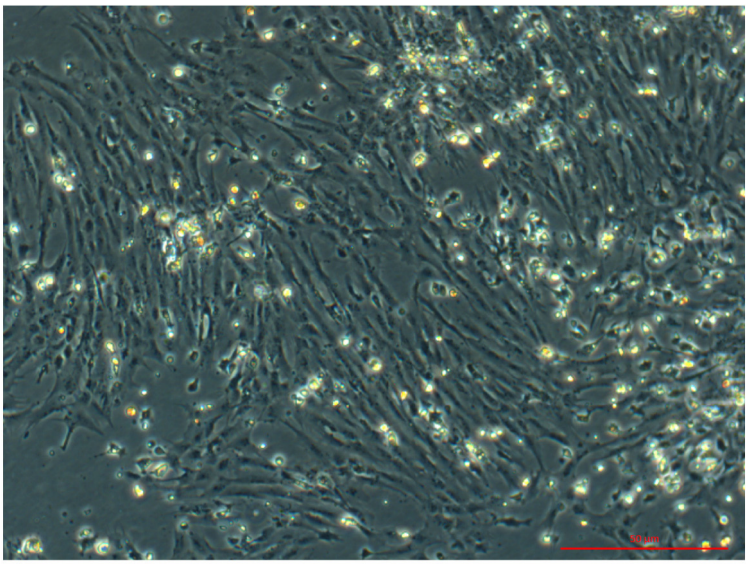

Figure 1) Primary culture of oyster mantle cells with multiple morphologies and high confluency. Scale bar = 100 μm

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Discussion and future work

The methods developed have produced primary cell cultures that can potentially be used for a range of applications, including the establishment of an immortalised cell line in the future. There are multiple approaches for immortalisation, but these are generally optimised for mammalian cell culture. The immortal gastropod mollusc *Biomphalaria glabratra* cell line was established by careful maintenance and sub-culturing of primary cells (Hansen 1976), which is currently underway with the *Crassostrea gigas* lines. Furthermore, these primary cultures present an opportunity to replicate OsHV in the laboratory, which will be trialled in the future. It is anticipated that these cell cultures will provide tools to improve our understanding of host response and genetic resistance to OsHV in Pacific oysters.

References

Dégremont L., Maurouard E., Ledu C., Benabdelmouna A., 2019. Synthesis of the "PLAN DE SAUVEGARDE" using selected all-triploid oysters to reduce the shortage of spat in France due to OsHV-1–associated mortality in Crassostrea gigas. Aquaculture, 505, 462-472

Hansen E. L., 1976. A cell line from embryos of *Biomphalaria glabrata Pulmonata* Establishment and characteristics.

Yoshino T. P., Bickham U. & Bayne C. J., 2013. Molluscan cells in culture: primary cell cultures and cell lines. Canadian Journal of Zoology, 91**,** 391-404.

GROWTH PERFORMANCE OF MEAGRE JUVENILES (*Argyrosomus regius*) AT TWO TEMPERATURES FED WITH DIFFERENT SOURCES AND PROTEIN CONTENTS

S. Sousa e Brito[1], M. Barata[1], A. Candeias-Mendes[1], S. Castanho[1], J. Dias[2] and P. Pousão-Ferreira[1]*

1. Portuguese Institute for the Ocean and Atmosphere (IPMA), Aquaculture Research Station (EPPO), Av. Parque
2. Sparos Área Empresarial de Marim, Lote C. 8700-221 Olhão, Portugal
E-mail: sara.sousabrito@gmail.com

Introduction

Temperature is the environmental factor that most influences aquatic organisms, which directly interferes with their metabolic activity, feed intake and growth rate.

Meagre (*Argyrosomus regius*) is a species with high commercial value which, under appropriate temperature conditions, presents interesting attributes for a more sustainable and diversified aquaculture practice in the Southern Europe[1]. This species can be produced in a (recirculating system) RAS system at higher and constant temperatures when compared with fish produced in sea cages or earthen ponds.

As carnivorous species with high protein requirements, meagre has been the subject of numerous studies to determine their ideal dietary protein levels and how particular ingredients can boost its growth, in order to turn this species increasingly appealing to the industry, from an economic and environmental point of view[2,3].

This study aimed to evaluate the combined effect of the water temperature and the diet protein source and content on the growth of meagre juveniles.

Materials and Methods

A homogeneous group of fish (initial body weight: 30.17 ± 5.58g) were distributed in triplicates in twelve 1,5m^3 tanks. Four treatments (LTCP55, HTCP50, HTCP55, and HTCP55ALT) were performed at two temperatures LT (22°C) and HT (26 °C), and fish were fed with three isolypidic diets (16.2%), consisting on the specifications shown on the table 1, during a period of 56 days.

Results

When comparing treatments with different water temperatures (LT22º CP55 and HT26º CP55), it was found that individuals under 26°C obtained a notoriously superior growth performance when compared under individuals at 22ºC, demonstrating a final weight of 134.53g and 109,16g respectively and feed conversion ratio (FCR) 0,80 and 0,85 (fig.1 and 2).

Considering the juveniles that were fed with different sources and protein contents (HT CP50, HT CP55, and HT CP55 ALT), the only difference observed was the FCR, showing that, although fish fed with 55% CP did a better conversion at temperatures of 26 °C, the source and protein contents did not influence their growth performance (fig.1 and 2).

Table 1: Composition of experimental diets (CP50, CP55 and CP55ALT).

	Diets		
	CP50	**CP55**	**CP55ALT**
Ingredients (%)			
Fish meal	24.5	28.0	7.0
Vegetable meals	25.5	27.0	21.0
Poldry meal	0.0	0.0	27.0

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Conclusions

The results of this work demonstrated that temperature is an extremely important and a predominant factor in the culture of meagre juveniles in combination with appropriate protein level.

Moreover, the growth of juveniles of this species was not significantly affected by the replacement of fish meal by poultry and/or vegetable meals.

Acknowledgements

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References

1) Ribeiro, L., Soares, F., Quental-Ferreira, H., Gonçalves, A., & Pousão-Ferreira, P., 2013. Portuguese Research Studies on Meagre Production In Earthen Ponds. Global Aquaculture Aldvocate, 16, 38–40.

2) Matias, A.C.; Dias, J.; Barata, M.; Araujo, R.L.; Bragança, J.; Pousão-Ferreira, P., 2020. Taurine modulates protein turnover in several tissues of meagre juveniles. Aquaculture, Vol.528. https://doi.org/10.1016/j.aquaculture.2020.735478

3) Saavedra, M.; Pereira, T.G.; Candeias-Mendes, A.; Carvalho, L.; Pousão-Ferreira, P. & Conceição, L.E.C., 2018. Effect of increased dietary protein level in meagre (Argyrosomus regius) juvenile growth and muscle cellularity. Aquaculture Nutrition 24:1153-1159. DOI: 10.1111/anu.12654

TESTING THREE MICROALGAE DIETS FOR STONY SEA URCHIN (*Paracentrotus lividus)* LAMARCK, 1816) LARVAL CULTIVATION

J. Araújo, A. Candeias-Mendes, Ivo Monteiro, N.M. Bandarra and P. 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
Email: joao.araujo@ipma.pt

Introduction

Sea urchins, *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: Echinoidea) is a marine resource considered a delicacy food in many parts of the globe, being particularly appreciated in Asian countries such as Japan and South Korea. Due to the growing demand and consequent capture, a decrease of the natural stocks has been observed (FAO, 2020) leading to the interest on echinoid aquaculture, which greatly increased in the last two decades. The echinoderms aquaculture is a challenge, mainly due to the existence of different stages throughout these animals' life cycle and to the complex biotic relationships that occur during the phase transitions of larvae and post-larvae. The quality of the microalgae provided is a primordial factor on growth, competence (capacity for settlement) and survival. In the last years, several microalgae species have been tested in order to optimize the larvae culture (Ahmed et al., 2016; Carboni et al., 2012; Castilla-Gavilán et al., 2018). The main objective of this work was to evaluate three microalgae diets, such as *Skeletonema costatum*, *Phaeodactylum tricornutum* and *Emiliania Huxley* on *P. lividus* larvae development and survival. In addition the synergistic effect of the combination of these microalgae was also evaluated.

Material and methods

Wild sea urchin *P. lividus* were collected from intertidal rocks in the South of Portugal coast, transported and kept at EPPO (Aquaculture Research Center). Diet was based on maize (*Zea mays*) and mainly fresh *Ulva sp*. The spawning induction was performed by injecting 1 ml of KCl 0.5 M into the coelom via the peristomial membrane. Fertilized eggs were hatched in a 220 L cylindrical fiberglass tanks with soft aeration and covered with shader, to reduce environmental light exposure, for approximately 48h. The larval cultivation experiment was carried out in fifteen 6L flat bottom flasks (four replicates for each treatment) without water inlet and with a reduced aeration. The initial cultivation density was approximately 2800 larvae per liter. Partial water renewals of 30% of the total water volume were performed daily using a siphon with reverse filtration trough a filter of 30 μm. Three tests were carried out to evaluate the use of two diatoms (*S. costatum*, *P. tricornutum*) and a cocolitophoro *E. Huxley*. The daily ration was calculated according to the larval stage of development, method described by Carboni et al. (2012). Larval survival, development stage and biometric sampling were assessed during the experiments.

Results and Discussion

Larvae fed with a single microalgae diet had lower survival at 15 DAH (Days After Hatching) comparing to multiple microalgae species diet. Larvae fed exclusively with diet Sc (*S. costatum*), diet Pt (*P. tricornutum*) and diet Eh (*E. Huxley*) had a survival of 55.8, 55.0, and 74.9, respectively at 15 DAH. Greater survivals were found on larvae fed with diet Sc+Eh (75.8%) and Sc+Pt (76.0%) at 15 DAH. The survival recorded in diets with multispecies is greater than the observed in similar studies (Ahmed et al., 2016; Carboni et al., 2012; Castilla-Gavilán et al., 2018). In general, the mixture of microalgae is also responsible for faster development, comparing with single specie diet. It was observed that larvae fed with diet Eh showed a relatively lower development than the other diets and at 8 DAH 84.6% of the larvae were still on 4-arms phase. At 15 DAH only 55.6% of the larvae were on 8-arms stage. However, when *E. huxley* was associated with *S. costatum* (Sc+Eh) had a development than the other diets tested. At 15 DAH 100% of *P. lividus* already reached the 8-arms development phase. The lipid analyzes of three microalgae studied show significant differences both in terms of total fatty acid content and specific profile. These lipidic nutritional differences and the synergistic effect of combination are the main reason for the differences in the results obtained in these tests (Araújo et al., 2020).

Conclusions

The use of different microalgae diets has a great influence on the success of the development and survival of *P. lividus* larvae with the association of different species, with different nutritional characteristics, being the best strategy for the successful cultivation of sea urchins.

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References

- Ahmed H.O., Elmasry E., El-Syaed A.M., Rasek A. 2016. Larval growth and metamorphosis of South Eastern Mediterranean Sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different microalgal diets. Journal of Fisheries and Aquatic Science 11 : 287-295
- Araújo, J, Candeias-Mendes, A, Monteiro, I, Teixeira, D, Soares, F, Pousão-Ferreira, P. The use of diatom Skeletonema costatum on aquaculture-produced purple sea urchin (Paracentrotus lividus) larvae and post-larvae diet. Aquac Res. 2020; 51: 2545– 2554.
- 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. https://doi.org/10.1016/j.aquaculture.2011.10.037
- Castilla-gavilán, M., Buzin, F., Cognie, B., Dumay, J., Turpin, V., Decottignies, P. 2018. Optimising microalgae diets in sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification. Aquaculture, 490(February), 251–259.
- FAO. 2020. Fishery and Aquaculture Statistics. Global production by production source 1950-2018 (FishstatJ). In: FAO Fisheries Division [online]. Rome. Updated 2020.

DEVELOPMENT OF TECHNIQUES TO CULTIVATE NEW MICROALGAE STRAINS AND INCREMENT THEIR NUTRITIONAL VALUE - *Tetraselmis* sp. IMP3 AND *Phaeodactylum tricornutum*

Monteiro[1], M. Angelo[1], J. Araújo[1], C. Cardoso[1], F. Soares[1], J. Varela[2]; N.M. Bandarra[1], P. Pousão-Ferreira[1*]

[1] Portuguese Institute for the Ocean and Atmosphere (IPMA), Aquaculture Research Station (EPPO), Av. Parque Natural da Ria Formosa S/N, 8700-194, Olhão, Portugal

2 CCMAR –, University of Algarve, campus de Gambelas, 8005-139 Faro, Portugal
E-mail: ivojmonteiro@gmail.com

Introduction

Microalgae biomass is considered a potential source of a wide spectrum of products to feed, food, pharmacy and crucial to the animal aquaculture industry. Currently, it is estimated that there are only about 30,000 species of microalgae described and from those, an even smaller number are used in aquaculture despite their nutritional value and therapeutic activities. Microalgae may play an important role on aquaculture, especially in fish larvae production and, accordingly, bioprospecting for novel microalgae strains is important but not enough, as it is necessary to develop protocols and techniques to improve their applications on aquaculture. This is essential not only achieve microalgae's best growth performance but also to trigger the production of essential and high valuable biomolecules like β-carotene, astaxanthin and PUFA, among others. Therefore, the objective of this study was the development of a new protocol to cultivate microalgae strains in the laboratory.

Materials and Methods

Two concentrations of the Phytobloom® culture medium was used: High (5 $mL.L^{-1}$) and Control (1 $mL.L^{-1}$) as recommended by the manufacturer, to evaluate the growth (cellular density using a Neubauer chamber and dry weight using 0.45µm celulose filters), biomass production and nutrient composition of the microalgae species: *Tetraselmis* sp. IMP3 and *Phaeodactylum tricornutum*. Cultures were grown in 500 mL Erlenmeyer's inside of a climatic chamber at 20ºC, with a photoperiod 16:8 (d/n, 100 photons. s-1. m^{-2}), aerated with compressed air, for 12 days. Samples were collected daily to count and, to detect eventual changes in the biomass, dry weight and nutritional analyses, at different growth stages: day 2 (exponential), day 6 (exponential/stationary) and day 12 (stationary). All experiments were carried out in triplicates.

Results and Discussion

For *P. tricornutum* the cultures displayed similar growth rates during the first 4 days (FIG 1), but the high treatment grew for more 6 days, reaching the maximum cell concentration at day 11 ($8.8\pm1.1x10^4$ $cel.mL^{-1}$), approximately 2.5 times more than the maximum cell concentration found in control conditions ($3.4\pm0,5x10^4$ $cel.mL^{-1}$). At the end of the incubation, day 12, it was possible to harvest twice as much biomass (TAB I) in the the treatment with higher nitrogen concentration (High), (0.97 ± 0.11 $g.L^{-1}$) then in control cultures (0.97 ± 0.11 $g.L^{-1}$). According to Xiu et al (2017) nitrogen can be a restriction factor of microalgae growth. No significant difference was observed in IMP3 growth rate between treatments (FIG 1b) whereas that the cultures reached the stationary phase at day 10 (FIG 1). In terms of weight, it was possible to harvest almost the doble of biomass, at day 12, in high treatment (TAB 1). This seems to have occurred due to the increase in cell biomass as shown in FIG 2.

Conclusions

The results of this work demonstrated that both species are able to grow in Phytobloom® culture medium with good performance, particularly in the high concentration (5ml. L^{-1}) that leads to a greater amount of biomass than in control conditions.

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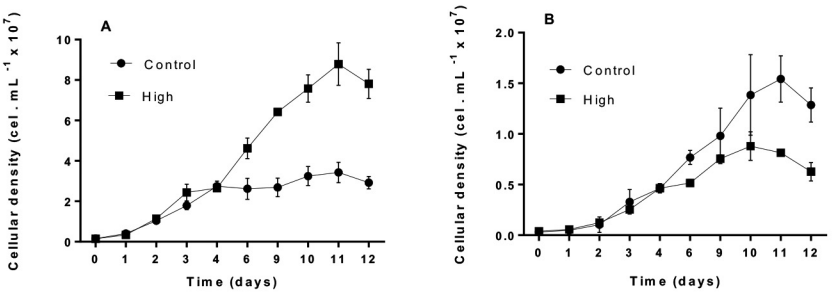

Figure 1. Cellular density of (A) *Phaeodactylum tricornutum* and (B) *Tetraselmis* sp. in different culture medium concentrations.

Table I. Dry weight of *Phaeodactylum tricornutum* and *Tetraselmis* sp. in different culture medium concentrations.

Dry Weight (g . L^{-1})				
	P. tricornutum		**IMP3**	
Day	Control	High	Control	High
3	0.40 ± 0.04	0.39 ± 0.05	0.31 ± 0.04	0.30 ± 0.11
7	0.62 ± 0.06	1.29 ± 0.15	1.15 ± 0.10	1.50 ± 0.09
13	0.97 ± 0.11	2.16 ± 0.25	1.61 ± 0.31	2.97 ± 0.64

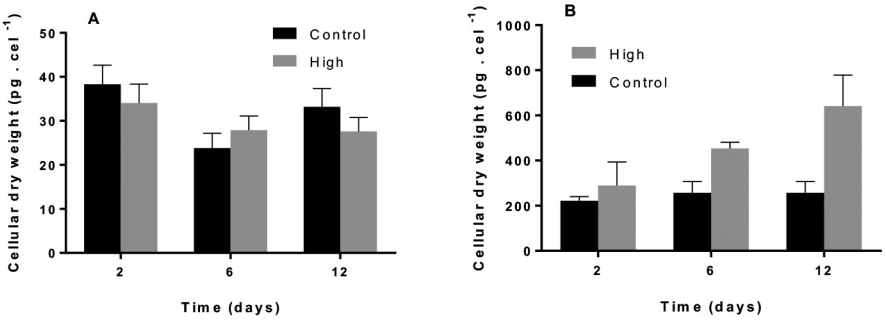

Figure 2. Cellular dry weight of (A) *Phaeodactylum tricornutum* and (B) *Tetraselmis* sp. in different culture medium concentrations.

Acknowledgements

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References

Xie, T., Xia, Y., Zeng, Y., Li, X., Zhang Z. 2007. Nitrate concentration-shift cultivation to enhance protein content of heterotrophic microalga Chlorella vulgaris: over-compensation strategy.Bioresour. Technol. 233, 247-255.

Hu, q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A. I. 2008. Microalgal tracylglycerols as feedstock for biofuel production: perspectives and advances. The plant Journal. 54, 621-639.

Monteiro, I. 2009. Estudo do efeito da salinidade e concentração de ferro no crescimento e conteudo lipidico da estirpe de microalga CTP4. MSc Thesis. Universidade do Algarve.

Schulze, P., Carvalho, C., Pereira, H., Katkam, G., Schüler, L., Santos, T., Varela, J., Barreira, L. 2016. Urban wastewater treatment by Tetraselmis sp. CTP4 (Chlorophyta). Bioresource Technology. 223

DEVELOPMENT OF A COMMERCIAL SCALE LARVAL REARING TECHNIQUE FOR THE NORWAY LOBSTER, *Nephrops norvegicus*

Adam Powell[a,c*], D. M. Cowing[bcd], J.W.S. Scolding[a], T. Shepherd-Waring[a], M.L. Johnson[b], R.J. Shields[a], D. Gowland[d], S.P. Eriksson[c]

[a]Centre for Sustainable Aquatic Research, College of Science, Swansea University, Singleton Park, Swansea, SA2 8PP, UK
[b]Centre for Environmental and Marine Sciences, University of Hull, Filey Road, Scarborough, YO11 3AZ, UK
[c]Department of Biological and Environmental Sciences - Kristineberg, University of Gothenburg, SE-451 78 Fiskebäckskil, Sweden
[d] North Bay Shellfish Ltd, Horries, Deerness, Orkney, KW16 2QL, UK

*Email: adam.powell@stirling.ac.uk

Introduction

Nephrops norvegicus (Norway lobster) represent a significant wild fishery with the majority of the annual 60,000 tonne landings taken from the N.E. Atlantic Ocean across their European range. The high value of the fishery (*ca*. EURO 200 million) has driven an historic research effort which has included reproductive biology and fecundity, larval recruitment and settlement of *N. norvegicus*. This has increased in recent years, particularly with rising awareness of the potential effects of ocean acidification, disease, pollution and fishing practices across the industry (Ungfors et al., 2013; Eriksson et al., 2013; Styf et al., 2013).

Safeguarding lobster fisheries has been a key driver to seek, validate and foster uptake of new technologies, one of which is the release of hatchery reared lobsters for ranching, restoration or remediation. Established European lobster (*Homarus gammarus*) culture has produced scalable hatchery facilities, equipment and know-how, promoting survival of captive Zoea larvae through three pelagic stages. Following metamorphosis to stage IV post-larvae and beyond, many tens of thousands of juveniles are released at appropriate local fishing grounds (Burton, 2003).

Whilst the ecology and reproductive biology of both *Homarus sp*. and *N. norvegicus* is broadly similar, there are key differences that demand modifications to rearing larvae successfully. Powell and Eriksson (2013) found scant accepted best practice regarding *Nephrops* culture techniques or a scalable rearing system. Research and development of Norway lobster larviculture apparently began in the early 1970s and has continued infrequently until the current time, at lab scale, with varying degrees of success. Generally larval survival from hatching to metamorphosis has been limited to approximately 5% (Powell and Eriksson, 2013).

Results and Discussion

A pilot scale hatchery programme was developed considering key aspects of rearing a novel species, such as feeding ration, feed type, rearing vessel hydrodynamics, water temperature and larval density. In particular, the presentation will provide an overview of specific broodstock husbandry and transportation, larval rearing system technical design (modifications to maintain satisfactory larval circulation and filtration) and approaches for juvenile rearing (Cowing et al., 2015; Powell et al., 2017; 2020). The overall results of the iterative hatchery development programme over 3 seasons culminated in *ca*. 30-50% survival from early Zoea 1 stage to metamorphosis, using scalable 70 litre upwelling systems routinely used for *Homarus sp*. hatcheries.

The knowledge developed in the project provides a solid foundation to produce copious larvae and young juvenile *Nephrops* for future commercial and research operations, and transferable techniques for other fragile aquatic invertebrate species that may be similarly investigated, e.g. to safeguard food security, ecological restoration, and the effects of climate change. The hatchery techniques are now freely available to the public in an easily digestible format, as a Hatchery Handbook (Powell et al., 2020).

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References

Burton, C.A., 2003. Lobster hatcheries and stocking programmes: an introductory manual. Sea Fish Industry Authority Aquaculture Development Service. Seafish Report, SR552.

Cowing, D.M., Powell, A., Johnson, M. L. 2015. Evaluation of different concentration doses of eugenol on the behaviour of *Nephrops norvegicus*. Aquaculture. 442, 78-85.

Eriksson S.P., Hernroth B, Baden S.P. Stress Biology and Immunology in Nephrops norvegicus. 2013. Chapter 5, Advances in Marine Biology: The Ecology and Biology of *Nephrops norvegicus*. Johnson M.L and Johnson M.P. (Eds). Pp 149-200.

Powell, A., Eriksson S.P. 2013. Reproduction: Life Cycle, Larvae and Larviculture. Chapter 6, Advances in Marine Biology: The Ecology and Biology of *Nephrops norvegicus*. Johnson M.L and Johnson M.P. (Eds). Pp 201-246.

Powell, A., Cowing, D.M., Eriksson, S.P., Johnson, M.L. 2017. Stress response according to transport protocol in Norway lobster, *Nephrops norvegicus*. Crust. Res. 46, 17-24.

Powell, A., Cowing, D.M., Scolding, J.W.S., Shepherd-Waring, T., Eriksson, S.P., Lupatsch, I., Johnson, M.L., Shields R.J., Gowland D. 2020. *Nephrops norvegicus:* Hatchery Handbook. Deliverable 4.1., *NEPHROPS* project, EU Framework 7 Project no. 286903.

Styf, H.K., Sköld, H.N., Eriksson, S.P. 2013. Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature Ecol Evol. 3, 5055–5065.

Ungfors, A., Bell, E., Johnson, ML., Cowing, D., Dobson, N.C., Bublitz, R., Sandell, J. 2013. Nephrops Fisheries in European Waters, Chapter 7, Advances in Marine Biology: The Ecology and Biology of *Nephrops norvegicus*. Johnson M.L. and Johnson M.P. (Eds). Pp 247-314.

IMPROVING THE FEEDING REGIME AND SUSTAINABILITY OF EARLY LIFE STAGE EUROPEAN LOBSTER, *Homarus gammarus*

Adam Powell[a]*, J. Hinchcliffe[ab], K. Sundell[a], M. Langeland[c], A. Vidakovic[c], I. Undeland[b], N-G. Carlsson[b], S.P. Eriksson[a]

[a] Department of Biological and Environmental Sciences - Kristineberg, University of Gothenburg, SE-451 78 Fiskebäckskil, Sweden
[b]Department of Biology and Biological Engineering - Food and Nutrition Science, Chalmers University of Technology, Gothenburg, Sweden
[c]Department of Animal Nutrition and Management, SLU, Uppsala, Sweden

*Email: adam.powell@stirling.ac.uk

Introduction

One approach to safeguard the European lobster (*Homarus gammarus*) fishery has involved the production and release of hatchery reared juvenile lobsters into local fishing grounds. *Homarus spp*. culture was initiated in the late 19th century and has since developed the technology and know-how to support reliable hatchery operations (Nicosia and Lavalli 1999). Wild gravid broodstock are maintained to hatch pelagic zoea larvae, which are cultured through three pelagic stages using live feeds such as *Artemia salina,* or recently more convenient sterilised plankton feed. Although larvae are highly cannibalistic, *ca*. 20% reach metamorphosis to stage IV post-larvae and beyond (Burton, 2003), with many tens of thousands of benthic juveniles released over appropriate fishing grounds per year.

In the UK alone there are at least 7 hatcheries operating on a partly charitable or grant assisted basis. They aspire to improve the quantity and quality of lobster juveniles in a cost effective and sustainable manner, working closely with local fishermen, the public and other stakeholders. A Swedish initiative (the Nomaculture project) identified *H. gammarus* as a suitable species and culture methodology to develop sustainable marine aquaculture, culminating in two feed development studies for lobster larvae and young juveniles (Powell et al., 2017; Hinchcliffe et al., 2020).

Results and Discussion

For larvae, we initiated four progressive feeding experiments to determine any effect on larval performance (growth, development and survival). Firstly, to improve hatchery feed storage, delivery and feeding regime, we demonstrated that a commercially available "dry" feed pellet did not significantly alter performance compared to a conventional "wet" plankton feed of similar ration and size grade (both within 600–1000 μm). Further experiments found that the same rations of dry feed offered six times per day (every 4 hours, using a feeder) and small-grade dry feed (particles: 250–360 μm) improved performance over larger grade feed offered by hand during "office hours".

Larvae were also fed different proportions of dry feed and/or defrosted larval conspecifics in communal (or individual rearing systems, preventing cannibalism via segregation). Individually reared larvae, fed only conspecifics, displayed significantly higher survival (80%) and rapid development to post-larvae. Unprecedented analysis of *H. gammarus* larval composition identified deficiencies in ash and carbohydrate in typical lobster feeds, underlining the impact of cannibalism on survival and nutrition in *H. gammarus* larviculture (Powell et al., 2017).

For young juveniles, we initiated three feeding experiments to investigate the suitability of feed pellets incorporating by-products, compared to a standard reference feed (fresh shrimp, *Pandalus borealis*), by monitoring performance of stage IV to stage V post-larvae. Screening an array of locally produced, novel protein sources (fishmeal, herring protein isolate alone or supplemented with astaxanthin or glucosamine, and mussel meal) found that a novel type of shrimp meal, produced from waste factory water floc and subsequent freeze drying, promoted best lobster performance of any experimental feed and was comparable to the standard shrimp reference feed (Hinchcliffe et al., 2020).

The results from both larval and juvenile studies demonstrate promising avenues for developing a sustainable and convenient dry feed using local by-products, provided they are specifically formulated and processed appropriately. These results and knowledge transfer have ultimately supported a pilot lobster release programme set to release in excess of 4,500 juveniles off the Swedish west coast, alongside pilot sea-based container culture.

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References

Burton CA. 2003. Lobster hatcheries and stocking programmes: an introductory manual. Sea Fish Industry Authority Aquaculture Development Service. Seafish Report, SR552.

Hinchcliffe J, Powell A, Langeland M, Vidakovic A, Sundell K, Eriksson SP. (2020). Comparative survival and growth performance of European lobster *Homarus gammarus* post-larvae, reared on formulated dry feeds. Aqua. Res. 51 (1) 102-113.

Nicosia F, Lavalli K. 1999. Homarid Lobster Hatcheries: Their History and Role in Research, Management, and Aquaculture. Mar. Fish. Rev. 61(2), 1-57.

Powell A, Hinchcliffe J, Sundell K, Carlsson N-G, Eriksson, SP. 2017. Performance of European lobster larvae, *Homarus gammarus*, reared on dry feed and conspecifics. Aqua. Res. 48 (10) 5300-5310.

SIMPLIFIED METHOD FOR GENETIC SLAUGHTER YIELDS IMPROVEMENT IN COMMON CARP UNDER EUROPEAN POND CONDITIONS

M. Prchal[1]*, J. Zhao[1], D.Gela[1], J. Kašpar[1], P. Lepič[1], V. Kašpar[1] and M. Kocour[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
Email: mprchal@frov.jcu.cz

Introduction

Common carp slaughter yields are usually offered as chilled headless carcass or fillets with skin at the markets and their preference is becoming increasingly important. Therefore, slaughter yields are interesting traits for a breeding program. In our previous studies with market size common carp, we observed high phenotypic and genetic potential of slaughter yield models and predictors based on 3D digitization of morphological landmarks and ultrasound measurements (Prchal et al., 2020). Alternatively, ultrasound predictor defined as ratio of abdominal fillet thickness (E8) to the abdominal depth (E23) also suggested a strong potential for fillet yield improvement on rainbow trout (Haffray et al., 2013; Vandeputte et al., 2019). However, all recent studies on slaughter yields were conducted on market size fish and information about possibilities to select younger and smaller breeding candidates is still missing. In the present study, we investigated genetic potential of simple slaughter yield predictor defined as ratio of E8/E23 or alternatively E8/2D in two-year old Amur mirror carp with comparison to market size fish. Thus, we aimed to i) estimate genetic variation of simple slaughter yield predictors, basic phenotypes and slaughter yields, ii) estimate genetic correlations of simple yield predictors to the real slaughter yields iii) calculate expected genetic gains based on simple yield predictor recorded on two- and three-year old fish and iv) suggest simplified methodology of carp breeding program.

Materials and methods

The experimental stock was established by a partial factorial design of 27 dams and 29 sires of broodstock of Amur mirror carp. The stock was reared communally until market size under semi-intensive pond conditions, and 987 progenies were assigned to their parents using 12 microsatellites. Fish were phenotyped (after second wintering and at market size) for body weight, standard length, muscle fat content, biometrical indicators, Fulton's condition factor and slaughter yields (headless carcass and fillet yield). Likewise, 2D abdominal depth (2D_2) was recorded as a distance between lateral line and ventral part of body at the vertical from base of ventral fins and was studied as a "quickly-to-measure" alternative to ultrasound measurement E23. Two internal measurements (E8 and E23) were collected through ultrasound imagery (SonoScape E2, 10 MHz using curved array 2 – 6 MHz). Slaughter yields were calculated as log-log residuals of the regression with body weight. Heritability estimates and genetic correlations were calculated using an animal model with software DMU with a fixed effect of sex. Genetic gains were calculated using breeder's equation (Falconer and McKay, 1996) for theoretical mass selection (MS), full-sib selection (FSS) and indirect selection (IS) with 10% selection intensity of selected individuals using simple yield predictors.

Results

Heritability estimates of all studies traits were significantly different from zero and achieved moderate to high values in the range of 0.27 – 0.60. The predictors recorded on market size fish showed slightly better heritability (0.41, 0.44), genetic correlations to the slaughter yields (0.78 – 0.86) and expected genetic gains (1.29%– 1.54%) of the slaughter yields. Still, predictors recorded on two-year old fish showed a solid genetic potential for slaughter yields improvement (h^2 = 0.27 – 0.40; r_g = 0.64 – 0.72, expected genetic gain = 0.93% – 1.35%).

Discussion and conclusion

A simple slaughter yield predictor defined as ratio of ultrasound values (alternatively ultrasound value and 2D abdominal depth) has a strong potential for genetic improvement of slaughter yields in common carp. Moreover, such predictor can be efficiently applied either on market size fish or on two-year old fish. It suggests that the indirect selection for improved slaughter yields could be performed using simple and quickly-to-measure yield predictors, also applicable in two-year old fish that are much easier for handling and manipulation.

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In conclusion, our new finding might lead to a significant simplification of yield predictor phenotyping and its use in a sustainable and long-term breeding program of common carp. However, the efficiency of simple slaughter yield predictors needs to be verified by real response to selection in genetically improved generations. Moreover, monitoring of other performance traits (e.g. harvest weight, relative head length, body shape, muscle fat) shall be remembered to see real effect of selection on such traits.

Acknowledgements

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References

Falconer, D.S., MacKay, T.F.C., 1996. Introduction to Quantitative Genetics. fourth ed.Longman Scientific & Technical, Harlow, UK, pp. 464.

Haffray, P., Bugeon, J., Rivard, Q., Quittet, B., Puyo, S., Allamelou, J.M., Vandeputte, M., Dupont-Nivet, M., 2013. Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 410-411, 236-244.

Prchal, M., Kocour, M., Vandeputte, M., Kause, A., Vergnet, A., Zhao, J., Gela, D., Kašpar, V., Genestout, L., Bestin, A., 2020. Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program. Aquaculture, 734993.

Vandeputte, M., Bugeon, J., Bestin, A., Desgranges, A., Allamellou, J.-M., Tyran, A.-S., Allal, F., Dupont-Nivet, M., Haffray, P., 2019. First evidence of realized selection response on fillet yield in rainbow trout *Oncorhynchus mykiss*, using sib selection or based on correlated ultrasound measurements. Front. Genet. 10, 1225

ASSESSING LONG-TERM VIABILITY OF THE MARINE MICROALGA *Tetraselmis chuii* UNDER STORAGE IN HIGH CELL DENSITY ALGINATE MICROSPHERES

A. Prieto*, C. Pérez, C. Fernández-Díaz, V. Anguís, and JP. Cañavate.

Instituto de Investigación y Formación Agraria Pesquera, Centro IFAPA El Toruño
E-mail:anampb22@hotmail.com

Introduction

Tetraselmis chuii (Chlorophyta, Prasinophyceae) is a widely used microalga as a hatchery and nursery food source. It is basically supplied as a single live cell suspension in most instances in order to support nutrition of filter-feeding and suspension-feeding aquaculture organisms. Extending microalgae nutritional applications to other aquaculture species and developmental stages exhibiting different feeding behavior could be accomplished if live microalgal cells are packaged within stable particles with an adequate size. One option to achieve such goal is that of encasing high cell density of live microalgae in alginate microspheres, which is an economic and ready to use procedure. Alginate has been amply used for immobilizing living cells in beads, allowing for maintaining cell metabolic activity under cold and dark storage (*Chen*, 2007). However, information regarding the effects of high microalgae density on cell viability and biochemical stability upon storage is lacking. This kind of knowledge is basic before any further nutritional application can be claimed for microalgae. Thus, the goal of this study was that of assessing how cell density affects survival and physiological stage of *T. chuii* preserved during long term storage in submillimeter alginate microspheres.

Materials and methods

Tetraselmis chuii was grown in 6 l glass round beakers at a salinity of 20 psu, constant irradiance of 200 μM photon m^{-2} s^{-1}, temperature of 20ºC, and continuous aeration enriched with 1% CO_2. A modified f/2 (*Guillard*, 1975) medium was used (1.8 mM nitrate, 0.1 mM phosphate). *T. chuii* was harvested at the end of the exponential phase. Cells were concentrated by centrifugation (4000 rpm, during 10 min at 4ºC). The alginate was prepared in a salinity of 20 psu using the method described by *Moreira et al*. (2006). Two cell densities were tested within the microspheres, $12*10^6$ cells ml^{-1} and $120*10^6$ cell ml^{-1}. The percentage of alginate in the microspheres was 1.2%. Microspheres of around 300 μm in diameter were produced using a Buchi (B-390) encapsulator and hardening in a 0.2 M $CaCl_2$ solution for 30 min. Microspheres were stored in a glass bottle with autoclaved 20 psu seawater at 4ºC and darkness for 6 months. Seawater was removed and replaced every week. Sampling was carried out on days 7, 14, 30, 60, 90,120 and 180. At sampling, some microspheres were dissolved in a 5% solution of sodium polyphosphate at 20 psu salinity in order to disperse the cells to medium and determine their cell viability. Cell viability was determined by using a vital dye (Evans-blue vital dye), chlorophyll *a* variable fluorescence measurement, and cell growth under standard conditions after storage. Variable chlorophyll *a* fluorescence was used to identify the influence of the preservation conditions on cell physiology along the storage and was performed with a pulse amplitude modulated chlorophyll fluorometer (Phyto-PAM II, Heinz Walz). The maximum quantum yield (*Fv*/*Fm*) and non-photochemical quenching (*qNP*) of chlorophyll *a* fluorescence was measured. *Tetraselmis* cells were grown for 7 d and the exponential growth rate was calculated.

Results

In microspheres including $12*10^6$ cells ml^{-1} around 95% of the cells remained alive during the first 60 d of storage. During this time, the fluorescence parameters, *Fv*/*Fm* and *qNP* remained stable at around 0.6 and 0.2, respectively, indicating that photosynthetic performance of *T. chuii* was unaffected by the preservation conditions (4ºC and darkness) for up to two months. Similarly, the average growth rate when stored cells were transferred to fresh medium and standard growth conditions was very similar to that obtained before storage (0.28±0.01 d^{-1}). Prolonged storage from 60 to 180 d resulted in a lower survival (50-60%), a decreased *Fv*/*Fm* (0.45) and an increased *qNP* (0.3). These values reflect some alterations in the photosynthetic apparatus. In denser microspheres including $120*10^6$ cells ml^{-1} the percentage of live cells dropped to 70% and physiological indicators remained unchanged only during the first 30 d of storage. Despite of cell damage, cells recovered and grew at the same growth rate as did before the storage (0.28 ± 0.01 d^{-1}). In these higher cell density microspheres, cell survival decreased to 5% after 30 to 90 d of storage and fluorescence parameters were close to 0. It is thought that higher mortality in denser cell microspheres could be induced by anoxic conditions derived from excessive oxygen consumption in the dark.

Conclusion

High cell density within microspheres significantly affects the preservation of living cells of *T. chuii* inmobilizated in alginate. Nevertheless, using a cell density which is above that normally achieved in phototrophic cultures, allows encapsulating *T. chuii* in alginate microspheres preserving living cells with good physiological conditions for long periods.

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References

Chen Y.C., 2007. Immobilization of twelve benthic diatom species for long-term storage and as feed for post-larval abalone *Haliotis diversicolor*. Aquaculture 263:97-106.

Guillard, R.R.L.,1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.,). Culture of Marine Invertebrate Animals.Plenum Publ. Corp, New York, pp. 29-60.

Moreira S.M., Moreira-Santos M., Guilhermino L., and Ribeiro R., 2006. Immobilization of the marine microalga *Phaeodactylum tricornutum* in alginate for in situ experiments: Bead stability and suitability. Enzyme and Microbial Technology 38: 135-141.

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IMMUNE-RESISTANT CARP CROSS “SURSKIY MALOKOSTNY”

G.I. Pronina

Federal State Budgetary Scientific Institution All-Russian Research Institute of Irrigation Fish Breeding
Noginsk, Dekabristov, 3-b, 49. Moscow region, Russia
e-mail: gidrobiont4@yandex.ru

The industrial fish farming is followed by lower immune status of cultured specimens in connection with high stocking density, handling and other technological factors. Specific immune-modulating substances or selection for higher immune status could be used for solving of this problem. The most dangerous multifactorial infection in commercial carp farms is red spot disease (RSD) caused by *Pseudomonas*, *Aeromonas*, and Spring Viremia of Carp Virus *Rhabdovirus carpio*. The authors had obtained a new commercial mirror hybrid carp “Surskiy Malokostny” (with lower number of bones) characterized by high rates of weight growth, survival and immune resistance. Selection achievement number 8057049. Cross showed high true heterosis in a number of zootechnical indicators. The high immune resistance of new hybrid is connected with characteristics of one of parent lines, Angeline mirror carp exposed to long-term selection after outbreak of RSD more than 60 years ago. The leucogram of new hybrid was characterized by high levels of myeloid segmental cells suggested full development of inborn cell immunity. The lower level of lysosomal cation protein in neutrophils of new hybrid before winter comparing with control full-scaled carp of commercial line is the feature of high immune resistance. In addition, cross carp “Surskiy Malokostny” has a small number of small intermuscular bones. This quality increases its consumer properties.

EVALUATION OF GROWTH, BIOCHEMISTRY, IMMUNOLOGICAL STATUS, AND ACTIVITY OF DIGESTIVE ENZYMES IN TWO MULLET'S SPECIES (*Liza aurata)* AND (*Chelon labrosus*), FEED AT TWO DIFFERENT FEEDING FREQUENCIES

Quirós-Pozo Raquel*[1], Moyano Francisco[2], Bainour Khalida[1], Ramírez-Bolaños Sara[1], Ventura-Castellano Anais,[1] Robaina Lidia[1]

[1] Grupo de Investigación en Acuicultura, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Spain
[2]Universidad de Almería, Spain
Email address: raquel.quiros101@alu.ulpgc.es

Introduction
The optimization of feeding management is one of the major measures to minimize the cost and environmental impacts of aquaculture activity. Additionally, species diversification is an important strategy to promote both aquaculture's economic diversification and sustainable development. The Mugilidae family presents great potential due to its eurythermal, euryhaline, and low-trophic nature. However, there are many similar species, being its differences and cultivation optimal conditions quite unknown, mostly in intensive systems. For those reasons, this study aimed to evaluate two different feeding frequencies (once vs thrice times per day), in two different mullet species, *Chelon labrosus* and *Liza aurata*, to determine, on the one hand, the effects of feeding frequency on growth, use of feed, biochemical parameters, immunological response and activity of digestive enzymes, and, on the other hand, to characterize the main differences for these parameters between both species.

Experimental design
The general experimental design considered the two species under study and two feeding patterns (one or three meals/day). The study was developed through two different experiments aimed to 1) assess differences in digestive biochemistry, as well as to simulate the digestion in vitro for both species and 2) to assess differences in growth when fish were fed following the two feeding frequencies.

Experiment 1.
The experiment aimed to apply an in vitro model that could be used to assess the potential effect of different ration sizes resulting from different feeding patterns, on the hydrolysis of protein and carbohydrate fractions of the feed. A preliminary in vivo assay was performed to obtain both the information required to set operative conditions and the enzyme extracts used to run the assays with the in vitro model. Results were after compared to those obtained with the growth experiment developed with the mullet's juveniles.

Experiment 2.
For each species (*Liza aurata* and *Chelon labrosus*), 36 juveniles were distributed in 6 250 liters cylindrical tanks (tripled by treatment), using a seawater open system, in the facilities of the Technological Science Park Foundation (FCPCT) of Taliarte, Telde. Two treatments based on two different feeding frequencies were tested for each species, using the same type and dose of feeding (1% of biomass).

Results
Experiment 1:
Total activities of intestinal alkaline protease and amylase were higher for *Chelon labrosus* that for *Liza aurata*, irrespective of the feeding pattern or moment of sampling. Regarding the effect of the number of daily meals and sampling moment on the enzyme activities of each species, in *Liza aurata*, the protease activity was significantly decreased with time spent after the first meal, while amylase activity did not show significant variations related to the feeding frequency or sampling moment. This was also the case for protease and amylase activity in *Chelon labrosus*.

Regarding results obtained in the in vitro assays (Fig1), the release of amino acids after hydrolysis was significantly higher in *C. labrosus* than in *L. aurata*. No significant effect related to the feeding pattern was observed in the total release of amino acids for both species. In the case of carbohydrates, no significant effect of the species was observed when tested an E:S ratio adapted to simulate 1 meal, or in the case of *C. labrosus*, when using different E:S.

(Continued on next page)

Fig1. Release of amino acids and reducing sugars after hydrolysis, resulted from the *in vitro* assays.

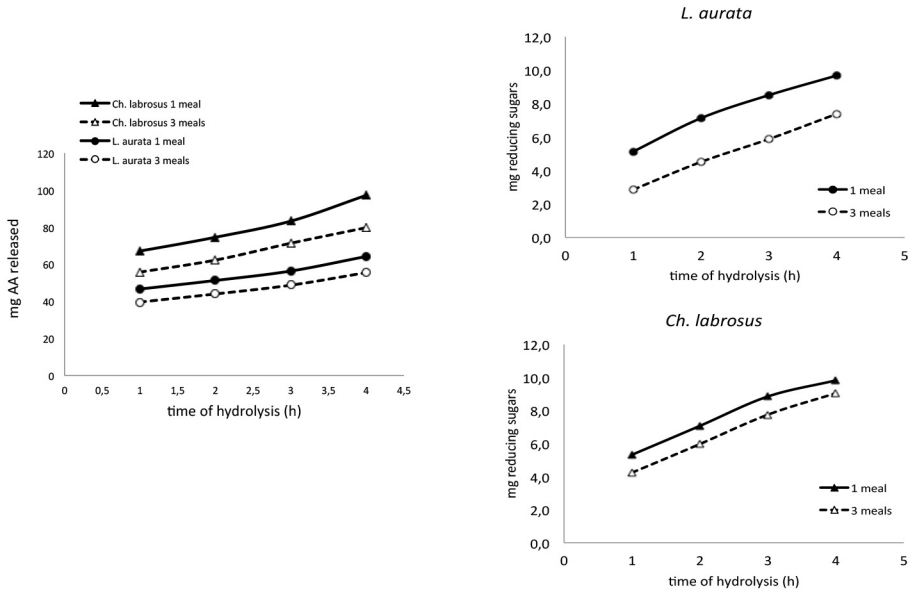

Table I: Effect of species and feeding pattern of growth and feed efficiency obtained in the experiment.

	Species		Feeding pattern			p valuesb		
	L. aurata	C. labrosus	One meal	Three meals	SEMa	S	F	SXF
Initial weight	32.25	32.93	32.03	33.14	0.20	0.12	-	-
Final weight	41.00b	43.51a	40.83B	43.68A	0.53	0.05	0.03	0.05
HSI	0.82b	1.01a	0.93	0.90	0.02	0.00	0.30	0.41
FCR	3.81	3.29	3.79	3.31	0.14	0.11	0.13	0.32
SGR	0.20	0.23	0.20	0.23	0.01	0.095	0.17	0.31

a Standard error of the mean, b Significant (P<0.05) differences among means for “species” are indicated by lowercase letters (a, b), while for “feeding pattern” are indicated by uppercase letters (A, B).

Experiment 2:

Regarding feeding frequency, higher growth was obtained when fish received the daily ration in three meals, although, there were no differences in biochemistry or chronic stress indicators. Between species, significantly higher muscle lipid content and eviscerated weight was measured for *Liza aurata*, while liver lipid deposition and serum bactericidal activity were higher for *Chelon labrosus*.

FIRST REPORT ON *Malaciobacter marinus* AS A POTENTIAL BACTERIAL PATHOGEN FOR PACIFIC OYSTER *Crassostrea gigas*

Faiz Ur Rahman[1 2*], Karl B. Andree[1], Margarita Fernandez-Tejedor[1], Maria J. Figueras[2,] Dolors Furones[1]

1. IRTA, Crtra. Poble Nou, Km 5.5, Sant Carles de la Rapita, 43540, Spain
2. Unit of Microbiology, Department of Basic Health Sciences, Faculty of Medicine and Health Sciences, IISPV, Universitat Rovira i Virgili (URV), Reus, Spain
* E-mail: faiz.urrahman@irta.cat

Introduction

The Pacific cupped oyster (*Crassostrea gigas*) is the 5th largest aquaculture species within the European Union accounting for 6.98% of the total production (EC, 2017). Since 2008 the impact of OsHV-1 has been felt in the European oyster industry. The overall production of oysters has declined (Segarra et al., 2010), while during the same period other bacterial, viral pathogens have become notable (Fleury et al., 2020). Several studies have reported that increase in water temperature changed the oyster microbiome composition and the abundance of *Arcobacter-like* spp., along with known pathogenic *Vibrio* species, increased with higher temperatures in *C. gigas* (Green et al., 2019; King et al., 2019). *Arcobacter-like* spp. have been reported associated with the spoilage of seafood including *C. gigas* (Madigan et al., 2014; Green et al., 2019). They have also been found in the hemolymph of diseased pacific oyster (Lokmer & Wegner, 2015). Although they are reported in several studies and found in abundance during mortality outbreaks in *C. gigas,* their role in pathogenesis and the virulence potential of *Arcobacter*-like spp. towards *C. gigas* are still unknown (Green et al., 2019). The detection of *Arcobacter*-like spp. during several mortality outbreaks and polymicrobial infections highlight the importance to study the role of these bacterial spp. and understand their role in mortality outbreaks in shellfish, specifically in *C. gigas*. The objective of this study was to characterize *Arcobacter*-like spp. from mortality outbreaks in *C. gigas* from the Ebro Delta, Spain. Thus, we isolated and identify potential etiologic agents among those species and evaluated their potential role in causing mortalities in *C. gigas* by experimental challenge.

Material and Methods

A summer mortality episode was reported in cultured adult diploid pacific oysters (*Crassostrea gigas*) in Fangar bay, Ebro Delta, Spain in June 2019. Affected animals (n=30) were collected and mortality was estimated. The oysters were individually sampled for bacterial isolation and tissue DNA extraction. The tissue DNA was screened for OsHV-1 microvar, *V. aestuarianus, V. splendidus* clade and *Arcobacter*-like spp. by qPCR assay (Webb et al., 2007; Saulnier et al., 2017; Salas-Masso et al., 2019).While 6 pooled samples (each pool contained 5 individual oysters) were homogenized with equal amount (w:v) of phosphate buffered saline (PBS). The homogenate was serially diluted (1:10) and aliquots plated on TCBS and marine agar for culture of *Vibrio spp*. *Arcobacter*-like species were isolated by enrichment of the homogenate (1:10) in 2 enrichment broth media supplemented with CAT supplement i.e. Arcobacter broth + 2.5% NaCl (Salas-Masso et al., 2016) and Arcobacter broth + 50% artificial seawater (ASW) (Rahman et al., 2020). Later, 100 μl of the enrichment broth were transferred to marine agar plates overlaid with filter papers of 0.45 μm pore size and 55 mm diameter (Millipore) for exclusion of large-cell species. After 30 minutes, the filter papers were removed, and the plates were incubated. Identification of *Arcobacter*-like isolates was done by *Arcobacter*-like 23S rDNA genus-specific qPCR (Salas-Masso et al., 2019). Genotyping of *Arcobacter*-like isolates was done by ERIC-PCR (Versalovic et al., 1991) and the presence of nine putative virulence genes was studied by the method of Douidah et al., (2012). The rpoB gene was amplified from *Arcobacter*-like isolates for representative exemplars of each ERIC genotype and sequenced for phylogenetic analysis and putative species identification using Neighbor-Joining (NJ) analysis in MEGA6 using a data set of 429bp from the rpoB gene from these collected taxa. As there was a majority of taxa grouping closely in one clade as a single species, a potential etiologic agent was therefore identified for use in a bacterial challenge. A bacterial challenge was carried out with two strains, IRTA-19-131 and IRTA-19-132, by injecting 0.1 ml of 10^8 cfu/ml to the posterior adductor muscle of *C. gigas* (n=20 each). The challenge experiment was carried out for 21 days at 23 ºC. Two co-habitation experiments (Co-hab A [no feeding] and Co-hab B [with phytoplankton feeding]) were carried out for 15 days in the same conditions with IRTA-19-132, by housing one moribund oyster from the previous earlier challenge for 24 hours with oysters. The moribund and dead oysters were processed, and bacteria identified as described above.

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Results and Discussion

The cumulative mortality in the diploid adult Pacific oysters collected in June 2019 was 17.5%. The tissue DNA was negative, by qPCR assays, for the pathogens: OsHV-1, *V. aestuarianus*, and *V. splendidus* clade, while 63.33% (19/30) of the samples were positive for *Arcobacter*-like spp. by qPCR. *Arcobacter*-like spp. were isolated from 50% of the pooled samples in Arcobacter-CAT broth +2.5% NaCl and 100% of pooled samples with Arcobacter-CAT broth +100% ASW. The efficiency of the latter broth medium was significantly higher (p value <0.05) in respect to the number of positive isolates and cfu/ml. These results agreed with our previous finding that Arcobacter broth +50% ASW is an improved enrichment broth for the isolation and enrichment of *Arcobacter*-like spp. from marine and shellfish sources. A total of 37 distinct isolates were recovered by direct culturing and enrichment in the 2 broth media. The genotyping by ERIC-PCR identified 9 genotypes. The rpoB gene sequencing revealed that 5/9 (55.6%) of the genotypes belonged to *M. marinus* species and 4/9 (44.4%) were potential novel *Arcobacter*-like spp. The 5 *M. marinus* genotypes represented 26/37 (70.3%) of total isolates. Only 11.54% of *M. marinus* isolates carried *ciaB*, *cj1349* and *tlyA* virulence genes, while 88.46% of the isolates had none of the virulence genes for which we assayed. Mortality results in the challenge experiment resulted in 10% and 25% with IRTA-19-131 (had no virulent gene) and IRTA-19-132 (had 3 putative virulent genes), respectively. There was no significant difference in mortality of the 2 strains and suggests that, although there is a slight increase in mortality with IRTA-19-132, there could be some other virulence factors involved. The 2 co-habitation experiments conducted with strain IRTA-19-132 resulted in 50% and 90% mortality, with Co-hab A and Co-hab B (feeding) respectively,. The Co-hab B challenge had significantly higher mortality compared to Co-hab A.

Conclusion

The study indicates that a potential new pathogen for *C. gigas*, *M. marinus*, is emerging, which may negatively impact the shellfish industry. Although marine *Arcobacter*-like spp. are not usually screened as pathogens of *C. gigas* in routine shellfish diagnostic labs, our study suggests that *M. marinus,* particularly, should be considered as a potential pathogen for adult *C. gigas*.

WELFARE IN *Danio rerio* EARLY STAGES

Juan Ramos Blasco * [1] , Lluis Tort[1]

Dpt. Cell Biology, Physiology and Immunology. Universitat Autonoma de Barcelona
juanramoblas@gmail.com

Zebrafish has become the most popular fish model as its exponential use in biomedical research since 2000. The small size, easy husbandry, translucent and big embryos, external fecundation and its high reproduction rate related with a short intergenerational time among other characteristics have made of *Danio rerio* an important tool for scientist for development, gene regulation, drug testing, regeneration…Also the European animal research law (2010/63 / EU) has included fish as social regarding about animal welfare grows. The development of indicators for evaluating fish welfare and a set optimal ranges of environmental parameters in order to preserve fish welfare and reability of scientific research.

Husbandry and experimental protocols could affect fish welfare specially during early stages of development but they are not standardized among the laboratories. Many scientific papers show that social and environmental interaction during zebrafish development and gametogenesis could affect normal development and cause transgerational effects in further generations. In order to make a proper standardization husbandry and experimental protocols should be evaluated taking in account water quality parameters, light, noise and vibrations, environmental enrichment and social interaction.

Introduction

As the use of zebrafish increase in the scientific community many protocols for experimental technique and husbandry, feeding reproduction has been developed (1,2). These variations in protocols could affect research results so an effort for a standardization has been done by different association. The implications of these variations is not still completely understood, but many papers have shown the implications of some parameter in a normal development and how they can affect to welfare of the fish and further generations.

Early stages of fish, till their independent feeding are not considered as research animal by European legislation. So little attention in welfare of early stages in zebrafish has been put as is not mandatory by law. In the other hand during early stages and gametogenesis fish are very sensitive to environmental conditions trying to cope them and avoid welfare alterations, modifying genetic expression and normal development.

Standard protocols will be a useful tool for avoiding these variations, but have to be implemented considering all the environmental and others factors that could interfere in the normal development. Influence in development and welfare has to be understood comparing different protocols and the influence in normal development.

Methods:

Different protocols for euthanasia, incubation and transport will be compared in order to understand different implications in welfare and stress of early stages of zebrafish.

Early stages of zebrafish have a cutaneous breathing at least till day 14. Common anesthetics used in euthanasia in overdose, block muscular contraction, so death come by affixation previous unconscious of the fish. So specific protocols for these stages are needed in order to euthanatize them properly. Comparing the effectiveness, genetic expression and aversion of different euthanasic protocols as lidocaine with ethanol, tricaine and clove oil aversion, genetic we can select the best protocol for euthanizing zebrafish early stages in a proper manner. (3,4).

After zebrafish eggs are spawned the embryos start to develop. Usually zebrafish egg is spawned in statics tanks where oxygen is low and ammonia is high and interferes in normal development of embryo, but they could be kept in these conditions for several hours. After collecting them they are usually placed in a petri dish but with different medias and density. Standard temperature for incubation of zebrafish is 28.5ºC, so incubators are usually used for incubation zebrafish eggs or embryos but no photoperiod control in the incubator is usually made (5,6).

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Many labs exchange zebrafish strains (transgenic or wild types ones) between them. During transport environmental conditions can not been properly controlled and is as stressful event for fish. Usually zebrafish facilities ship *Danio rerio* embryos as they are cheaper and has no legal restrictions because they are no legally research animals. There are not standard protocols for avoiding o minimizing environmental conditions. Thermal control, water quality, embryo density and photoperiod should be considered as conditions to be controlled because their influence in normal development is well known (7,8,9).

Conclusion:

As welfare is a continuous and measurable issue it has to be considered during all the lifetime in order to preserve it. Parental experience could be transmitted to offspring so could affect to many generations. Specially during the development, environmental conditions can modify genetic expression in a permanent way.

Welfare in early stages of development of fish should be considered as they have further implications in fish. As there is no legal obligation for it protocols have not been developed to preserve welfare and normal development. Many welfare problems in zebrafish adults could be solved during thei development stages.

Bibliography

1. Aleström P, D'Angelo L, Midtlyng PJ, Schorderet DF, Schulte-Merker S, Sohm F, Warner S. Zebrafish: Housing and husbandry recommendations. Lab Anim (2019) doi:10.1177/0023677219869037
2. Reed B, Jennings M. Guidance on the housing and care of Zebrafish Danio rerio. Res Anim Dep Sci Group, RSPCA (2011)
3. Strykowski JL, Schech JM. Effectiveness of recommended euthanasia methods in larval zebrafish (Danio rerio). J Am Assoc Lab Anim Sci (2015)
4. Collymore C, Kate Banks E, Turner P V. Lidocaine hydrochloride compared with MS222 for the euthanasia of zebrafsh (danio rerio). J Am Assoc Lab Anim Sci (2016)
5. Lin LY, Zheng JA, Huang SC, Hung GY, Horng JL. Ammonia exposure impairs lateral-line hair cells and mechanotransduction in zebrafish embryos. Chemosphere (2020) doi:10.1016/j.chemosphere.2020.127170
6. Sfakianakis DG, Leris I, Laggis A, Kentouri M. The effect of rearing temperature on body shape and meristic characters in zebrafish (Danio rerio) juveniles. Environ Biol Fishes (2011) doi:10.1007/s10641-011-9833-z
7. Villamizar N, Vera LM, Foulkes NS, Sánchez-Vázquez FJ. Effect of lighting conditions on zebrafish growth and development. Zebrafish (2014) doi:10.1089/zeb.2013.0926
8. Dhanasiri AKS, Fernandes JMO, Kiron V. Acclimation of zebrafish to transport stress. Zebrafish (2013) doi:10.1089/zeb.2012.0843
9. Barton CL, Baumann DP, Cox JD. "Export and transportation of zebrafish," in The Zebrafish in Biomedical Research: Biology, Husbandry, Diseases, and Research Applications doi:10.1016/B978-0-12-812431-4.00037-3

EVALUATION OF BODY COMPOSITION MODELS OF NILE TILAPIA

A. I. G. Raposo*[1,2], F. Soares[2], L. E. C. Conceição[2], L.M.P. Valente[1,3], T. S. Silva[2]

[1] ICBAS - Abel Salazar Biomedical Sciences Institute - University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto

[2] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal.

[3] CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal

E-mail: andreiaraposo@sparos.pt

Introduction

The proximal body composition analysis is an important measure to evaluate fish condition and understand their nutritional requirements. Usually, this type of analysis is performed using standard methods (AOAC 1995), but it requires animal sacrifice, involves toxic chemicals and can be expensive and time-consuming for fish farmers. The use of indirect methods to estimate body composition can be useful tools to overcome some of these problems. Such methods may also allow farmers to estimate the environmental impacts (e.g., N and P waste) using their own data (e.g., body weight, FCR), without additional costs. Prediction tools based on such models can also be used to reduce feed waste, improve water quality and feed efficiency. The most common models use linear regression analysis to predict the body composition of animals, through the relationship between body weight and body components (e.g., moisture, crude protein, crude lipid, ash) (Dumas et al. 2007)"ISSN":"00448486","abstract":"Current animal growth models rely on accurate quantitative description of body composition and nutrient deposition. The objectives of the study were to: (1. The use of this method implies either an isometric relationship with additive noise (if data is not transformed) or an allometric relationship with multiplicative noise (if data is log-transformed). However, in the specific case of Nile tilapia (*Oreochromis niloticus*), the choice of model type and calibration method to be used is often subjective (e.g., based on visual evaluations, calibration error metrics or simply convenience).

In this work, we have used a large tilapia body composition dataset to compare different models and calibration methods, using objective criteria based on cross-validation to determine the best combination for this species. The usefulness of the resulting optimal models is demonstrated by comparisons against published tilapia body composition models, using an independent validation dataset.

Materials and methods

Nile tilapia whole-body composition and respective whole-body weight data were collected from 142 publications (973 measurements), covering fishes from 0.01 g to 1470 g. Regression analyses were performed for each body component and both isometric and allometric models were tested with different calibration methods. The "model + calibration method" combinations were evaluated with qualitative (diagnostic plots) and quantitative methods (RMSE, MAPE and CRM). Then, all combinations were subjected to different cross-validation methods (leave-one-out cross-validation, repeated k-fold cross-validation with k=10, k=5 and k=2) to objectively evaluate their predictive capacity. After identifying the optimal "model + calibration methods" for tilapia, such models were calibrated using all data. The selected calibrated models were then compared against published tilapia body composition models, using an independent validation dataset.

Results

Results show that model predictions are greatly affected not only by the type of model, but also by the calibration method used (e.g., with the assumptions of the type of noise present). In particular, models calibrated under the assumption of multiplicative noise, seem to have a better prediction capability than those where noise was assumed to be additive (Figure 1), displaying more reasonable and stable predictions for body weights below 100 g. This suggests that performing the regression in "log space" is not only convenient, but advisable, even for isometric models.

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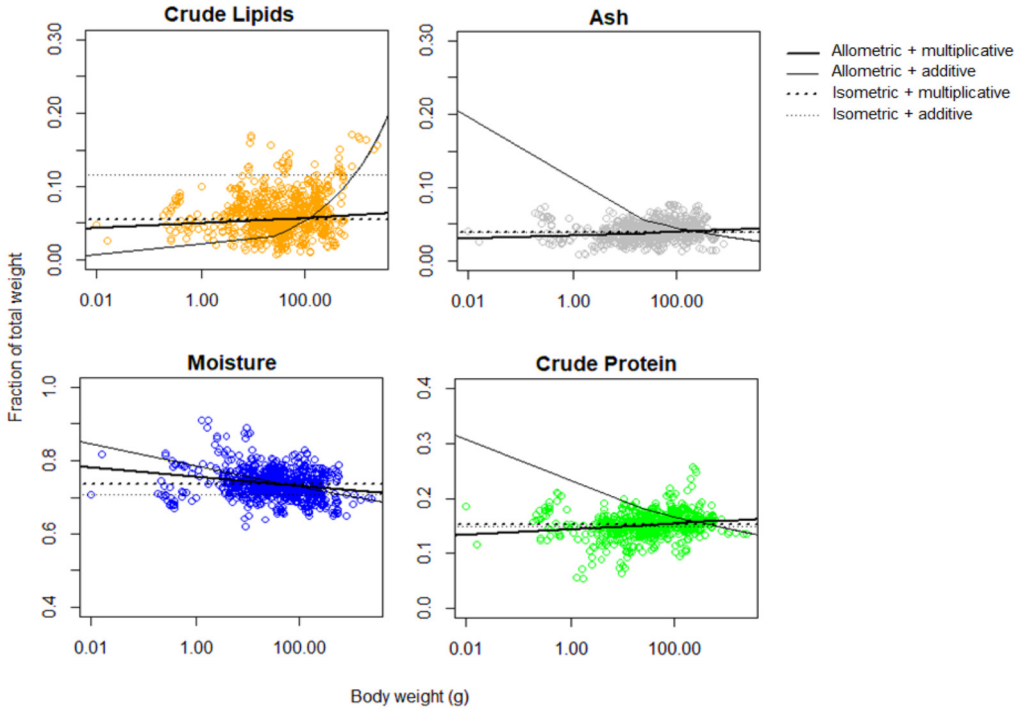

Figure 1 – Scatter plots showing the dependence of each component (in relative terms) as a function of body weight (log scale). Points represent measured data, while lines represent the predictions of isometric and allometric models.

Discussion and Conclusion

This study shows the importance of testing body composition models and their assumptions, in order to ensure high predictive capacity, and suggests that some of the currently used approaches may be suboptimal. Furthermore, it provides optimal body composition models calibrated for tilapia, and predictions are coherent with published data.

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References

Dumas, A., de Lange, C. F. M., France, J., & Bureau, D. P. (2007). Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss)*. *Aquaculture*, *273*(1), 165–181.

COMPARATIVE ANALYSIS OF THE STRESS RESPONSIVE HEPATIC PROTEOME AND GENE EXPRESSION: IDENTIFICATION OF STRESS BIOMARKERS

Cláudia Raposo de Magalhães*, Denise Schrama, Chatsirin Nakharuthai, Surintorn Bunanantanasarn, Dominique Revets, Annette Kuehn, Sebastién Planchon, Marco Cerqueira, Raquel Carrilho, Ana P. Farinha, Pedro M. Rodrigues

Centre of Marine Sciences, CCMAR, Universidade do Algarve, Campus de Gambelas, Edifício 7, 8005-139 Faro, Portugal
csraposo@ualg.pt

Introduction

Managing fish stress in captivity is crucial to ensure a sustainable aquaculture production. The physiological stress response, specifically high plasma levels of cortisol, glucose and lactate are universally used in a research context to assess fish welfare. However, their reliability in cases of long-term stressors has been questioned due to high biological variability and fish adaptation processes. An integrated multi-omics approach can be used as a tool to discover more robust fish welfare biomarkers, since it can offer the possibility of understanding the complete flow of information in the fish biological system from the gene to the metabolite. Our aim is to integrate different omics technologies to achieve a global picture of the fish response to stress. In this work, the liver was analyzed due to its importance in the energy metabolism.

Methods

Sparus aurata was reared under different stressful conditions: overcrowding, repetitive net handling, and hypoxia, using fish reared under optimal conditions for the species as control. By the end of the trial, fish were sampled, and protein extracts were prepared from liver samples. Proteins were separated by 2D-DIGE and identified by MALDI-TOF/TOF MS. Specific proteins were then chosen based on their stress-related function, fold-change and score, and used for primer design. Total RNA was extracted from liver samples using Trizol reagent with DNase I treatment and used for cDNA synthesis. The mRNA levels of the target genes were assessed by RT-qPCR, calculated based on a standard curve from linearized cDNA plasmids, and normalized by endogenous controls. Cortisol, glucose and lactate were measured from blood samples, to assess their consistency as stress indicators, and glycogen stores were assessed in the liver.

Results and conclusions

MS analysis identified in the liver a total of 75 differential abundant proteins between stressed and control fish, from which 20 were implicated in the response to stimulus, based on functional gene ontology annotation and KEGG pathway analysis. Thirteen corresponding genes were chosen for their transcription level analysis. Quantitative gene expression analysis reveals that the transcripts' levels of 2 heat-shock proteins (HSPs) were modulated, corresponding to the expression profile observed in proteomics results. Liver glycogen stores were significantly decreased in stressed fish, suggesting that the glycogenolysis pathway was activated. This joint analysis provides a starter point for the development of more reliable fish welfare assessment measures to improve aquaculture sustainability.

A COLLABORATIVE ECOSYSTEM FOR ATLANTIC AQUACULTURE

E. Ravagnan[1]*, T. Li Chen[2], P. O'Donohoe[3], U. Pérez Sautu[4], P. Cousin[5], S. Dupont[6], M. Stanley[7], L. Poersch[8], M. E. Smith[9], T. Chalde[10], M. Desoche[11], J. Bolton[12], N. Beaume[13], E. Hannisdal[14], N. Loubser[15], P. E. Anyanwu[16], B. Macey[17], O. Denoual[18]

[1] NORCE, Bergen, Norway
[2] AIR Centre, Praia da Vitória, Portugal
[3] Marine Institute, Galway, Ireland
[4] LEITAT, Terrassa, Spain
[5] EGM, Valbonne, France
[6] BIOCEANOR, Valbonne, France
[7] SAMS, Dunbeg Oban, UK
[8] FURG, Rio Grande, Brazil
[9] CSIR, Pretoria, South Africa
[10] CADIC-CONICET, Ushuaia, Argentina
[11] Crowdhelix, Cork, Ireland
[12] UCT, Rondebosch, South Africa
[13] PMBA, Plouzane, France
[14] M&O Partners, Rio De Janeiro, Brazil
[15] Viking Aquaculture, Hermanus, South Africa
[16] NIOMR, Lagos, Nigeria
[17] DEFF, Cape Town, South Africa
[18] TQC,

elra@norceresearch.no

New sustainable and profitable value chain in IMTA systems will be developed in the Atlantic regions.

ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) is a HORIZON 2020 project recently financed under the Blue Growth programme; the project will contribute to the implementation of the Belém Statement to develop a strategic partnership on marine research and it will participate in building the All Atlantic Ocean Community.

The project main goal is to increase value and sustainability for integrated multi-trophic aquaculture (IMTA) production by developing new, resilient, and profitable value chains. In IMTA production, multiple aquatic species from different trophic levels are farmed together. Waste from one species are used as inputs (fertilisers and food) for another species. The IMTA process will be used at four 'labs' in Scotland, South Africa, Brazil and Ireland; these sites will grow species such as fish, scallops, lobsters, oysters, urchins and seaweed. A prospective IMTA lab will also be assessed for future production in Argentina.

ASTRAL goals include the increase of circularity and the achievement of zero-waste aquaculture systems, as well as the creation of appropriated business models to increasing profitability. Potential climate risks and emerging pollutant (microplastics, harmful algae blooms, pathogens) 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.
The ASTRAL consortium assembles a multidisciplinary and multisectoral team of experts as SMEs, industrial clusters, intergovernmental organizations and other relevant stakeholders from several countries along the Atlantic Ocean.

circRNA, miRNA AND mRNA MUSCLE TRANSCRIPTOMES INDICATE THEIR ROLE IN GROWTH REGULATION IN NILE TILAPIA

Rbbani, G.M*., Nedoluzhko, A.V., Siriyappagouder, P., Raeymaekers, J.A.M., Galindo-Villegas, J., and Fernandes, J.M.O

Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway
Email: golam.rbbani@nord.no

Introduction

Nile tilapia (*Oreochromis niloticus*) is a commercial teleost fish species cultured in numerous countries worldwide. Genetic improvement of its production traits could have substantial potential to strengthen the farming of Nile tilapia. More in-depth knowledge about the regulation of key genes involved in muscle growth is essential to understand the observed phenotypic variation between strains and culture conditions. Competing endogenous RNA (ceRNA) is a mechanism of post-transcriptional gene regulation in which the expression of a specific RNA transcript may be reduced due to ceRNA (Salmena et al., 2011). CeRNAs include encoding mRNAs, long non-coding RNAs and genes that crosstalk by competing for shared miRNAs. Circular RNAs (circRNAs) are stable, mostly non-coding RNAs produced during pre-mRNA splicing. They are categorized into three types, namely exonic, intronic and exon-intronic based on their biogenesis. They have an important role in regulating gene expression both at transcriptional and post-transcriptional levels. CircRNAs function as miRNA sponges through binding to the complementary miRNA, thus promoting expression of its target mRNAs. Overexpression of circLMO7 sponges out miR-378a-3p and increases the abundance of HDAC4 mRNA (Wei et al., 2017). In turn, expression of HDAC4 inhibits myoblast differentiation and promotes cell survival in skeletal muscle development (Wei et al., 2016). CircRNAs also regulate host gene expression in the nucleus through interaction with RNA polymerase II and U1 small nuclear ribonucleoprotein at the promoter of their parental genes. It has been shown that the circRNA regulatory network plays a vital role in the growth of vertebrates (Jiang et al., 2020; Ling et al., 2020) but little is known regarding their characteristics and regulatory roles in fish. Hence, we investigated mRNA, miRNA and circRNA expression in muscle to better understand the mechanisms of growth in Nile tilapia.

Materials and methods

Fast muscle samples were collected from adult fish with different growth rate and body size (small: 11-17 cm and big: 27-33 cm standard length) from the same age and genetic background. Total RNA was isolated from each individual using the Direct-zol RNA kit (Zymo Research, California, USA). RNA purity was assessed on a NanoDrop ND-1000 spectrophotometer (Thermofisher Scientific, USA) and its quantity and quality were determined with Qubit (Thermofisher Scientific). RNA integrity was evaluated using TapeStation (Agilent Technologies, USA). The samples with an RNA integrity number (RIN)≥7 were used for library preparation. RNAs were treated with RNAse R (Lucigen, USA) and NEBNext® rRNA Depletion kit (New England Biolabs, Ipswich, MA, USA) before circRNA library preparation. circRNA and mRNA multiplexed libraries were prepared using the NEBNext Ultra™ RNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA). miRNA multiplexed libraries were prepared using NEXTFLEX® Small RNA-Seq Kit (PerkinElmer, Massachusetts, USA). The barcoded libraries were then pooled and loaded at 1.4 pM on the Illumina NextSeq 500 sequencer (Illumina, San Diego, CA, USA) with the NextSeq 500/550 High Output Kit for paired-end (circRNA and mRNA) or single-end (miRNA) sequencing at the Nord University genomics platform (Bodø, Norway).

Results

We generated a minimum of 18 million reads per mRNA, microRNA circRNA library. Raw reads were cleaned and mapped onto the Nile tilapia reference genome. Back spliced junctions were identified from circRNA sequencing data using CIRI2. The number of circRNAs ranged from 440 to 632 between small and big fish group. Most circRNAs were derived from exons and had minimum and a maximum length of 49 and 336,496 base pairs, respectively. A total of 13 miRNAs and 3,143 mRNA transcripts were differentially expressed between big and small fish from miRNA and mRNA sequencing data. Interestingly, oni-miR-202, oni-miR-21, oni-miR-217, oni-miR-34, oni-miR-731, oni-miR-99b were significantly upregulated in small fish. Besides, several growth-associated protein-coding genes, including *igf2bp2*, *tgfbi*, *myod1*, *myf5*, *eftud2*, *adgrg2 ptger3*, *ppp1r3b*, *uchl1*, *zdhhc2*, *mapk15*, and *mfap2*, were differentially expressed with size. The differentially expressed genes were enriched in 953 GO terms. These include genes for glycerophospholipid metabolic process, muscle fiber development, striated muscle cell development and ubiquitin-dependent ERAD pathway. KEGG pathway enrichment analysis revealed that genes differentially expressed with size were associated with spliceosome, protein processing in endoplasmic reticulum, ribosome proteasome.

(Continued on next page)

Conclusion

This study provides the first view of circRNA, miRNA and mRNA co-expression as a possible network with functional implications in teleost fish. Our results suggest that epigenetic regulation by non-coding RNAs plays a role in Nile tilapia growth.

Acknowledgements

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References

Jiang, R., Li, H., Yang, J., Shen, X., Song, C., Yang, Z., Wang, X., Huang, Y., Lan, X., & Lei, C. (2020). circRNA profiling reveals an abundant circFUT10 that promotes adipocyte proliferation and inhibits adipocyte differentiation via sponging let-7. *Molecular Therapy-Nucleic Acids*, *20*, 491-501.

Ling, Y., Zheng, Q., Zhu, L., Xu, L., Sui, M., Zhang, Y., Liu, Y., Fang, F., Chu, M., & Ma, Y. (2020). Trend analysis of the role of circular RNA in goat skeletal muscle development. *BMC Genomics*, *21*(1), 1-12.

Salmena, L., Poliseno, L., Tay, Y., Kats, L., & Pandolfi, P. P. (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell*, *146*(3), 353-358.

Wei, X., Li, H., Yang, J., Hao, D., Dong, D., Huang, Y., Lan, X., Plath, M., Lei, C., & Lin, F. (2017). Circular RNA profiling reveals an abundant circLMO7 that regulates myoblasts differentiation and survival by sponging miR-378a-3p. *Cell death & disease*, *8*(10), e3153-e3153.

Wei, X., Li, H., Zhang, B., Li, C., Dong, D., Lan, X., Huang, Y., Bai, Y., Lin, F., & Zhao, X. (2016). miR-378a-3p promotes differentiation and inhibits proliferation of myoblasts by targeting HDAC4 in skeletal muscle development. *RNA biology*, *13*(12), 1300-1309.

PROFILING MICROBIAL COMMUNITIES FOR HEALTHIER OYSTER HATCHERIES

Tim Regan[1], Michaela Byrom[2], Tim P. Bean[1]

[1]The Roslin Institute, University of Edinburgh, UK
tim.regan@roslin.ed.ac.uk
[2]Guernsey Sea Farms Ltd. Parc Lane, Vale, Guernsey GY3 5EQ

Pacific oyster cultivation (worth £7.5M per annum to the UK) provides high quality produce to a global market and skilled employment in rural areas. Benefits of water clarification and increased local biodiversity associated with these filter-feeders is well documented. The biggest threat to Pacific oyster aquaculture at present is the devastating Ostreid herpesvirus (OsHV-1). Unlike neighbouring countries in Europe, UK waters remain largely free from this pathogen. As such, the majority of UK oyster farmers must source their oysters from disease-free oyster hatcheries in the UK, of which there are currently two.

Hatcheries commonly experience significant sporadic early life mortalities, which are suspected to be associated with microbial contamination. Possible sources of contamination include, for example, the seawater intake (via compromised filtration systems), the adult oysters (simultaneously with gametes) or the algal culture system. To elucidate the perturbations associated with mortality events, we designed a sampling strategy to capture microbial genomic DNA at all stages of the hatchery process: pre- and post- water filtration, the larvae themselves and their environment at various stages of development. By sampling through multiple spawning cycles, we have captured samples from larvae cohorts with and without major early life mortality events. These samples are sequenced using a metabarcoding approach for prokaryotic and eukaryotic microbes with the Nanopore MinION system. Microbial diversity and abundance has been correlated against metadata, comprising local weather conditions, water quality analysis, hatchery water conditions (temperature, algal content, salinity and pH), in addition to the number of larvae present. Selected samples will be processed for whole genome sequencing to describe the core microbiome of the hatchery and to fully characterise interesting microbes.

To date, we have described microbes associated with normal functioning hatchery water systems. By continuing to analyse the microbial components of these samples, we will describe variations in the hatchery system, identify its compromised areas, and provide the first atlas of microbes associated with healthy/unhealthy larval culture.

INFLAMMATORY RESPONSES OF ZEBRAFISH TO SOYBEAN AND EFFICACY OF YEAST BETA-GLUCAN TO MAINTAIN INTESTINAL HOMEOSTASIS

S. Rehman[1] *, A. H. Gora[1], P. Siriyappagouder[1], S. Brugman[2], J. M. O. Fernandes[1], J. Dias[3], V. Kiron [1]

[1]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
[2]Department of Animal Sciences, Wageningen University, Wageningen, the Netherlands
[3]SPAROS Lda, Olhão, Portugal

* Presenting author: S.Rehman
Email: saima.rehman@nord.no

Introduction

Soybean contains anti-nutritional factors that can induce inflammation in the gut of several farmed fish, and the reported alterations include widening of lamina propria, shortening of intestinal villi and reduced feed utilization. To overcome these diet-induced problems, the aquaculture industry can employ anti-inflammatory feed additives such as Beta-glucan. Studying both soybean-induced enteritis and the effect of anti-inflammatory feed additives will provide valuable information to devise strategies to manage inflammation. We employed a transcriptomic approach and histomorphological analysis to present a holistic picture of soybean meal-induced alteration and the combined effect of yeast-derived Beta-glucan and soybean meal on the intestine of a juvenile zebrafish model.

Materials and Methods

Healthy AB zebrafish juveniles, reared in 4 replicate tanks, were fed three experimental diets for 30 days. A reference zebrafish diet (CZ) served as the control while a plant-based diet (CP) that had 50% soybean meal was intended to induce inflammation. Another diet (PM) identical to CP but supplemented with MacroGard® was considered as the inflammation alleviating diet. At the end of the 30-day feeding period, distal intestine samples (n=4) were collected from each treatment group for the transcriptomic analysis and histological analysis.

Results

The intestine transcriptome analyses revealed a total of 101 differentially expressed genes ($|\text{Log}_2$ fold-change$| \geq 1$, q-value < 0.05); based on the comparisons, CP vs CZ and PM vs CP. We detected 29 up-regulated and 44 down-regulated genes in the CP group compared to the CZ group. Gene ontology analysis of the upregulated genes revealed molecular functions like transferase activity, GTP binding and oxidoreductase activity. The intestinal barrier function in the CP group was also affected, as indicated by the modulation of mucin genes and genes linked to junction proteins. The PM vs CP comparison revealed 9 upregulated and 19 downregulated genes in the PM group. Among the upregulated genes in the PM group were neutrophil protease *elastase* 2 (*ela2*), *actin-related protein 2/3 complex subunit 4 like* (*arpc4l*), autophagy-related gene *receptor-interacting serine-threonine kinase 2* (*ripk2*). The most downregulated gene in the PM group was *chemokine C-C motif ligand 36* (*ccl36.1*). Histological analysis revealed a plausible reduction in goblet cell numbers and widening of lamina propria in the CP group compared to the CZ group. The width of lamina propria in the PM group was significantly reduced by 34 % compared to the CP group, which had apparently shorter villi compared to the PM group (47 % reduction).

Conclusion

The intestinal transcriptome of juvenile zebrafish fed soybean-based feed had altered GTP-binding genes, mucin genes and metabolic genes related to antioxidant capacity and lipid metabolism. The yeast Beta-glucan is likely to regulate immune responses and autophagy, and strengthen the mucosal barrier to maintain intestinal homeostasis in zebrafish.

IN VIVO STUDIES OF [1-^{14}C] FATTY ACID METABOLISM IN ARTEMIA (*Artemia* sp.) AND ROTIFER (*Brachionus plicatilis*)

Diana B. Reis[1]*, José A. Pérez[1], Daniel Ramírez[1], Nieves G. Acosta[1], Salvador Jerez[2], Juan C. Navarro[3], Covadonga Rodríguez[1]

[1]Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain.
[2]Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Santa Cruz de Tenerife, Canary Islands, Spain.
[3]Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Castellón, Spain.
*E-mail: dbotelho@ull.edu.es

Introduction

Despite the recent progresses in the development of inert diets, the rearing of early life stages of aquatic organisms still depends on the use of live feeds. Within live feeds, *Artemia* sp. and rotifer (*Brachionus plicatilis*) are widely used in the rearing of marine larvae due to their high availability and acceptance by a large number of species. Nonetheless, both live preys naturally possess low content of long-chain polyunsaturated fatty acids (LC-PUFA) such as 20:5n-3 (EPA), and 22:6n-3 (DHA), which are essential fatty acids (EFA) for proper development of marine fish larvae. In this respect, enrichment of live preys is used to tailor its lipid composition towards the nutritional needs of marine larvae. To improve the design of live prey enrichment protocols, it is advisable to unveil the metabolic fate of fatty acids (FA) during the enrichment process. Therefore, the aim of the present study was to determine the *in vivo* capability of *Artemia* sp. metanauplii and rotifers to incorporate and transform unsaturated FA.

Materials and methods

Artemia sp. (Artemia Cysts EG - INVE AQUACULTURE, Belgium) metanauplii obtained according to Sorgeloos (2001), were incubated in 6-well flat-bottom tissue culture plates (Sarstedt AG & Co., Nümbrecht, Germany) in 10 mL of filtered seawater during 5 hours, at a density of 10,000 metanauplii per incubation well (n=4). On the other hand, rotifers (*B. plicatilis*) strain S1, cultured in the hatchery facilities of the Instituto Español de Oceanografía (IEO) – Centro Oceanográfico de Canarias and fed with baker's yeast (*Saccharomyces cerevisiae*), were incubated in 75 cm^2 tissue culture flasks (Sarstedt AG & Co.) in 50 mL of filtered water (20 ppt), at a density of 75,000 rotifers per incubation flask (n=4). 40 μM of [1-^{14}C]FA including 18:1n-9 (OA), 18:2n-6 (LA), 18:3n-3 (LNA), 20:4n-6 (ARA), 20:5n-3 (EPA) or 22:6n-3 (DHA) were added to individual incubation well/flask. A control group of each prey (n=4) was also maintained under the same experimental conditions without the addition of labelled FA. After incubation, total lipid (TL) extraction was performed as described by Christie (2003). Incorporation of radioactivity into TL (pmol.mg pp^{-1}.h^{-1}) and FA transmethylation and separation using TLC plates were performed as previously described by Reis et al. (2017). Developed TLC plates were placed in closed exposure cassettes (Exposure Cassette-K, BioRad, Madrid, Spain) in contact with a radioactive-sensitive phosphorus screen (Imagen Screen-K, Biorad), scanned with an image acquisition system (Molecular Imager FX, BioRad) and quantified in percentage by "Quantity One" image software.

Table 1 – Incorporation of radioactivity into total lipid (pmol ^{14}C fatty acid mg protein^{-1} h^{-1}) of *Artemia* sp. and rotifers.

	18:1n-9	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Artemia sp.	15.6±2.8^{a}	18.3±2.5^{a}	13.6±3.9^{ab}	19.4±2.6^{a}	13.4±2.7^{ab}	6.8±0.8^{c}
Rotifers	16.6±3.0^{cd}	29.5±2.7^{a}	26.8±3.5^{ab}	25.9±3.7^{ab}	21.3±4.4^{bc}	9.6±3.4^{d}

Results represent means ± SD; n=4. Different letters in superscript within the same row represent significant differences among fatty acids ($p<0.05$).

(Continued on next page)

Results

Most notably, the incorporation of [1-^{14}C]DHA into live preys TL was approximately half to third of the incorporation of all other radiolabeled FA substrates ($p<0.05$).

Most of the radioactivity incorporated into TL of both live preys was recovered as unmodified substrate (over 70%). While [1-^{14}C]OA was the most transformed substrate in rotifers, a higher catabolism of [1-^{14}C]DHA was detected in *Artemia*, with almost 30% of incorporated radioactivity being recovered in shorter chain-length FAs (14, 16 and 18 carbons). Interestingly, this was the only detected metabolic fate of incubated FA in *Artemia* metanauplii, since no elongation or desaturation products were recovered from any of the substrates assayed. By contrast, rotifers presented an active metabolism over incorporated substrates where the synthesis of EPA and DHA from all [1-^{14}C]C18 FA (OA, LA and LNA), and that of ARA from [1-^{14}C]OA and [1-^{14}C]LA was detected.

Discussion

Similarly to that reported by Lubzens et al. (1985), the results of the present study showed the capacity of rotifers to elongate and desaturate dietary FA, being this organism able to biosynthesize LC-PUFA from their C18 FA precursors. Besides, when [1-^{14}C]OA was added to the incubation media, the biosynthesis of not just LA or LNA, showing Δ12 and Δ15 desaturase enzymes activity, but also of ARA, EPA and DHA, indicating the activity of other ϖ3 desaturases (Δ17 and Δ19) over C20 and C22 FA substrates, was detected. These results agree with those of Kabeya et al. (2018), who showed the existence of Δ12 and ϖ3 desaturase enzymes, in the rotifer *Adineta vaga* by functional characterization. This capacity would theoretically favor the accomplishment of a high n-3 LC-PUFA content in rotifers' tissues, and the consequently potential attainment of DHA and EPA requirements of marine fish larvae, even when enriched with vegetable oils (rich in LA and LNA). In contrast, the null capacity of *Artemia* metanauplii to biosynthesize LC-PUFA and its preferent catabolism of DHA (Guinot et al., 2013; Reis et al., 2017), highlights again the reported difficulties for an efficient enrichment protocol of *Artemia* as food for marine organisms.

References

Christie, W.W. (2003). Lipid Analysis, third ed. The Oily Press, Bridgewater, UK, pp. 205-224.

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. (2018). Genes for *de novo* biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. Science Advances 4, eaar6849.

Lubzens, E., Marko, A., Tietz, A. (1985). *De novo* synthesis of fatty acids in the rotifer, *Brachionus plicatilis*. Aquaculture 47, 27-37.

Reis, D.B., Acosta, N.G., Almansa, E., Navarro, J.C., Tocher, D.R., Andrade, J.P., Sykes, A.V., Rodríguez, C. (2017). Comparative study on fatty acid metabolism of early stages of two crustacean species: *Artemia* sp. metanauplii and *Grapsus adscensionis* zoeae, as live prey for marine animals. Comparative Biochemistry and Physiology Part B 204, 53-60.

Sorgeloos, P., Dhert, P., Candreva P. (2001). Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 155, 231-247.

Guinot, D., Monroig, Ó., Hontoria, F., Amat, F., Varó, I., Navarro, J.C. (2013). Enriched on-grown *Artemia* metanauplii actively metabolise highly unsaturated fatty acid-rich phospholipids. Aquaculture 412, 173-178.

PASSIVE ACOUSTIC FEEDERS AS A TOOL TO ASSESS FEED RESPONSE AND GROWTH IN SHRIMP POND PRODUCTION

João Reis*, Alexis Weldon, Samuel Walsh, William Stites, Melanie Rhodes, D Allen Davis

School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University, AL, 36849
Jzt0062@auburn.edu

Shrimp production has been one of the most important sectors of aquaculture for the last few decades for both its market value and acceptance. As production systems move towards increasing intensification, animal nutrition takes a central role as an important growth, environment and health factor. It is well reported that shrimp are opportunistic grazers that eat a wide variety of organisms, yet diets with low or no inclusion of fish meal are often still regarded as of lower quality and a potential cause of less than ideal growth and production. As the majority of shrimp feeding protocols in typical production setups rely in a combination of feed trays and predetermined feed plans, direct assess of shrimp appetite and feed preferences can be complicated. However, for the last decade, development of passive acoustic monitoring has allowed a much more direct measurement of shrimp feed intake by capture and integration of clicking sounds produced by shrimp while eating. Tying this to a automated feeding systems has allowed the development of on demand feeding for shrimp. Hence, this technology is a potential tool to help understand feed preferences when the feeding protocol is based on real time demand for feed rather than predetermined quantities. Building on previous research, the goal of this trial was to use passive feedback acoustic feeders as a tool to evaluate if shrimp prefer commercial diets with different protein sources when given the option to eat as much as requested. This 13-wk trial was performed in 16, 0.1 ha outdoors ponds, stocked at 30 shrimp/m^2 and equipped with the AQ1 acoustic feeding system. All ponds were fed the same predetermined protocol during the first month after which acoustic systems were initiated and four treatments were assigned with a 35% crude protein commercial diet with different protein sources: all-plant, 8% poultry meal (PM), 8% fish meal (FM) and 12% FM. Results for this trial are summarized in Table 1. We did not find any differences in statistical differences in any of the main production parameters. Results of this study indicate that shrimp did not clearly favor a particular diet. Hence suggesting that when well balanced commercial feeds and feed quantity are not a limiting factor, shrimp growth was not highly impacted.

Table 1 - Pacific White Shrimp response to four commercial diets with varying protein sources

Treatment	g/week	Final mean weight (g)	Feed Input (Kg/ha)	Percent Survival	Yield (kg/ha)	FCR
All Plant	1.64	21.02	7898	91.66	5355	1.54
8% PM	1.67	21.54	8084	88.03	5725	1.48
8% FM[1]	1.72	22.59	7596	92.95	6276	1.21
12% FM	1.64	21.49	7631	80.92	5227	1.50
P-value	0.770	0.7604	0.492	0.798	0.607	0.608
PSE[2]	0.06	0.73	17.46	8.13	540.5	0.14

[1]n=3
[2]PSE: Pooled Standard Error

MACROALGAE IN EUROPE: A QUANTITATIVE AND QUALITATIVE ANALYSIS OF TRENDS AND FUTURE OPPORTUNITIES

Luís Resende[1], Cláudia Moreira[1,2], A. Carla Garcia[1], A. Cristina Rocha[1,2]

[1] University of Coimbra, Marine and Environmental Sciences Centre (MARE-UC), Incubadora de Empresas da Figueira da Foz, Parque Industrial e Empresarial da Figueira da Foz (Laboratório MAREFOZ), Rua das Acácias Lote 40A, 3090-380 Figueira da Foz, Portugal
[2] University of Coimbra, Faculty of Sciences and Technology, Department of Life Sciences, 3000-456 Coimbra, Portugal
Email: luis.resende@uc.pt

Introduction

Macroalgae are an important resource around the world, being currently applied in many human activities such as food production, phytopharmaceuticals and soil fertilization (Buschmanna et al 2017). In 2015, the total amount of seaweed produced in the world was 30.4 million tonnes (29.4 million cultivated and 1.1 million harvested), with most of the production being directed for the food industry (Ferdouse *et al* 2018). Global production is mainly dominated by Asian countries, such as China and Japan, which are also the main consumers of macroalgae (Ferdouse *et al* 2018). On the other hand, in Europe, the cultivation of seaweeds is still very limited considering the production rates of other regions, showing however a significant contribution in wild harvesting (Camia *et al* 2018). Nevertheless, the interest and the need for seaweed as raw material, especially for hydrocolloid industry, have been growing in Europe over the last decades. Thus, macroalgae production and application is currently an evolving economic sector (Ferdouse *et al* 2018). Aiming at identifying significant producers and consumers of this resource and the major trends over the last twenty years and foreseeing futures opportunities in this emerging sector, a thorough survey of the literature and information available regarding macroalgae production, industry, distribution and research, in European context, was performed and is presented herein.

Material and methods

Information on research and development projects and companies related to macroalgae production, application or distribution funded or operating, respectively, from 2000 to end of 2019 in Europe, was collected from several databases from governmental and private entities. Specifically, searches were conducted in: i) funding programs at European and national level as European Commission – Cordis Europe (EU) research Results database, Interreg Europe database, European Regional Development Fund (ERDF); ii) national research entities as National Research Agency (ANR, France)), Foundation for Science and Technology (FCT, Portugal); iii) research institutions databases iv) EMODnet human activities database; v) Netalgae database; vi) companies' websites. The keywords used were a combination of the base words macroalgae (macro-algae) and seaweed with terms like: production, cultivation, biomass production, projects, company(ies), research & development (r&d), investments, financed projects, IMTA projects, delimited by "in Europe or identical denominations (EU, European Union and European). The collected information strictly derived from open access data; therefore, restricted information was not included. Data was compiled, compared and analysed. A diverse number of variables, such as, production focus (harvesting, aquaculture or IMTA), overall investment from financial institutions, derivative products and spatial distribution of the activities, were taken into account.

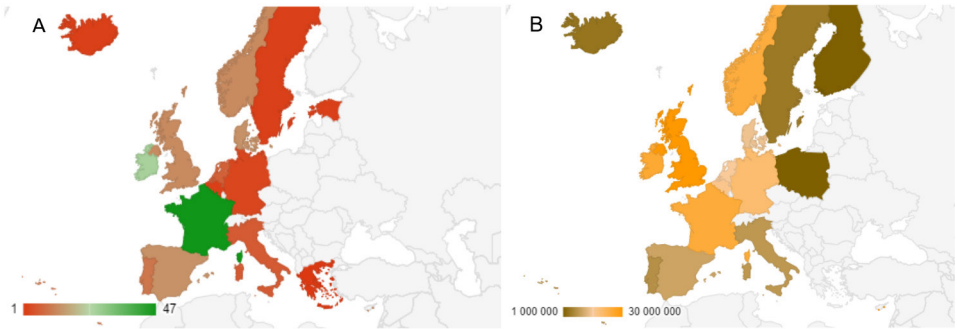

Figure 1 A & B. Fig 1A Representation of the number of companies per European country; Fig 1B Representation of funds distribution per European country in Euros (€)

(Continued on next page)

Results and Discussion

One hundred and sixty-two companies were found within a total of fifteen European countries and which presented one or more economic activities related to macroalgae aquaculture, harvesting, transformation, importation, consultancy and research. France, Ireland and Denmark contribute to more than half the total value of companies. On the contrary, Belgium, Estonia, Iceland, Sweden and Greece show the lowest number of companies handling macroalgae (Fig1.A). Specifically, 61% of the companies exploit macroalgae as their main activity, through aquaculture (37%) and wild harvesting (75%) but only 33% of them supply the market with raw biomass (20% of total companies). 50% of the companies dedicate their activity to macroalgae transformation in refined products such pharmaceutical & cosmetics, fertilizers and food (31% and 17%, respectively) and food (57%).

With regard to research and development, 154 projects were funded in Europe since 2000 to 2019, increasing significantly in the last decade. The main aims of research have been related to biotechnology development, fundamental scientific knowledge and food and feed development. The United Kingdom, Ireland and France presented higher values of funds (Fig.1.B) and that, generally, led more projects than other European countries. From the data collected, since 2000, the total investment in research has been around 206 Million Euros, the funding being mainly supported by European Union, followed by domestic and private funding.

Overall, the funding of research and the establishment of companies related to macroalgae have been augmenting, especially, over the last ten years. This progression reflects the increasing interest in macroalgae products and applications. On the other hand, European macroalgae production seemed not to have followed this trend, since the macroalgae biomass output is still constrained (Ferdouse *et al* 2018). This throughout analysis of the gathered data will be completed and correlations among data will be searched and presented with the purpose of disclosing the major trends and futures opportunities within macroalgae production and transformation in Europe.

References

H. Buschmanna, C. Camusa, J. Infanteb, A Neoric, Á. Israele, M. Gonzáleza, S. Pereda, J. Pinchettic, A Golberg, N Shalev and A Critchley, 2017. Seaweed production: overview of the global state of exploitation, farming and emerging research activity. European journal of phycology, vol 52, no 4, 391-406

F. Ferdouse, S. Lovstad Holdt, R. Smith, P. Murua, and Z. Yang (2018). The global status of seaweed production, trade and utilization. Volume 124. Rome, Italy: Food and

Agriculture Organization of the United Nations FAO.

A. Camia, N. Robert, R. Jonsson, R Pilli, S. Garcia-Condado, R López-Lozano, M. van der Velde, T Ronzon, P. Gurría, R M'Barek, S Tamosiunas, G Fiore, R Araujo, N Hoepffner, L Marelli, J Giuntoli , 2018. Biomass production, supply, uses and flows in the European Union. First results from an integrated assessment. JRC Science for Policy Report. EUR 28993.

DIET INFLUENCE ON *Paracentrotus lividus'* SOMATIC AND GONADAL GROWTH

M. Ressurreição, A. Bóia, T. Verdelhos

Affiliation: University of Coimbra, Marine and Environmental Sciences Center, Department of Life Sciences, Calçada Martins de Freitas, 3000-456 Coimbra, Portugal
Email: martaressurreicao@gmail.com

Introduction:
The edible sea urchin *Paracentrotus lividus* is the most commercially exploited echinoid in Europe (Baião et al., 2019). Considered a gourmet delicacy and has been commercially harvested in many areas with a substantial detrimental impact on the local populations and consequently on the ecosystem (Cirminna et al., 2020). Another threat *P. lividus* is facing is the global rise in water temperature (Shpigel et al., 2018). Aquaculture is recognized as a possible solution to mitigate harvesting pressure on wild sea urchins (Cirminna et al., 2020). Most research related to sea urchin's aquaculture has been developed in two parallel lines: one focusing in the larval and juvenile breeding for restocking and full cycle aquaculture production; and another targeting the gonad enhancement of stocks collected from nature (McBride, 2004). In practice, the echinoculture is still largely dependent of growing out wild-caught sea urchins fed with a variety of diets for gonad enhancement (Phillips et al., 2010). For this it is necessary of a diet that maximize sea urchin somatic growth reducing time to market and lead to high gonad quality and yields. The available results concerning the best dietary protein and energy levels able to simultaneously ensure somatic growth and promote gonad enhancement in *P. lividus* are often contradictory, mainly due to the limited range of dietary protein levels and variable energy sources tested in each study.

The present study assessed the effect of different diets (high lipid content *vs* high protein content) on *P. lividus*, evaluating somatic and gonad development on two different size classes, on an experimental recirculating aquaculture system.

Methods:
The experimental design consisted of one recirculating aquaculture systems (RAS) with 6 tanks (each divided in two separate compartments), equipped with aeration, mechanical and biological filtration (e.g. sponge filters; bio-balls), an air-cooled water chiller, and a water pump.

Sea urchins and macroalgaes were collected at Buarcos beach (Figueira da Foz, Portugal), transported to the laboratory, processed (cleansed with distilled water, litter and epiphytes were removed, and biometrics were determined – weight (g) and diameter (mm)), and placed in the system for acclimatization during one month at 21ºC; 37 salinity, 8 pH and 12D:12L photoperiod.

After acclimatization, sea urchins were measured, separated in different size classes (20 to 30 mm; 31 to 45 mm) and distributed by the experimental tanks (individuals of each size class were placed on each tank compartment). Two different diets were tested – Diet A, with high lipid contend diet (3 tanks); Diet B, with high protein diet (3 tanks), during 3 months.

In order to monitor and ensure water quality during the assay, temperature, pH, dissolved oxygen and salinity were daily measured using a multiparameter probe, and water samples were collected weekly for the determination of ammonia, nitrite and phosphate concentrations, by autoanalyser method.

P. lividus performance during the assay and response to the different treatments was assessed determining survival rates, growth – measuring diameter (mm) and weight (g) variations, and estimating gonad weight and gonadal index. Moreover, lipid, proteins and carotenoids content was analysed on the assay organisms and on the used diets.

Results:
At this stage, only preliminary results were analysed (corresponding to the 1st month of the trial). Total results of the 3 month assay will be analysed subsequently and presented on the congress.

During acclimation, weight reduction was observed in organisms of both size classes (Table 1), while during the first month they recovered part of the lost weight on both diet treatments (Table 2).

(Continued on next page)

Table 1 – Sea urchins' biometrics before and after acclimatization.

Size Class	Before acclimatization Weight (g)	Diameter (mm)	After acclimatization Weight (g)	Diameter (mm)
20 – 30 mm	7,64 ± 0,05	25,48 ± 0,06	5,76 ± 0.21	25,47 ± 0,17
31 – 45 mm	20,79 ± 0,11	36,78 ± 0,09	18,89 ± 0,14	36,78 ± 0,13

Table 2 – Preliminary results after the 1st month of the assay.

	Diet A 20 – 30 mm	31 – 45 mm	Diet B 20 – 30 mm	31 – 45 mm
Diameter (mm)	25,48	36,77	25,48	36,77
Weight (g)	5,96	19,01	5,96	18,99
SGR (%)	0,11	0,02	0,11	0,01
DGR (%)	6,98	3,91	6,87	3,80
GI	3,33	4,8	3,51	4,96

Smaller individuals (size class 20 – 30 mm) presented higher growth rates on both treatments, with SGR = 0,11 % and DGR almost reaching 7 %, while larger individuals (size class 31 – 45 mm) showed significant lower values. On the other hand, the obtained results indicate higher gonad development on individuals of the size class 31 – 45 mm, showing higher GI values, almost reaching 5, while smaller individuals exhibit GI between 3,3 and 3,5.

The organisms submitted to Diet A showed higher growth rates when compared to organisms submitted to Diet B. However, GI values were higher on Diet B treatment.

Discussion:

At this stage, we consider that is premature to draw conclusions from preliminary results, being necessary to analyse the complete test results in order to perform a thorough discussion and draw robust conclusions. Nevertheless, some important indications were observed: i) as expected, the acclimatization period promotes a general weight loss, as a result of the required adaptation of individuals changing from natural field to laboratorial conditions; ii) smaller individuals seem to grow faster than larger ones, following the typical growth pattern of natural populations with higher growth rates during the early stages of the life cycle; iii) gonads are more developed on larger individuals, as expected as well; iv) the different tested diets led to different results – Diet A, richer in lipid content, promoted higher somatic growth; while Diet B, richer in protein content, seem to promote higher gonad development.

References:

Baião, L., rocha, F., costa, M., Sá, T., Oliveira, A., Maia, M., Fonseca. A., Pintado, M., Valente, L. (2019) Effect of protein and lipid levels in diets for adult sea urchin Paracentrotus lividus (Lamarck, 1816), Aquaculture 506 127-138

Cirminna, L., Signa, G., Vaccaro, A., Messina, C., Mazzola, A., Vizzini, S., (2019) Formulation of a new sustainable feed from food industry discards for rearing the purple sea urchin Paracentrotus lividus, Aquaculture Nutrition 00, 1-12

Santos, P., Ferreira, S., Albano, P., Raposo, A., Costa, J., Pombo, A. (2019) Can artificial diets be a feasible alternative for the gonadal growth and maturation of the sea urchin Paracentrotus lividus (Lamarck, 1816)? J World Aquacult Soc. 1–25.

Shpigel, M., Shauli, L., Odintsov, V., Ben-Ezra, D., Neori, A., Guttman, L., (2018) The sea urchin, Paracentrotus Lividus, in an Integrated Multi-Trophic Aquaculture (IMTA) system with fish (Sparus Aurata) and seaweed (Ulva lactuca):

EFFECT OF DIETARY β-GLUCAN ON GROWTH, SURVIVAL, BLOOD STATUS AND SKINMUCUS MICROBIOME OF THE SEA TROUT (*Salmo trutta* L.)

O. Revina[1,2]*, D. Cīrule[1] and A. Valdovska[2]

[1] Institute of Food Safety, Animal Health and Environment „BIOR"
[2] Latvia University of Life Sciences and Technologies
[1]Lejupes street 3, Rīga, LV-1076, Latvia
[2]Liela street 2, Jelgava, LV-3004, Latvia
E-mail: olga.revina@bior.lv

Introduction

The sea trout (*Salmo trutta* L.) is a historically significant fish species for Latvia and for decades has been artificially reared to improve the natural salmonids sources (Purvina *et al*., 2019).

The goal of this study was to define the effects of dietary supplementation with βglucan products - a commercial product (Angel Yeast, China) of purified βglucan and a product (BGN-2) derived from yeast by enzymatic hydrolysis produced by JP Biotechnology (Latvia) on growth performance, survival, blood status and changes of the skinmucus microbiome and its antimicrobial resistance of the sea trout.

Materials and methods

Investigations occured at the state fish farm "Tome", hatchery "Pelci" of the Institute of Food Safety, Animal Health and Environment „BIOR" (56°55'16.3" N 21°58'28.6" E) in flow-through rearing system.

The experimental diets were prepared as follows: basal diet (D0), basal diet + 1 g kg^{-1} β glucan (D1), basal diet + 3 g kg^{-1} β glucan (D2), basal diet + 6 g kg^{-1} BGN2 (D3), basal diet + 14 g kg^{-1} BGN2 (D4). Feed was administered approximately 2% of body weight per day. Each group (n=3000) was placed into an identical 1.2 m3 tank.

For blood sampling was done once a month, during fivemonth period (n=5 individuals each group). Blood smears were stained with MGG Quick Stain. Leukocytes were classified as: lymphocytes, neutrophils, monocytes, eosinophils and basophils. Haematocrit was determined by the standard microhaematocrit method.

Table 1
Growth performance by diets

	D0	D1	D2	D3	D4
Initial weight (g)	2.50±0.21^{a}	2.59±0.17^{a}	2.67±0.21^{a}	2.67±0.17^{a}	2.75±0.16^{a}
Final weight (g)	14.42±0.63^{c}	15.83±0.65^{c}	18.40±0.67^{bc}	24.38±1.26^{ab}	26.60±1.45^{a}
Total final length (cm)	11.76±0.34^{a}	11.91±0.17^{a}	12.90±0.18^{ab}	13.86±0.27^{b}	14.08±0.17^{b}
Feed conversion ratio	2.35	2.11	1.78	1.29	1.17
Survival rate (%)	92.33	94.43	95.80	97.87	98.40

Mean ± SE / Mean; Means with different superscript letters in a row are significantly different (P<0.05),
according to Duncan's multiple range test.

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TThe material for bacteriological investigations was mucus samples from the skin and gills (n=5 individuals each group). Samples were cultured on a double set of plates with a culture medium consisting of Tryptone soya agar (TSA), Blood agar (BA) and MacConkey agar (MCA). Incubation was in aerobic conditions: one set at 22 °C and the other set at 37 °C for 24 - 48 h. After incubation, the different colonies were sub cultured onto TSA, MCA and BA, until the pure culture was obtained, further the identification and antimicrobial susceptibility testing to Amoxicillin (25 μg), Ampicillin (10 μg), Cefalexin (30 μg), Doxycycline (30 μg), Enrofloxacin (5 μg), Erythromycin (15 μg), Florfenicol (30 μg), Gentamycin (10 μg), Oxytetracycline (30 μg), Spectinomycin (100 μg), Streptomycin (10 μg), Tetracycline (30 μg) and Trimethoprim (5 μg). Multiple antibiotic resistance (MAR) index was determined by Krumperman, 1983 described procedure: MAR = a / b, where a represents the number of antibiotics to which the isolate was resistant and b is the total number of antibiotics to which the isolate was exposed.

Length and weight of 50 fish from each tank were measured every 30 days for a period of eight months. Mortalities were collected every day during the study: Survival rate (%) = (Final number of fish / Initial number of fish) × 100. Feed Conversion Ratio (FCR) was calculated using the following formulae:

FCR = Feed consumption / Gain in weight (Hopkins, 1992).

All statistical analyses were performed using R (version 3.6.2), R Studio software. Values are represented by means ± standard error (SE). The significance of haematological parameters was determined by the t-test. Significant differences of growth parameters were determined using one-way ANOVA, followed by Duncan's multiple range test. Differences were considered statistically significant when $P < 0.05$.

Results and Discussion

According to our study, dietary β-glucan did not affect leukocyte differential count significantly. There was no statistically significant difference between the groups with supplements compared to the control group. Mean haematocrit level was found to be significantly increased in January in D3 and D4 compared to the other groups– 33.8±5.25 and 33.0±5.00. For all groups the isolates mainly are presented by *Aeromonas sp*. and *Pseudomonas sp*. *Aeromonas sp*. in D2 present 20%, D3 – 20 % and D4 25 %. In D0 *Pseudomonas sp*. present 75%, D1 – 100 %, D2 – 80 %, D3 – 60 % and D4 – 75%. *Janthinobacterium lividum* was isolated in one case from D0 and consisted 25%. Furthermore, in one case was isolated *Yersinia intermedia* from D4 (20%).

All isolates displayed 100% resistance to Ampicillin, Amoxicillin, Erythromycin, Cefalexin and 100% susceptibility to Gentamycin. There was no significant difference in MAR index among diet groups. MAR index was above 0.2 in all isolates. MAR index value higher than 0.2 are high potential for contamination.

Betaglucan does not affect the skin-mucus microbiome of sea trout.

Our research proved the beneficial effects of the β-glucan dietary supplement on the growth performance and survival rate of sea trout, especially product BGN-2 in dosage 14 g kg^{-1}.

References

Hopkins K. 1992 − Reporting Fish Growth: A Review of the Basics − J. World Aquac. Soc. 23: 173-179.

Krumperman P.H. 1983 − Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods − Appl. Environ. Microbiol. 46: 165-170.

Purvina S., Medne R., Bajinskis J., Kondratyeva N. 2019 – Success of long term salmonid resocking in Latvia – Environmental and Experimental Biology. 17: 31-32.

ASSESSMENT OF A "FUTURE PRACTICAL" DIET ON GUT MICROBIOTA OF GENETICALLY SELECTED OR NOT SELECTED STRAIN OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

S. Rimoldi1*, S. Torrecillas2, D. Montero2, A. Serradell2, R. Fontanillas3 and G. Terova1

1 Department of Biotechnology and Life Sciences, University of Insubria, Via J.H.Dunant, 3, 21100 Varese, Italy
2 Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Telde, Las Palmas, Canary Islands, Spain,
3 Skretting Aquaculture Research Centre, Stavanger, Norway

E-mail: simona.rimoldi@uninsubria.it

Introduction

In the last decades, the aquaculture industry has progressively reduced its dependence on traditional marine-origin ingredients, such as fishmeal (FM) and fish oil (FO) in favour of more sustainable alternatives, such as plant proteins and oils, terrestrial animal by-products, single-cell proteins and oils, insects, and microalgae. Enormous research efforts have been devoted to new diets with low levels or devoid of FM and FO (see also results from the ARRAINA project). However, it is still difficult to use high or total replacements of FM and FO with alternative sources in some carnivorous marine fish diets, without having negative effects on fish growth and gut health in particular, in European sea bass. Most of these problems have been overcome by formulating more balanced diets and supplementing them with different functional additives, such as probiotics, prebiotics, organic acids, and phytogenics (Torrecillas et al., 2019).

Besides improving diet formulation, breeding programmes exist for all major carnivorous **fi**sh species to improve growth, feed efficiency, and disease resistance when novel diets are used. However, any modification of the diet needs to be carefully evaluated since diet is one of the main factors shaping the intestinal microbiota, which in turn affects host metabolism, nutrition, growth, immunity, and disease resistance.

The present study aimed to investigate the benefits of using a strain of European sea bass selected for growth in a nutritional challenge with a "future" practical dietary formulation completely devoid of FO and with low percentage of FM. The effects on gut microbiome and selected genes related with gut associated lymphoid tissue (GALT) functionality were studied.

Materials and methods

The feeding trial was set at at Parque Científico-Tecnológico Marino of the University of Las Palmas de Gran Canaria (Telde), Canary Island, Spain.

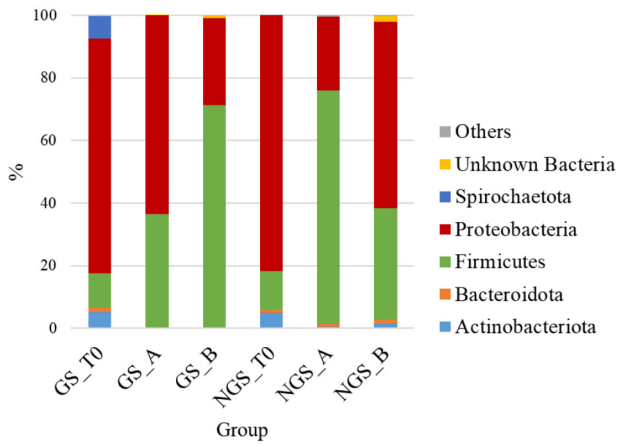

Fig 1. Relative abundance (%) of the most prevalent bacteria phyla in gut mucosa.

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Two *D. labrax* strains of 15 ± 0.5 g mean initial weight, one genetically selected for growth (GS), and the other one not selected (NGS) were fed with two experimental diets: a control diet (A) mimicking a commercial diet based on low fish meal (FM) and fish oil (FO), and a "future" diet (B) completely devoid of FO and with low percentage of FM (10%). Fish were fed for nine months in triplicate (3 tanks/diet/genotype). At the beginning (T0) and at the end of feeding trial, two fish per tank were sampled (6 fish/diet/genotype) and the entire intestine was aseptically removed and used for microbiota and gene expression analysis. Three aliquots from each feed were sampled for microbiota analysis, too. Bacterial DNA for microbiota analysis was extracted from three aliquots per each feed and from six samples of gut mucosa per each dietary group for a total of 42 samples. Then, the hypervariable region V4 of 16S rRNA gene was amplified. The 16S amplicon libraries preparation and sequencing were performed by the GalSeq srl company (Italy), applying the 16S Metagenomic Sequencing Library Preparation for Illumina MiSeq System (#15044223 rev. B) and using an Illumina MiSeq platform (PE 2 x 250), as described in Terova et al., (2021), and Rimoldi et al., (2020). The RT-qPCR was carried out using iTaq Universal SYBR® Green Supermix in a CFX96 real-time PCR instrument (Bio-Rad, Milan, Italy). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Results and Discussion

In terms of gene expression, responsiveness to the feeding trial was more relevant in distal portion of intestine. A significant interaction effect between diet and genotype was found in the expression of *cd4, il-1β, il-10, mhc ii,* and *tnf-α* genes in distal intestine. Specifically, pro-inflammatory cytokine *il-1β* was up regulated in distal intestine of NGS_A and NGS_B fish groups, whereas *tnf-α* was induced only in GS_B. *Il-10*, a potent anti-inflammatory cytokine, was more expressed in GS fish fed diet A. The induction of pro-inflammatory cytokines in response to experimental diets A and B was coherent with the reduced biodiversity and species richness found in the gut microbiome of these fish. The biodiversity reduction was more evident in GS than in NGS samples. As expected, Firmicutes and Proteobacteria were the most abundant phyla in the intestinal mucosa of sea bass (Fig. 1). At phylum level, statistical analysis showed significant differences ($p<0.05$) in the relative abundance of Firmicutes and Proteobacteria. Firmicutes phylum, mainly represented by Clostridia class, was enriched in GS_B and NGS_A fish as compared to T0. At genus level, *Lactobacillus* and *Streptococcus* were practically absent in GS fish fed experimental diets A and B, whereas they were detectable only in NGS sea bass regardless of the diet. These genera are lactic acid bacteria generally considered as beneficial; therefore, their reduction suggests a negative effect of the diet in genetically selected sea bass.

Acknowledgements

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References

Terova, G. et al., (2021). Journal of Animal Science and Biotechnology 12:30
Rimoldi, S. et al. (2020). PLoS ONE 15(4): e0231494.
Torrecillas, S. et al (2019) PLoS ONE 14(9): e0222063.

MODELLING SEA LICE DISPERSAL USING A STAGE STRUCTURED MOTILE PARTICLE TRACKING MODEL

Harrison Carmody[1], Mat Roberts[2]*, Michael Barry[1], Meadhbh Moriarty[3], Alexander Murray[3], Glenn Shiell[1], Louise Bruce[1,4]

[1]BMT Commercial Australia Pty Ltd, Level 5, 348 Edward Street, Brisbane QLD 4000, Australia
[2]BMT UK Ltd., 1 Park Road, Teddington, Middlesex, London, UK, TW11 10P
[3]Marine Scotland Science, 375 Victoria Rd, Aberdeen AB11 9DB, UK
[4]The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia
Email: mat.roberts@bmtglobal.com

Introduction

Productivity loss due to lice infestations has a major impact on the salmon aquaculture industry, which contributes £1.8 billion annually to the Scottish economy. Traditional treatment methods are also costly (Abolofia et al. 2017) and can have a detrimental impact on the marine environment (Burridge et al. 2010). With plans in Scotland to double production by 2030 (Burnett 2017), the industry needs innovative solutions for targeted and effective sea lice management.

Supported by the UK Seafood Innovation Fund, BMT are developing integrated modelling techniques to improve understanding of sea lice dispersion, infestation, the impact of chemical discharges on the marine ecosystem and benefits of targeted treatment methods. A fully integrated decision support system (AquaDEEP) incorporating hydrodynamic, particle tracking, water quality and aquatic ecosystem software will help farmers with site selection and operational management practices, reducing the commercial risks associated with expansion plans. At the same time, supporting the Scottish Environment Protection Agency's (SEPA) "beyond compliance" vision of sustainability.

Following a series of workshops with Scottish Sea Farms, Marine Scotland Science, Aquatera and SEPA, a sea lice motile particle tracking module has been developed, incorporating temperature dependent sea lice maturation and motility response to environmental cues. This module, integrated with the bath treatment ecosystem impact module, will enable operators to target effective lice treatment practices, minimise environmental impact while reducing treatment costs and production losses.

Methods

AquaDEEP uses the hydrodynamic model, TUFLOW FV (https://www.tuflow.com/), a powerful flexible mesh engine that solves the non-linear shallow water equations. TUFLOW FV determines the fate and transport of sea lice released from fish pens by simulating currents, light, temperature and salinity. The particle tracking module algorithms were developed to simulate sea lice motility in response to multiple environmental stimuli including light and salinity and include a temperature dependent rate of maturation. The model can simulate up to ten life cycle stages with a common application being two life stages: pre-infective nauplii and postinfective copepodid.

The sea lice motility algorithm was based on observations that sea lice will actively avoid salinities below 27-30 ppt (Brooker et al. 2018, Crosbie et al. 2019) and actively swim to the surface where there is more light available (Salama et al. 2018, Brooker et al. 2018). Parameters for minimum light and salinity to trigger motility response and swim speeds are set for each planktonic stage simulated by the model. Temperature dependent maturation algorithms have been adapted from the degree day equation from Stien et al. (2005) and experimental review tables from Brooker et al. (2018). Three alternative temperature dependent equations can be selected at each stage:

1. Linear $Hr = p1*T + p2$;
2. Exponential $Hr = p1.exp(-p2*T))$; or
3. Degree days $Hr = p1/(T-10+p1*p2)^2$.

Where Hr is the number of hours to maturation calculated at each model time step and used to advance the sea lice stage, p1 and p2 are constants based on best fit to observed data and T is the water temperature in °C. A constant mortality is set for each stage and a maximum stage duration can be applied to the infectious stage to remove particles from the simulation if they have not found a host.

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Results

Preliminary results have tracked sea lice particles through the water column responding as expected to change in environmental variables. Motile particles will move towards the surface layers where more light is available and away from fresher surface layers under stratified conditions. Maturation duration is dependent on alternative "fit" regression equations, for the pre-infective nauplii stage and the post-infective copepodid stages. Comparisons of simulations of inert and motile particle density distributions, highlight how different the particle responses can be when the full range of environmental conditions are modelled.

Discussion and Conclusions

An integrated approach to modelling sea lice using motile particle tracking has been proposed. This approach can be used to study optimal mitigation strategies for preventing sea lice infections; de-risk farm site selection to reduce risk of sea lice infection; and model infestation pressure on wild salmon and the associated risk of re-infestation from wild salmon populations into aquaculture salmon populations. Further developments to this integrated approach such as the diffusion of bath treatment chemicals and ecosystem response will be designed to inform salmon farmers on how to reduce sea lice losses and work towards a more sustainable management of the industry.

References

Abolofia, J., Wilen, J. E. and Asche, F. (2017) 'The cost of lice: Quantifying the impacts of parasitic sea lice on farmed salmon', Marine Resource Economics. MRE Foundation, 32(3), pp. 329–349. doi: 10.1086/691981.

Brooker AJ, Skern-Mauritzen R, Bron JE (2018) Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling, ICES J. Mar. Sci., Volume 75, Issue 4, Pages 1214–1234, https://doi.org/10.1093/icesjms/fsy015.

Burnett, S. (2017) Aquaculture Growth to 2030 A Strategic Plan for farming Scotland's seas.

Burridge, L., Weis, J.S., Cabello, F., Pizarro, J. and Bostick, K. (2010) 'Chemical use in salmon aquaculture: A review of current practices and possible environmental effects', Aquaculture. Elsevier B.V., 306(1–4), pp. 7–23. doi: 10.1016/j.aquaculture.2010.05.020.

Crosbie T, Wright DW, Oppedal F, Johnsen IA, Samsing F, Dempster T (2019) Effects of step salinity gradients on salmon lice larvae behaviour and dispersal. Aquacult Environ Interact 11:181-190. https://doi.org/10.3354/aei00303.

Salama NKG, Dale A, Ivanov VV, Cook PF, Pert CC, Collins CM, Rabe B (2018) Using biological–physical modelling for informing sea lice dispersal in Loch Linnhe, Scotland. J Fish Dis. 41: 901– 919. https://doi.org/10.1111/jfd.12693.

Stien A, Bjørn PA, Heuch PA, Elston DA (2005) Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. Mar Ecol-Prog Ser. 290: 263-275. 10.3354/meps290263.

Acknowledgements

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ONGROWING OF WRECKFISH JUVENILES (*Polyprion americanus*) BORN IN CAPTIVITY IN GALICIA (SPAIN)

J.L. Rodriguez*, B. Méndez, J.J. Sánchez, S. Díaz and F. Linares

Instituto Galego de Formación en Acuicultura (IGaFA). Niño do Corvo s/n 36626 Illa de Arousa, (Pontevedra), España
E-mail: xose.luis.rodriguez.villanueva@xunta.gal

Introduction

Among the new species to be exploited for commercial aquaculture, wreckfish (*Polyprion americanus*) is one of the most interesting ones. Because of its rapid growth, good adaptation to captivity (Machias *et al*.,2003, Papandrolakis *et al*.,2004; Rodriguez *et al*., 2014), late maturation (Sedberry *et al*., 1999), the quality of its flesh and its high commercial value, wreckfish has become one of the target species to be developed for aquaculture during the next years. In this paper, the ongrowing of the first wreckfish fry produced in captivity is presented.

Materials and methods

The experiment was carried in the facilities of the Instituto Galego de Formación en Acuicultura (IGaFA, Illa de Arousa, Pontevedra). It started with 9 individuals with an initial average weight of 427,36± 59g were kept in three rectangular tanks of 2x2x1 m. The individuals were produced in IGaFA from larvae of Instituto Español de Oceanografia (Vigo) and Aquarium Finisterrae (A Coruña). The fishes were fed with specific extruded feed designed by Sparos company seven days a week until are sated. The following culture parameters were daily recorded: oxygen, temperature, and salinity. The individuals were monthly sampled for size and weight- and phenoxyethanol was administered as anesthetic (0.2 ml / l) to be manipulated.

Results

At the end of the 598-day experiment, fish reached a weight of 3.066,5± 485.22g (Fig. 1). These results are slightly lower than those obtained in IGaFA in the growth phase from 1 to 3 kg (Rodriguez *at al*.,2014) with wild fish. The two largest specimens in the experiment reached an average weight of 3.752 g. One of the reasons for this less growth may be due to the fact that all the fish of the experiment are the result of the first wreckfish larval culture that had survival and no selection was made. It is important to highlight the growth slowdown that takes place from December to February, possibly due to the low temperature in the water that fluctuated between 12 degrees in winter and 19 degrees in summer. The average factor condition (FC) was 2.49 which was similar (2,41) to that obtained by Rodriguez *et al*.,2014. Throughout the experiment, one individual died, however no pathological problems were detected.

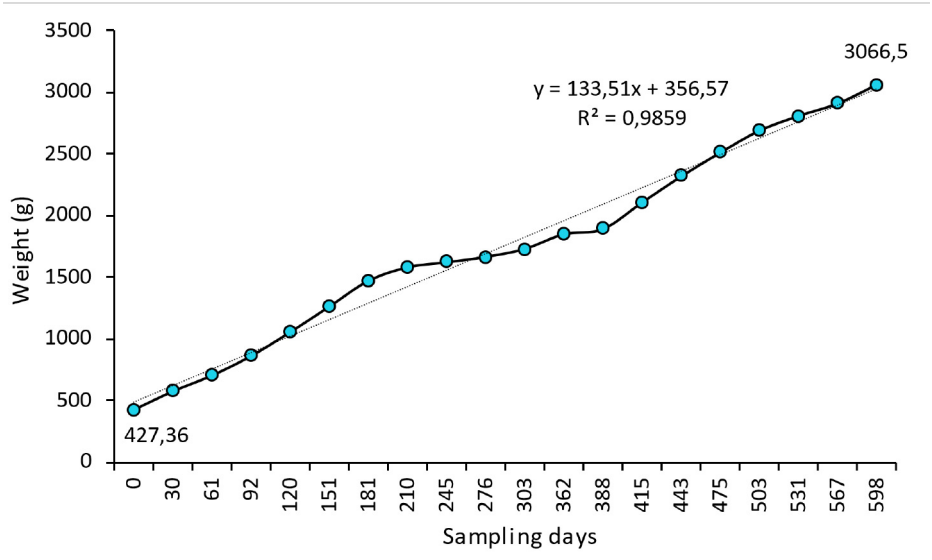

Figure 1. Growth of wreckfish in IGaFA facilities

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Discussion and Conclusion

Growth and adaptation results for the first juveniles wreckfish produced in captivity are very encouraging in terms of culturing viability, although the growth of culture specimens was lower than that obtained from wild specimens. Nevertheless, in order to develop the culture of this species at industrial scale, it is essential to deploy an important research effort to make a progress in aspects related to reproduction, larval culture, nutrition, etc., as very few data are available.

References

Machias A., Somarakis S., Papadroulakis N., Spedicato M.T., Suquet M., Lembo G., Divanach P. 2003. Settlement of the wreckfish (*Polyprion americanus*). *Marine Biology* 142: 45-52.

Papandroulakis N, M. Suquet, M.T. Spedicato, A.Machias, C. Fauvel and P Divanach. 2004. Feeding rates, growth performance and gametogénesis of wreckfish (*Polyprion americanus*) kept in captivity. Aquaculture International 12: 395-407.

Rodriguez J.L., Peleteiro J.B., Linares F. 2014. Growth of the wreckfish *Polyprion americanus* in Galicia (Spain). *Aquaculture Europea* 2014: pp. 1107-1108.

Sedberry G.R., Andrade C.A.P., Carlin J.L., Chapman R.W., Luckhurst B.E., Manooch C.S. III, Menezes G., Thomsen B., Ulrich G.F. 1999. Wreckfish *Polyprion americanus* in the North Atlantic: fisheries, biology and management of a widely distributed and long-live fish. *American Fisheries Society Sympsium*., nº 23, pp.27-50.

PREONGROWING OF TURBOT (*Scophthalmus maximus)* IN A RECIRCULATION SYSTEM

E. Murari*, E. Peláez, E. Pérez and J. L. Rodríguez

Instituto Galego de Formación en Acuicultura (IGaFA). Niño do Corvo s/n 36626, Illa de Arousa, Spain
E-mail: murari.egberto@gmail.com

Introduction

The ongrowing of turbot *Scophthalmus maximus* is normally carried out in open circuit systems. Recirculation systems have been greatly improved in recent years and are being applied in different culture stages and in many fish species. In this work, data on turbot preongrowing in a recirculation system are shown and compared with data from the open circuit literature.

Materials and methods

The experiment was carried out in the Instituto Galego de Formación en Acuicultura (IGaFA). We started off with three batches (A, B, C) with 100 samples each. The samples in batch A had in initial average weight of 6.54±1.83 g and an average size of 7.41±0.71 cm. Batch B had an initial average weight of 6.43±1.61 g and an average length of 7.47±0.63 cm. Batch C had an initial average weight of 6.22±1.69 g and an average length of 7.36±0,66 cm. During the whole study process, individuals were kept in 1.0x1.0x0.4 m flat bottom square tanks in recirculation system. The closed circuit system consists of a mechanical filtration, biological filter, sterilization by ultraviolet lamps and ozone, and a heat pump. Temperature, pH, redox, CO_2 were control with probes, which allowed to know the fluctuation of these parameters throughout the day. The levels of nitrites, ammonium and nitrates levels were recorded twice a week. The feeding consists in specific extruded feed which were supplied four times a day, during the daytime, through automatic feeders. The feeding rate throughout experience was 3%. Samplings of weight and length were done periodically. With the information obtained, we calculated the following growth index: condition factor (CF), specific growth rate (SGR) and feed conversion rate (FCR). Both partial (for each interval of the sampling) and global indexes were calculated. With the mean weight data of the three batches, a growth curve was elaborated which was compared to open circuit data from other research.

Results

At the end of the experience, which lasted 94 days, the three batches reached the similar weight. Batch A reached a weight of 78.98 g, batch B 79.55 g and batch C 88.68 g. The final average weight of three batches in recirculation system (RAS) was 82.40 g and this weight was slightly higher than obtained in the open circuit by Rodriguez *et al*., 2008 and Rodriguez (2012) (Fig.1). The index growth values were relatively similar in three batches: factor condition varied between 1.87 and 2.00, the average specific growth rate between 2.53 and 2.78 and the average feed conversion rate between 0.98 and 1.03 (Table 1). Growth parameters were kept constant throughout the experience: S‰ 36±2 (g/l), T 18±0.6 (°C), pH 7.27±0.21 y O^2 6.2±0.7 (ppm). The death was non-existent, and no pathological problem was detected during the experience.

Discussion and Conclusion

The growth during turbot preongrowing in a recirculation system maintained at 18 °C was slightly higher than in open circuit data from other experiments carried out in the same facilities and where the temperature varied between 12 and 19 °C. The index growth values were also better in the closed system. The recirculation system of turbot preongrowing could be an alternative to the current open circuit farming system.

Table I. Results obtained from the three batches in a recirculation system. CF: condition factor; SGR: specific daily growth; FCR: feed conversion rate.

Day	N	Average weight (g)			Average size (cm)			CF			SGR			FCR		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	300	6,54	6,43	6,22	7,41	7,47	7,36	1,61	1,54	1,56	-	-	-	-	-	-
16	300	12,29	12,36	12,47	8,79	8,79	8,76	1,81	1,82	1,85	3,71	3,84	4,09	0,48	0,46	0,42
31	300	19,19	19,27	20,94	10,20	10,01	10,17	1,81	1,92	1,99	2,97	2,96	3,46	0,75	0,75	0,62
52	300	34,79	35,38	38,42	12,04	11,96	12,36	1,99	2,07	2,03	2,83	2,89	2,89	0,74	0,72	0,72
73	300	57,36	58,14	62,85	14,09	14,15	14,68	2,05	2,05	1,99	2,38	2,37	2,34	0,93	0,93	0,94
94	300	78,98	79,55	88,68	15,94	14,48	16,36	1,95	2,62	2,03	1,52	1,49	1,64	1,63	1,63	1,46
						Average		1,87	2,00	1,91	2,53	2,65	2,78	1,03	1,03	0,98

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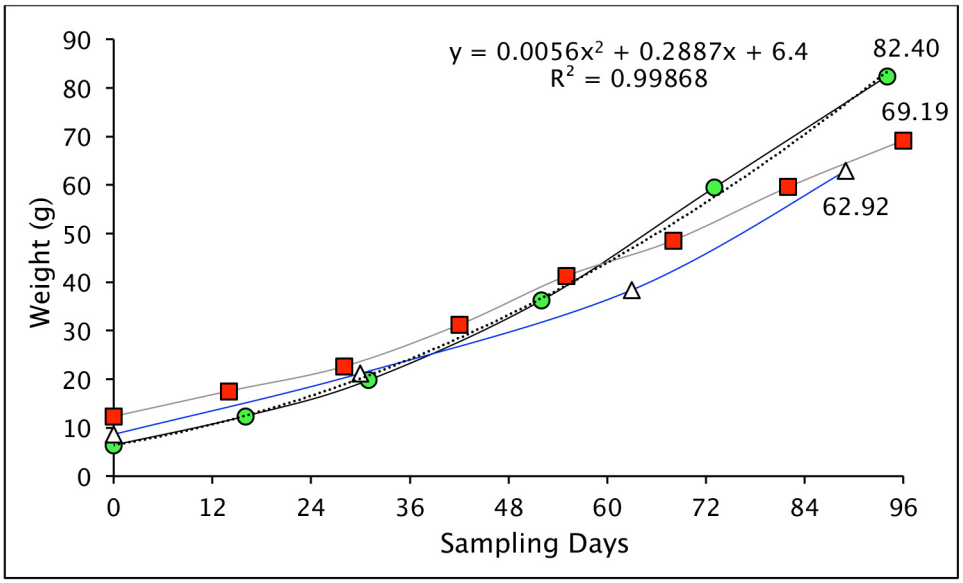

Figure 1. Growth curves in recirculation and open system: red line with squares corresponds to the weights obtained by Rodríguez *et al.*, (2008), blue line with triangles corresponds to the weights obtained by Rodríguez (2012), both in open system and green circles represents the mean of weights obtained in the current study.

References

Rodríguez, J.L. 2012. Effect of different feeding frequencies on turbot *Scophthalmus maximus* during preongrowing stage. Aqua 2012. Prague, Czech Republic.

Rodríguez, J.L., Souto, B.F., Figueiro, R., Quintáns, J.M., Canosa, M.C. 2008. Preongrowing of turbot *(Scophthalmus maximus*) in recirculation systems and in open circuit. Aquaculture Europe 08, Cracovia, Polonia.

PREONGROWING OF TURBOT (*Scophthalmus maximus)* SUBJECT TO THREE FEEDING RATE IN A RECIRCULATION SYSTEM

E. Murari*, E. Peláez, E. Pérez and J. L. Rodríguez

Instituto Galego de Formación en Acuicultura (IGaFA). Niño do Corvo s/n 36626 Illa de Arousa, Spain
E-mail: murari.egberto@gmail.com

Introduction

Feeding represents one of the main operational costs in turbot aquaculture (Aydin *et al*., 2011). Overfeeding results in waste of uneaten food and deterioration of water quality. Underfeeding results instead in sub-optimal fish growth. Identifying an optimal feeding rate is important for both economic and biological reasons. The aim of this study is to evaluate the growth of turbot *Scophthalmus maximus* juveniles in a recirculation system under different feeding rate.

Materials and methods

The experiment was carried out in the Instituto Galego de Formación en Acuicultura (IGaFA). We started off with three triplicate batches (A, B, C) of 100 fishes each. The samples in batch A, had an initial average weight of 6.49±1.70 g and an average length 7.64±0.66 cm, the feeding rate was 2%. Batch B had an initial average weight of 6.40±1.71 g and an average length of 7.41±0.67 cm, the feeding rate was of the 3%. Batch C had an initial average weight of 6.41±1.80 g and an average length of 7.41±0.72 cm, the feeding rate was of the 4%. The individuals were kept during the whole experience in flat bottomed square tanks of 1.0x1.0x0.4 m, in recirculation system. The feeding consisted in extruded feed specific for turbot, manufactured by Skretting, which were supplied four times a day, during the daytime, through automatic feeders. The physico-chemical parameters were kept constant throughout the experience: S‰ 36±2 (g/l), T 18±0.6 (°C), pH 7.27±0.21 and O^2 6.2±0.7 (ppm). The temperature, salinity and oxygen were registered on a daily basis and nitrates, nitrites, ammonium and CO_2 levels in the closed circuit tanks, were recorded twice a week.

Samplings of weight and length were done periodically. With the information obtained, we elaborated weight and growth curves, and we calculated the following growth values, condition factor (CF), specific growth rate (SGR) and feed conversion rate (FCR). Both partial (for each sampling interval) and global indexes were calculated. None of the individuals died during the experience.

Results

Throughout the experience, which lasted 94 days, the fish in batch A grew less than the fish in batch B and C, while there were no significant differences between batches B and C. The fish in batch A reached a final mean weight of 70.12±14.08 g with a net weight gain of 63.93 g, in batch B a weight of 82.40±15.03 g with a net weight gain of 76.01 g and in batch C fish increase their weight to 81.70±15.90 g; with a net weight gain of 75.29 g (Fig. 1). Mean SGR was similar in batches B (2.65) and C (2.68); and slightly less in batch A (2.51). Mean FCR in batch A was 0.67, showed a direct relationship with the feeding percentage. Mean FCR in batch B and C was 1.01 and 1.41, respectively (Table1).

Discussion and Conclusion

The best growth results of turbot juveniles in a recirculation system were obtained using a feeding rate of 3%. A feeding rate of 2% resulted in better FCR, but fishes lost growth potential and reached a lower final weight. A feeding rate of 4% led to a similar growth to that of a 3% feeding rate, but with worse FCR resulting in higher feeding costs.

Table I. Results obtained from batches A, B and C: (L) average length, (W) average weight, (CF) condition factor, (FCR) feed conversion rate and (SGR) specific growth rate.

Day	N	Average weight (g)			Average size (cm)			CF			SGR			FCR		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	900	6,49	6,40	6,41	7,64	7,41	7,41	1,55	1,57	1,57	-	-	-	-	-	-
16	900	10,70	12,37	12,65	8,53	8,78	8,82	1,77	1,83	1,84	3,10	3,88	4,01	0,41	0,45	0,58
31	900	17,49	19,80	20,30	10,00	10,13	10,20	1,75	1,91	1,91	3,09	3,13	3,15	0,48	0,71	0,93
52	900	29,10	36,20	37,51	11,48	12,12	12,22	1,93	2,03	2,06	2,43	2,87	2,92	0,60	0,73	0,95
73	900	46,98	59,45	60,82	13,44	14,31	14,44	1,94	2,03	2,02	2,28	2,36	2,30	0,65	0,93	1,29
94	900	70,12	82,40	81,70	15,18	15,59	15,96	2,01	2,20	2,01	1,91	1,55	1,40	0,82	1,57	2,37
						Average		1,82	1,93	1,90	2,51	2,65	2,68	0,67	1,01	1,41

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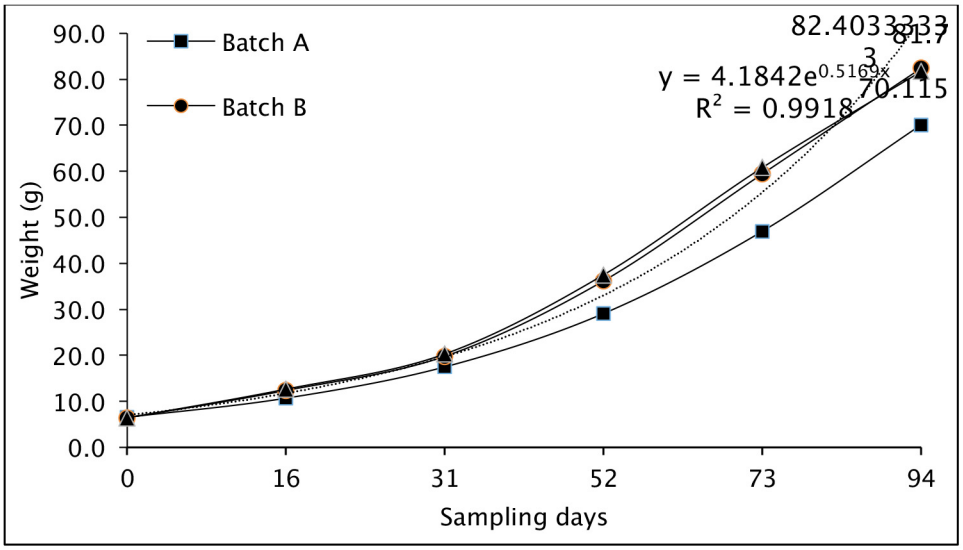


Figure 1. Growth curves of the different batches: Batch A: 2% rate feeding; Batch B: 3%; Batch C: 4%.

References

Aydın, İ., Küçük, E., Şahin, T., & Kolotoğlu, L. 2011. The effects of feeding frequency and feeding rate on growth performance of juvenile black sea turbot (*Psetta maxima*, Linneaus, 1758), *Journal of Fisheries Science*, 5(1). 35-42.

CALIBRATION AND ADJUSTMENT OF A PROXY FOR CARRYING CAPACITY OF MARINE AQUACULTURE IN THE SPANISH MEDITERRANEAN COAST FLOATING CAGES

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 Ctaqua-Fundación Centro Tecnológico Acuicultura de Andalucía, Muelle Comercial S/N, 11500, El Puerto de Santa María
Email: francisca.romero@ua.es

Introduction

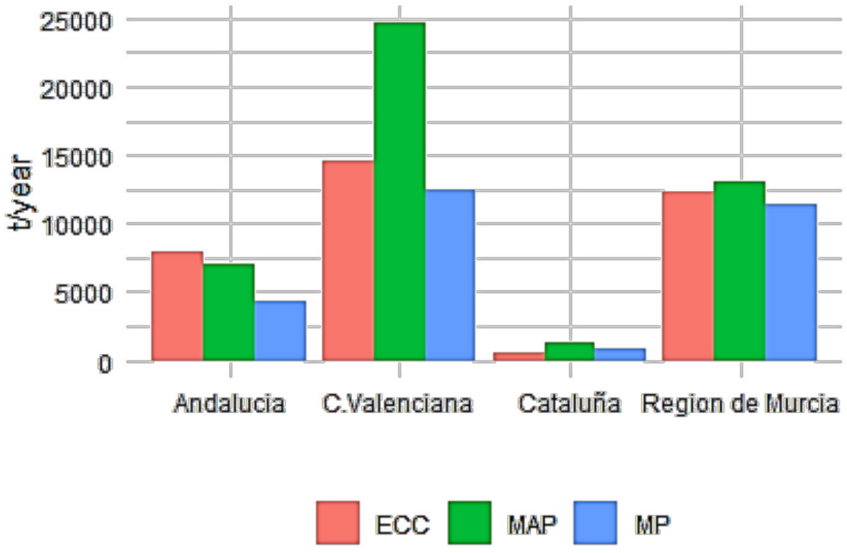
Carrying capacity models are one of the most valuable systematic tools for aquaculture management and planning, as they allow defining and limiting aquaculture production in open sea facilities. These models cover not only environmental aspects, but can also count with socio-economic factors, which can become crucial for an activity that takes place in the maritime public domain. A previous project (MIMECCA) developed a theoretical model aimed as a proxy of aquaculture carrying capacity in floating cages on the Spanish Mediterranean coast through a Delphi exercise. This study develops the first part of the validation of the model by applying it to gilthead seabream, sea bass and meagre installations. The objective is analysing possible mismatches and propose changes to improve it.

Material and Methods

The technical-productive, environmental, social and economic data sets of 25 fish farms were analysed, to estimate and compare their carrying capacity against their maximum permissible production and marketing production.

Results and Discussion

The results showed that, due to the lack of individualized production data for each facility, the most realistic comparison is that with marketing production data at the autonomous community level, as 75% of the regions analyzed have lower marketing productions (MP) than their estimated carrying capacities (ECC) (Figure 1). Although the model seems restrictive, there is ample room for improvement by choosing new socioeconomic factors and modifying some of the established backdrops. In addition, the model finds that carrying capacity could be increased through improved installation management, site selection and a more accurate calculation of currents (Figure 2). These changes together with in-situ validation of the model are crucial to improving model reliability.



Fig[1]. Comparison. Estimated Carrying Capacity (ECC) vs Maximum Allowable Production (MAP) and ECC vs Marketing Productions (MP) for each autonomous communit, 2019.

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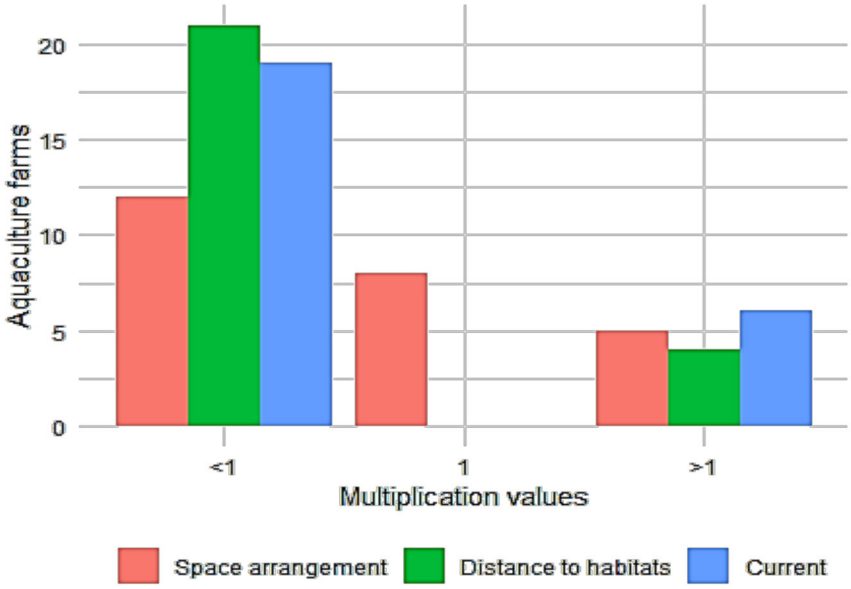

Fig[2]. Multiplication values. Selection of the multiplication values that limit final output of the model.

WHAT IS THE BEST SAMPLING METHOD FOR AQUACULTURE EMP? CHOOSING ACCORDING ENVIRONMENTAL AND ECONOMIC PARAMETERS

E. Carbonell-Garzon[1], V. Fernandez-Gonzalez[1], P. Sanchez-Jerez[1], F. Romero*[1], G. Martínez Millán[2], M.M Agraso[2] and K. Toledo-Guedes[1]

[1] Department of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain
[2] Ctaqua-Fundación Centro Tecnológico Acuicultura de Andalucía, Muelle Comercial S/N, 11500, El Puerto de Santa María
E-mail: estela.carbonell@ua.es

Introduction

Fish farming has negative effects on the benthos due to the high loads of organic matter resulting from fish production (Martinez-Garcia *et al*., 2018)in which they proposed the implementation of site-specific "Environmental Monitoring Plans" (EMPs. As all human activities carried out in marine environment, an effective Environmental Monitoring Plan (EMP) is necessary to develop sustainable production. However, there is a large variety between countries in monitoring programmes and indicators used, and differences in a range of approaches and conclusions about the severity of these effects (Read & Fernandes, 2003). In this way, in 2012 the Spanish National Advisory of Mariculture (JACUMAR) suggested an initiative to unify monitoring methodologies between different regions within Spain (Aguado-Gimenez et al., 2012). Therefore, the aim of this study was to make progress in this direction and evaluate the spatial variability and economic effort related to the use of two sampling methods such as scuba diving, with quadrats and corers, and Van-Veen grab.

Material and methods

The study was carried out in two fish farms on the Spanish Mediterranean coast in summer 2019, each one using a different sampling method. According to JACUMAR protocol, in each location polychaete assemblages, total free sulphides (TFS) and the finest sediment fraction (<63 μm) were analysed. To estimate spatial variability associated to each methodology, Variance Component Analysis (VCA) was carried out, emphasizing variability at the scale of meters.

Results and discussion

Results showed higher data homogeneity for TFS using corers while both polychaeta assemblage and the finest sediment fraction obtained less variability with Van Veen grab. Corers contributed very little to the TFS total variation (5.93%), probably due to the best conservation of sediment characteristic because this method prevent movement and oxidation of the samples during the sampling work (Choi *et al*., 2020 ; Guerra García & García-Gómez, 2008). Whereas, in the case of the finest sediment fraction, lower variability was directly related to the sampling method, but it be taken into account that, grab sampling could underestimate the finest sediment fraction when higher clasts prevent from closing the grab properly (Guerra García & García-Gómez, 2008). Finally, the high variability for polychaeta assemblages in scuba-diving sampling was related to the collected sediment weight (ANOVA $p < 0.05$), probably due to different handling and experience between divers.

Regarding economic analysis of both methodologies, sampling work with Van Veen grab entailed lower total investment and also reduce the risk situation for workers. Specifically, in terms of the material costs, Van-Veen grab sampling seems to be more expensive. However, scuba diving methods showed higher costs due to the greater number of sampling days and more workers that are specialized. According to these results, grab sampling reflected better results considered ecological and economic factors for aquaculture EMP.

This study is part of the project "Modelos aplicados de capacidad de carga a la acuicultura marina (MACCAM)", which 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.

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References

Aguado-Gimenez, F., Carballeira-Ocaña, A., Collado-Sánchez, C., González-Henríquez, N. and Sanchez-Jerez, P. (2012). Propuesta metodológica para la realización de los planes de vigilancia ambiental de los cultivos marinos en jaulas flotantes. Junta Nacional Asesora de Cultivos Marinos (JACUMAR).

Choi A., Kim B., Mok J.-S., Yoo J., Kim J. B., Lee W.-C., & Hyun J.-H. (2020). Impact of finfish aquaculture on biogeochemical processes in coastal ecosystems and elemental sulfur as a relevant proxy for assessing farming condition. Marine Pollution Bulletin, **150**, 110635.

Guerra García J., & García-Gómez J. (2008). Core manual versus draga van Veen en sedimentos intraportuarios: Estimación de sus eficiencias en el estudio de comunidades macrobentónicas. Zoologica baetica, **19**, 57-70.

Martinez-Garcia E., Fernandez-Gonzalez V., Aguado-Giménez F., Sánchez-Lizaso J. L., & Sanchez-Jerez P. (2018). From paper to practice: an initial approach to implementation of the environmental monitoring plan for fish farming proposed by JACUMAR. Scientia Marina, **82** (1), 27.

Read P., & Fernandes T. (2003). Management of environmental impacts of marine aquaculture in Europe. Aquaculture, **226**, 139-163.

COMPARING SELECTION RESPONSE AND INBREEDING LEVEL IN FAMILY AND GROUP MATING DESIGNS OF SEA BREAM AND SEA BASS

Chantal Roozeboom*, Hans Komen, John W.M. Bastiaansen

Department of Animal Breeding and Genomics, Wageningen University and Research,
Droevendaalsesteeg 1, 6708 PB, Wageningen (The Netherlands)
Email: chantal.roozeboom@wur.nl

Introduction

Genetic improvement programs use selective breeding to increase the performance of farmed fish species. European aquaculture breeding programs mostly implement a family design (Janssen et al., 2017). However, in breeding programs of seabream and seabass, group mating is also frequently applied. The objective of this study is to compare breeding programs that implement either groups mating or a family design. The genetic gain and rate of inbreeding will be predicted in both the breeding program using a family design and the breeding program with group mating. This work is part of the EU project AquaIMPACT.

Material and method

Two breeding programs are simulated for the fish species seabass and seabream: one implementing a family design and one using group mating. The traits simulated for selection are weight at pre-selection, harvest weight and survival (Table 1).

Fish in each generation were divided over 9 batches in the design with group mating and 4 batches in the family design. The base population consisted of 360 sires and 180 dams for both designs. In the family design, a 1 dam x 2 sires mating scheme was applied, producing 90 full sib families per batch. 50% of the selection candidates was assumed to be mature and fertile at the time of producing offspring. With group mating, each batch included 20 dams and 40 sires. 50% of the sires and dams contributed offspring with proportions according to a Gamma distribution: $\Gamma(0.75,0.11)$. EBVs for the survival trait were simulated based on testing sibs of selection candidates. 10 fish per full sib family (family design) or 400 fish per batch (group mating) were tested.

Selection was in three steps. For each generation: at 35 grams, 5400 fish were preselected out of 18000 candidates based on phenotypic weight at pre-selection; at 450 grams, 1620 fish were selected out of surviving candidates based on EBV for harvest weight and survival; sires and dams for each new batch were selected out of these 1620 selected fish based on EBV and optimal contributions with a targeted increase in coancestry of 0.01 each generation, implemented using AlphaMate (Gorjanc and Hickey, 2018). Both designs were simulated for 10 generations and evaluated for inbreeding rate and rate of genetic improvement in harvest weight and survival.

Table 1 Parameters for simulation

Trait	Mean*	σ^2_p	h^2 and r_g			c^2
Weight at pre-selection	35	40	0.25			0
Harvest weight	450	11250	0.71	0.35		0.06****
Survival	0.40	$\mu(1-\mu)$**	0	0.17	0.18***	0

** Mean of the traits in the base population, ** μ = mean of selected parents, *** Observed heritability in the base population (Visscher et al., 2008), **** Common environmental effect only applied to family design.*

Table 2 Results of the simulations

	ΔG/ ΔF in group mating	ΔG/ ΔF in family design	Difference (family design -group mating)
Harvest weight	60.3 grams	61.3 grams	1.7%
Survival	0.042 points	0.055 points	31%
Rate of inbreeding	0.32%	0.25%	-22%

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Results

With group mating, the genetic gain per generation was on average 60.3 grams for harvest weight and 0.042 points for survival (Table 2). In generation 10, the simulated fish had on average a weight of 939 grams and a survival of 0.75. In the family design, the genetic gain per generation was on average 61.3 grams for harvest weight and 0.055 points for survival (Table 2). In generation 10, the simulated fish had on average a weight of 1012 grams and a survival of 0.87. The rate of inbreeding was 0.32% in the group mating design and 0.25% in the family design.

Discussion and conclusion

Genetic gains in the family design were 1.7% and 31% higher for the traits harvest weight and survival compared to the group mating design. A bigger difference was found for the inbreeding, with a 22% lower rate of inbreeding in the family design. In both designs the rate of inbreeding is at a quite low level. More inbreeding could be allowed, especially in the family design. Comparison of breeding program designs is best done at equal rates of inbreeding. However, allowing more inbreeding in the optimal contribution selection does not automatically increase the rate of inbreeding.

The family design and group mating design differ in various aspects. The selection efficiency in the family design can be reduced due to the common environmental effects. In the group mating design, not every selected parent contributes to the next generation. Additionally, the size of full sib families are unequal, which will negatively affect the accuracies of EBV, especially for sib traits. Besides the rates of inbreeding and genetic improvement, other factors play a role in choosing a specific design. Implementing a family design is more labor intensive and requires more infrastructure than a group mating design. Reproductive characteristics of a species may limit the choice to group mating designs.

Comparing breeding program designs that depend on a large number of genetic, reproductive and other biological constraints is now possible with the developed stochastic simulation software. In the current study, the family design gives a higher rate of genetic improvement, especially for the survival trait. Group mating designs can perform almost as good as family designs if the target traits is only body weight gain. Both designs have low inbreeding rates.

Acknowledgement

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References

Gorjanc, G., Hickey, JM (2018). AlphaMate: a program for optimizing selection, maintenance of diversity and mate allocation in breeding programs. Bioinformatics. 375

Janssen, K., Chavanne, H., Berentsen, P., & Komen, H. (2017). Impact of selective breeding on European aquaculture. Aquaculture. 472. 8-16.

Visscher, P. M., Hill, W. G., Wray, N. R. (2008). Heritability in the genomics era—concepts and misconceptions. Nature reviews genetics. 9(4). 255-266.

THE EFFECT OF TEMPERATURE ON THE SURVIVAL AND FILTRATION RATE OF THE SEA SQUIRT *Ciona intestinalis*

F.O. Borges*[1], R. Cyrne[1], M. Franzita[1], M. Deleja[1], T. Repolho[1], R. Rosa[1].

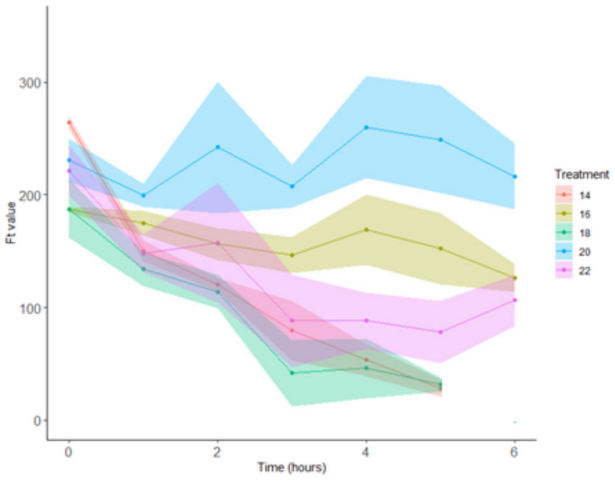
[1]Marine and Environmental Sciences Centre (MARE), Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Cascais, Portugal
Email: fdborges@fc.ul.pt

Introduction

The sea squirt, *Ciona intestinalis*, is a solitary epibenthic ascidian with a worldwide distribution. This species is known for its fast colonization of immersed structures, leading to vast negative economic impacts in marine activities which depend on the availability of surface space for colonization and fixation by other target species. One such example is mussel farming, in which sea squirts have been shown to quickly overrun mussel farm structures and hindering mussel fixation and development. In order to prevent losses, farmers have to spend increased amounts of time and effort in removing these invaders from their structures, with no economic benefit other than attempting to save their production. In fact, despite some localized examples, like in Oriental cuisine, ascidians have relatively few uses that could come as a profitable destination following their removal. A potential destination could be their use as potential supplement in fish rations for aquaculture production. However, the use of these species for aquaculture feeds would necessarily rely on controlled production to ensure product quality, which requires prior research on the correct maintenance parameters for this species. In this sense, the present study aimed to evaluate the effect of temperature, a key parameter for aquaculture production, on the short-term survival (i.e. 30 days) and filtration rate of *Ciona intestinalis* collected from an overrun mussel farm in Portugal.

Material and methods

Adult sea squirts (*Ciona intestinalis*) were collected in Portuguese Western Coast, namely Figueira da Foz, and then transported to the aquaculture facilities of Laboratório Marítimo da Guia (Cascais, Portugal). Upon arrival, the animals gently cleaned and were distributed across 25 recirculating tanks (5 treatments x 5 replicate tanks, 5 individuals per replicate tank). Sea squirts were slowly acclimated to laboratory conditions (15ºC, 8.0 pH and 35 salinity) over the course of a week. Afterwards, each treatment tank was adjusted to the final desired temperature (i.e. 14ºC, 16ºC, 18ºC, 20ºC, 22ºC), to which the animals were exposed for a total of 30 days. During the experimental period, specimens were fed daily with a mixture of lyophilized algae (Necton) and fish feed (Sparos). Water quality parameters were measured daily, and water renewed to a total of 5x turnover per replicate tank a day. Tanks were also cleaned from food and debris leftovers from feeding, and individuals checked for mortality. Survival was assessed daily, following four criteria: i) siphon state; ii) changes in tunic colour; iii) siphon contraction; and iv) smell. At the end of the experimental period, individuals of each treatment were gently placed in individual aerated containers (400mL each), each receiving a slow input of new water (at the respective treatment parameters). The animals were left to acclimate to the new containers for a minimum of 18 hours, upon which time the water input was closed and the filtration rate sampling begun. A previously prepared sample of living micro algae (*Phaeodactylum tricornutum*) at a concentration of 1 million cells per mL was taken from a microalgae culture, and injected into each experimental container (plus one control container with no animal per treatment). At that time (T0), a



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handheld diving PAM fluorometer was used to measure Chlorophyll fluorescence yield (Ft) of 3 1mL samples from each individual container. This process was repeated on an hourly basis up to a total of 6 hours (T0-T6), during which the syphon status (i.e. open or closed) of each *Ciona*, as well as the correct aeration of each container (in order to ensure homogenous distribution of the microalgae) were closely monitored. Following this procedure, all individuals were gently rinsed and weighted before freeze storing at -80ºC.

Results

Survival was impacted by increasing temperature, with mortality occurring at the two highest temperatures (i.e. 20º and 22ºC, with a total of 8 and 2 dead individuals per treatment, respectively). Regarding filtration, higher rates of algae consumption (indicated by decreasing Ft values over the 6 hours) were seen for 14 and 18ºC, followed by 16 and 22ºC and, lastly, 20ºC exhibiting almost constant microalgae levels. 14 and 18ºC treatments reached minimum values after 5 hours and reached Ft values near to 0 at T6.

In the 16ºC treatment, not only was mortality the highest, but individuals also exhibited closed syphons for the majority of the trials (approximately 50% individuals being closed). Closed syphons were also observed for the 16ºC treatment at time of filtration trials (20%).

Conclusions

Temperature can be one of the main factors impacting ascidian survival in aquaculture productions. Temperatures of 20 degrees and over were responsible for a mortality of between 8 and 32% regarding treatment population. No clear effect of temperature by itself was obtained in the present study regarding the filtration trial, in particular due to the status of the animal's syphons at the 16º and the 20º treatments. However, the lower temperature of 14ºC was the one where the filtration rate was higher. Further trials should be conducted in order to ascertain the optimal temperatures for *Ciona* growth in an aquaculture environment and to evaluate the species potential as fish feed as one possible solution to overgrowth in mussel farms and other human structures.

EFFECTS OF THERMAL ENVIRONMENT ON THE PROXIMATE CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF THE SEA SQUIRT *Ciona intestinalis*

R. Rosa[1*], F.O. Borges[1], R. Cyrne[1], T. Repolho[1], N. Bandarra[2]

[1]Marine and Environmental Sciences Centre (MARE), Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Cascais, Portugal
Email: rrosa@fc.ul.pt

[2] Division of Aquaculture and Upgrading, Portuguese Institute for the Sea and Atmosphere, Avenida de Brasília, 1449-006, Lisboa, Portugal

Introduction

Ocean warming has emerged as a significant environmental threat to healthy marine ecosystems, and, consequently, prospects of its impact on marine biota are of concern. Temperature is recognized to be a key environmental factor that shapes the physiology of marine poikilotherms. Moreover, temperature shapes their way of life in various climates and ecosystems, ultimately shaping their biogeography. The sea squirt, *Ciona intestinalis*, is a solitary epibenthic ascidian with a worldwide distribution. This species is known for its fast colonization of immersed structures, leading to vast negative economic impacts in marine activities which depend on the availability of surface space for colonization and fixation by other target species. Within this context, the present study aimed to evaluate the effect of temperature, a key parameter for aquaculture production, on the proximate chemical composition and fatty acid profile of *Ciona intestinalis*.

Material and methods

Adult sea squirts (*Ciona intestinalis*) were collected in Portuguese Western Coast, namely Figueira da Foz, and then transported to the aquaculture facilities of Laboratório Marítimo da Guia (Cascais, Portugal). Upon arrival, the animals gently cleaned and were distributed across 25 recirculating tanks (5 treatments x 5 replicate tanks, 5 individuals per replicate tank). Sea squirts were slowly acclimated to laboratory conditions (15ºC, 8.0 pH and 35 salinity) over the course of a week. Afterwards, each treatment tank was adjusted to the final desired temperature (i.e. 14ºC, 16ºC, 18ºC, 20ºC, 22ºC), to which the animals were exposed for a total of 30 days. During the experimental period, specimens were fed daily with a mixture of lyophilized algae (Necton) and fish feed (Sparos). Following the experimental period, proximate chemical composition was determined according to the AOAC (2005). Fatty acid methyl esters (FAMEs) were prepared by acid-catalyzed transesterification using the methodology described by Cohen et al. (1988). Samples were injected into a Varian Star 3800 CP gas chromatograph (Walnut Creek, CA, USA), equipped with an auto sampler with a flame ionization detector at 250°C.

Results and Discussion

Warming did not induce a significantly change in moisture (varied between 92.2 and 93.6% ww; $p>0.05$), ash (between 44.3 and 45.0% dw; $p>0.05$), protein (between 24.2 and 26.4% dw; $p>0.05$), and lipid (between 2.98 and 3.22% dw; $p>0.05$) contents. On the other hand, the contents of saturated fatty acids (SFA, including C16:0 and C18:0), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA, including EPA and DHA), $\Sigma\omega3$, $\Sigma\omega6$, and $\Sigma\omega3/\Sigma\omega6$ revealed significant changes with temperature ($p<0.05$). The FA results showed that the warming negatively affected the fatty acid composition of the ascidian, particularly EPA+DHA and $\Sigma\omega3/\Sigma\omega6$, which may jeopardize the ascidian nutritional quality and potential as supplement in fish rations for aquaculture production in a changing warmer ocean. Thus, further trials are needed to investigate the potential of this ascidian species as fish feed in the ocean of tomorrow as one possible solution to overgrowth in mussel farms and other human structures.

References:

AOAC. (2005). Official methods of analysis of the association of official analytical chemists international (18th ed., p. 473).

Cohen, Z., Vonshak, A., & Richmond, A. (1988). Effect of environmental conditions on fatty acid composition of the red algae Porphyridium cruentum: Correlation to growth rate. Journal of Phycology, 24, 328–332.

BREEDING FOR LICE RESISTANCE IN NORWEGIAN FARMED ATLANTIC SALMON: AN IGNORED (OR INSIGNIFICANT) PATH FOR SUSTAINABLE INNOVATION?

G. Kristin Rosendal* (FNI) and Ingrid Olesen (Nofima)

The Fridtjof Nansen Institute (FNI), PB 326, 1326 Lysaker, Norway
krosendal@fni.no

Introduction

Lice is a persistent and increasing problem in the aquaculture sector with serious environmental impacts and reducing income from the salmon industry (Holan et al, 2017). This article discusses whether there is an untapped potential in breeding for improved lice resistant salmon roe and fish, and if so, what factor may explain the gap. To this end, three sets of factors that may impact the state of breeding for lice resistance are examined: market-based factors, regulation of breeding technologies, and policy instruments.

Methods and materials

Methodologically, our data material relies on the literature on breeding for lice resistance. This body of literature is not very large, so we are able to review most of it, as well as identify gaps in the knowledge. Adding to this, we conducted in-depth interviews with key actors in Norwegian salmon farming and salmon breeding programs. We conducted interviews with all four companies involved in breeding programs in Norway; therefore, the results from these interviews are fully representative. The main challenge concerning results from these interviews is our respondents' requests for confidentiality, and thus our need to carefully handle sensitive information regarding corporate breeding strategies. In accordance with data-management practices, all interviewees were granted full anonymity and were not cited without consent.

Results

We found well documented selection response for lice resistance in salmon (AquaGen, 2016, 2017; Hillestad et al., 2017). Regarding the first set of explanatory factors, our data material indicates that market-based factors will hardly stimulate lice resistance in breeding. This is because genetic lice resistance is predominantly a collective or public good and partly due to the polygenic nature of lice resistance not easily lending itself to patenting and hence a private good. Second, the regulation of and consumer perceptions of gene editing technologies is in flux (NBAB, 2020), which creates uncertainty about investing in technologies that could handle the polygenic challenges of lice resistance. Finally, policy instruments aimed at stimulating relevant innovation has been applied generously for other types of innovation to deal with the lice problem (such as mechanical innovations). However, none of the policy instruments has targeted or stimulated breeding measures so far (Greaker et al., 2020).

Discussion

The current level of lice resistance selection is not strong enough to significantly reduce the number of delousing treatments: the maximum presence of 0.5 adult female lice per fish makes it difficult to document or measure the possible favourable effects from a less-than-perfect lice-resistant salmon by the farmers. As the farmed salmon are increasingly and severely stressed by delousing treatments, many big fish become susceptible to death from cardiomyopathy syndrome (CMS) close to slaughter (Veterinærinstituttet, 2017, p. 18). In effect, farmers are now increasingly asking breeders for CMS-resistant salmon roe, able to tolerate the tough delousing treatments. If breeders start earning more from delivering CMS-resistant roe than from lice-resistant roe, a paradoxical situation may arise that exposes more salmon to stressful mechanical treatments, while the lice situation and threat to wild salmon remains the same. Seen from a social and environmental point of view this could paradoxically lead to increased demand for fish that better endures harsh delousing treatments rather than demand for more lice resistant fish. Policy instruments such as the generous *Development licencing* contribute significantly to stimulate mechanical innovations aimed at reducing the lice problems in aquaculture through on-land and off-shore installations, but no policy instruments have been applied to stimulate breeding for lice resistance. The sum of policy instruments could ultimately weaken the competition of rural aquaculture industries operating along Norwegian fjords.

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References

AquaGen, 2016. Effective lice control through breeding and genetics. https://aquagen.no/en/2016/08/15/effective-lice-control-through-breeding-and-genetics/

Greaker, M., Vormedal, I., Rosendal, K., 2020. Environmental policy and innovation in Norwegian fish farming: Resolving the sea lice problem? *Mar. Policy*. 117, 1-10.

Hillestad, B., Thorland, I., Vela, S., Johansen, H., 2017. Avl for redusert lusetetthet hos Atlantisk laks. Tekna Konferanse Frisk Fisk 1. og 2. februar, Bergen.

Holan, A.B., Roth, B., Breiland, M.S.W., Kolarevic, J., Hansen, Ø.J., Iversen, A., Hermansen, Ø., Gjerde, B., Hatlen, B., Mortensen, A., Lein, I., Johansen, L.-H., Noble, C., Gismervik, K., Espmark, Å.M., 2017. Beste praksis for medikamentfrie metoder for lakseluskontroll (MEDFRI) Faglig sluttrapport. Nofima Rapport 10/2017. 111 pp.

NBAB, Norwegian Biotechnology Advisory Board (NBAB/Bioteknologirådet). 2020. Norwegian consumers' attitudes towards gene editing in Norwegian agriculture and aquaculture. https://www.bioteknologiradet.no/filarkiv/2020/04/Report-consumer-attitudes-to-gene-editing-agri-and-aqua-FINAL.pdf

Veterinærinstituttet, 2017. Kardiomypatisyndrom (CMS) hos laks. Norwegian Veterinary Institute.

A BLEND OF ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS EXERTED ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES DURING AN INFLAMMATORY CHALLENGE IN EUROPEAN SEABASS (*Dicentrarchus labrax* L.)

B. Rossi*, J. Dias, 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. The outbreak of diseases is not merely related to the causing agents, but it is instead an association among pathogens, environment and the host fish. 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 European seabass (*Dicentrarchus labrax* L.) short-term immune-response, during an inflammatory challenge.

Materials and methods

Four hundred and eighty European seabass specimens of an average weight of 62 g were randomly distributed into 12 tanks (40 fish per tank) at SPAROS Lda (Portugal). The following 4 diets were tested in triplicate tanks: 1) control (CTR), 2) AviPlus®Aqua 1000 ppm (D1000), 3) AviPlus®Aqua 1500 ppm (D1500) and 4) AviPlus®Aqua 2000 ppm (D2000). Fish were fed for 71 days and then subjected to an inflammatory challenge by injecting them intraperitoneally (i.p.) with either 100 μl of UV-killed Photobacterium damselae subsp. piscicida (Phdp) (CTR+, D1000+, D1500+, D2000+) or Hanks' Balanced Salt Solution (HBSS) (CTR-, D1000-, D1500-, D2000-). Upon injection, fish were redistributed into new tanks according to diet and injection stimuli. Four hours post-injection, fish injected with UV-inactivated Phdp (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). Data were analyzed with a two-way analysis of variance, with diet inclusion and injection type (Phdp and HBSS) as variables.

Results

Four hours after injection, Phdp-injected fish had marked differences on immune humoral criteria compared to those injected with HBSS, showing a significant decrease in PA and a significant increase in ROS and NO ($P<0.05$) (Fig. 1). Considering the challenged groups, fish fed D1000+, D1500+ and D2000+ showed significantly lower ROS and NO and higher PA, than fish fed CTR+ ($P<0.05$) (Fig. 1). Additionally, D2000+ showed significant lower ROS and NO and a higher PA than D1000 + and D1500 + groups ($P<0.05$) (Fig. 1).

Conclusion

In conclusion, European seabass fed diets supplemented with the AviPlus® Aqua for 71 days showed a mitigation of the over production of ROS and NO during the early stages of inflammation in a dose-dependent manner, displaying a higher capacity to cope with an inflammatory condition and a beneficial immunostimulatory effect.

THE LIFE CYCLE OF *Octopus vulgaris* IN CAPTIVITY: GROWTH, REPRODUCTION AND LIFESPAN

A. Roura*[1,2], M. Martínez-Pérez[1,2], A. Castro-Bugallo[1,2]

[1] Instituto de Investigaciones Marinas de Vigo (IIM-CSIC), Eduardo Cabello 6, Vigo
[2] Estación de Ciencias Marinas de Toralla, CIM, Universidade de Vigo

Introduction

The common octopus, *Octopus vulgaris*, is doubtless the best-known octopod, with written records of this species going back to Aristotle. However, there are aspects of their life cycle like longevity, growth, and reproduction that are still poorly known. While beak analysis from spent specimens suggest a 1-year life cycle in Mauritanian waters (Perales-Raya et al. 2014), the analysis of growth curves obtained using tagging studies estimated a longevity of 14-17 months for females and 18-20 months for males (Domain et al. 2000). The lack of a consistent ageing method has resulted in the majority of our understanding of octopus growth coming from laboratory studies, but to date there is only one study carried in captivity that obtained two adults of 1.55 and 1.8 kg in 339 and 356 days, respectively (Iglesias et al. 2004). Forsythe and Van Heukelem (1987) showed a trend of two-phase growth in cephalopods, with an initial rapid exponential phase followed by a slower power growth phase, yet to be confirmed in *O. vulgaris*. Likewise, many reproductive behaviours remain unknown owed to the difficulty of growing this species from egg to adults or the lack of field studies. In this work we describe the complete life cycle of *O. vulgaris* in captivity, from egg up to 829 days, which will help to understand unknown features of the biology of this species.

Methods

525 recently hatched paralarvae obtained from a wild female were transferred to three 50L dark green fibre glass tanks provided with filtered seawater (1μm) and a central outlet with 500μm filter. An open water system with 150% renovation per day was used with mean water temperature 19.5ºC (18.1-21.4), salinity 35.4 (34,8-36.2) and a 14:10 h light cycle provided with LED lights. The bottom of the tanks was siphoned every day. Live diet consisted of sub-adult Artemia (1-3 mm TL) at a concentration of 0.1-0.05 ind/ml, cultivated at 25ºC with a phytoplankton mix. Pre-settled paralarvae (n=52, between 55 and 70 days old) were transferred to two 75L cylindrical grey tanks with natural shelters (shells) and adult artemia >5 mm as food. From days 75-157, 8 settled juveniles were kept together in a 150L tank at 18ºC and fed with live prey consisting of small crustaceans, mussels, and clams. Cannibalism recorded at day 157 (d157) forced the individualization of the juveniles (11.6-30.5g) in 150L cylindrical tanks. After a weaning period of 1 month, subadults were fed with frozen crabs and fish and wet weight was recorded quarterly during the on-growing stage (d120-500). From day 280, all octopuses (4 females and 3 males, 632-1034g) were placed in couples to allow reproduction until spawning behaviour was observed (d512). Reproduction behaviours (mating, spermatophore transfer, egg laying, egg care and spawning) were filmed and notes were taken to quantify these poorly known behaviours.

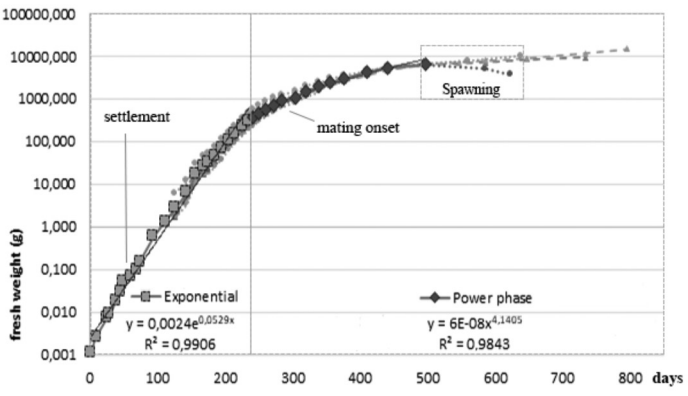

Fig. 1. Growth curves obtained from specimens reared in captivity for up to 829 days. Growth in *O. vulgaris* can be efficiently modelled with an exponential initial phase (light grey), followed by a slower power growth phase (dark grey) for sub-adults and adults.

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Results and Discussion

Survival rates of the planktonic stage ranged between 6.6-13.2% (d55). Settlement took ~ 20 days, starting with transparent swimming paralarvae with ~22-28 suckers that palpate the walls and bottom of the tank and finishing with truly benthic juveniles (>32 suckers) covered with pigmented cells hidden within shells. Survival rate during this poorly known phase was 15.4% (d55-75). After this critical stage, no mortality was recorded apart from a cannibalistic event at d157, recorded on video, and two escapes at d315 (female, 1,1 kg) and d436 (male, 4.6 kg). Males grew bigger (9.6-14.6 kg) and lived longer (d796-829) than females (7.2-10.1 kg, d560-638), the largest and oldest specimens ever recorded.

Octopus vulgaris growth curve can be effectively modelled with an initial exponential phase (d0~240), followed by a slower power growth phase (equations shown in Fig. 1). Remarkably, the inflexion point between both phases matched with the development of enlarged suckers in males and the onset of mating behaviours (~220-420g). Contrary to that found in other octopods (Forsythe and Van Heukelem, 1987; Semmens et al. 2004), growth was similar for females and males during both the exponential (SGR 4±1.5% and 4.2 ±1.4%) and power phase (SGR 1.4±0.7% and 1.4 ±0.6%), despite males reached a higher maximum weight at the end of their life, due to their superior longevity and absence of parental care.

Up to 52 copulations were observed in 7 months. Spermatophore "pumping" speed (i.e. the transfer from the penis until the end of the hectocotylus, sensu Wells & Wells, 1972) was estimated to be around 0.36 m/s. Spawning started from d512 to d546 at sizes ranging between 6.3-7.7 kg. Egg laying took between 16 and 21 days at 18ºC and 14ºC, respectively, while 241 and 175 egg strings were collected. Incubation took 49 days at 18ºC and 63 days at 14ºC. Hatching lasted for up to 25 days. The dissection of the oviducal glands revealed live sperm more than 4 months after the last recorded copulation.

Acknowledgements

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References

Domain, F., Jouffre, D. & Caverivière, A. 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. Journal of the Marine Biological Association of the United Kingdom. 80:699–705.

Forsythe, J. W., and Van Heukelem, W. F. (1987). Growth. In 'Cephalopod Life Cycles, Vol. II: Comparative Reviews'. (Ed. P. R. Boyle.) pp. 135–156. (Academic Press: London, UK).

Iglesias, J., Otero, J. J., Moxica, C., Fuentes, L., & Sánchez, F. J. (2004). The completed life cycle of the octopus *(Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using Artemia and zoeae, and first data on juvenile growth up to 8 months of age. Aquaculture International, 12, 481–487.

Perales-Raya, C., Jurado-Ruzafa, A., Bartolomé, A., Duque, V., Carrasco, M.N. & Fraile-Nuez, E. 2014. Age of spent *Octopus vulgaris* and stress mark analysis using beaks of wild individuals. Hydrobiologia. 725:105–14.

Semmens, J.M., Pecl, G.T., Villanueva, R., Jouffre, D., Sobrino, I., Wood, J.B. & Rigby, P.R. 2004. Understanding octopus growth: Patterns, variability and physiology. Marine and Freshwater Research. 55:367–77.

Wells, M.J. & Wells, J. 1972. Sexual displays and mating of *Octopus vulgaris* Cuvier and *O. cyanea* Gray and attempts to alter performance by manipulating the glandular condition of the animals. Animal Behaviour. 20:293–308.

OMEGA-3 LONG CHAIN POLYINSATURATED FATTY ACIDS DHA AND EPA DROVE THE FEEDING BEHAVIOUR OF JUVENILE RAINBOW TROUT

J. Roy, Y. Mercier, L. Tonnet, C. Burel, L. Larroquet, G. Corraze, F. Terrier, S. Panserat, S. Skiba-Cassy

INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, UMR1419 Nutrition Metabolism and Aquaculture, Aquapôle, F-64310 Saint-Pée-sur-Nivelle, France
Email : jerome.roy@inrae.fr

Introduction

The control of feed intake in fish in aquaculture requires the development of new techniques to improve diet composition, feed conversion efficiency and growth. The aim must be sustainability and an effective use of resources. The effect of replacing traditional aqua-feed ingredients (fishmeal and fish oil) by a 100% plant-based diet is known to drastically decrease fish performance (survival and growth). One disadvantage of the use of plant ingredients relies on the modification of the nutrient composition of the diet with totally absence of essential ω-3 long chain polyunsaturated fatty acids (ω-3 LC-PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In fish ω-3 LC-PUFAs are known to be essential in their life cycle, promoting optimal growth and survival, health, reproduction and offspring development. In farmed fish, except for a study about the effect on the origin of the fat source on feed selection in fish, the specific role of lipids and particularly ω-3 LCPUFA on feeding behavior (preference, food intake and uneaten food) has not yet been investigated. This information is crucial to understand if the decrease of survival rate and growth performance of fish fed with plant-based diets could be explained by the modification of feeding behavior due to the absence of ω-3 LC-PUFAs.

Materials and Methods

Using self-feeder, the present study examined the feed preference of rainbow trout *Oncorhynchus mykiss* for three diets containing distinct levels of omega-3 long chain polyunsaturated fatty acids (ω-3 LCPUFA): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (0% for low, 5% for medium and 20% for high, total fatty acid content). Feed preference values for each group (low *v.* medium ω-3 diets, medium *v.* high ω-3 diets and low *v.* high ω-3 diets) were observed using two self-feeders positioned at opposite sides of the tank. The hypothesis was that the decrease of fish growth and survival rate of fish fed with 100% plant-based diet could be explained by the absence of ω-3 LCPUFA relating to decrease of food intake. This could explain the tasting role of ω-3 LCPUFA in the feeding behavior of rainbow trout (which reflects the motivation to consume feed).

Results

The results showed that rainbow trout could discriminate between the diets containing different level of ω-3 LCPUFA even if unable to differentiate between level of 5% (no preference observed in low *v.* medium ω-3 diets). Overall they had a preference for diet high in ω-3 LCPUFA: 59.5% preference for high ω-3 diet in high *v.* low ω-3 diets, and 75.6% preference for high ω-3 diet in medium *v.* high ω-3 diets respectively.In parallel, the impact of dietary *ω*-3 LC-PUFAs on central molecular mechanisms regulating feeding behavior was determined. Overall transcript genes related to pro-inflammatory cytokines, inflammation, antioxidant status, cortisol pathway, serotoninergic pathways and dopaminergic pathways were down-regulated in the juveniles trout fed with the high ω-3 LC-PUFAs diet.

Conclusion

In conclusion, our data revealed that a diet rich in ω-3 LC-PUFAs drove the feeding behaviour of juvenile rainbow trout with affected a relatively high proportion of the brain function in rainbow trout through mechanisms comparable to those characterized previously in mammals.

ESTIMATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) RESPIRATION RATE WITHIN A COMMERCIAL RACEWAY USING A DATA ASSIMILATION APPROACH

Edouard Royer [(1)*], Roberto Pastres [(1),(2)]

1) Università Ca'Foscari, Campus scientifico, Via Torino, 155, 30170 Mestre, Venezia (Italy)
2) Bluefarm, s.r.l., Venice, Italy
E-mail: edouard.royer@unive.it

Introduction

Innovations and decrease in costs of sensors are making it possible to apply to aquaculture the concept of "Precision Livestock Farming", introduced in the agrifood sector in early 2000. The implementation of the "Precision Fish Farming" (PFF) framework (Fore et. al., 2018) is likely to revolutionize the aquaculture industry, leading to a new generation of softwares and decision support tools, based on dynamic data driven models. In a previous work (Royer et al., 2021), we proposed a PFF based model of oxygen mass-balance for rainbow trout within a commercial raceway, showing that it is possible to dynamically estimate hourly fish respiration rate in commercial farming condition and, on this basis, to improve current control systems. In this study, the estimation method was improved by introducing a data assimilation procedure (Kalman Filter) that allows one to correct the respiration rate as data acquisition goes by and, on this basis, to obtain more accurate short-term predictions of DO concentration.

The research leading to these results has received funding from the European Union's H2020 Framework Programmefor 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. The purpose of DA is two-fold: i) to correct the prediction of output variables, based on the information provided by any new datum; ii) to improve the estimation of the remaining state variables, which may include also some key model parameters. Key to that is the assumption that the state variables are regarded as stochastic ones: the dynamic of their expected values is driven by a set of governing differential equations, but their fluctuations are driven by the differences between model output and observations.

The PFF experimental setup is composed of a two probes YSI EXO2 (inlet and outlet) for water quality monitoring (Temperature and DO concentration), and a real-time biomass measurement system (Biomass Daily), tested for the first time in a trout farm.

The dynamic oxygen model includes contributions from water fluxes (inlet and outlet), liquid oxygen supply, fish respiration and reaeration (exchanges with the atmosphere).

The respiration rate was included in the state vector and, therefore treated as a stochastic variable. The Kalman Filter was applied, forcing the models with hourly values of DO and temperature for a one-month summer period.

Results and discussion

Results show that the model allows one to accurately predict short term variation of DO, thus providing relevant information for managing the oxygenation system, in order to avoid hypoxic conditions, maximizing fish welfare and minimizing energy cost (oxygen supply). Furthermore, the model provides an accurate estimation of the respiration rate (Figure 1), which is recursively estimated using the DA algorithm, showing a a daily pattern in line with light and feed entrainment oscillators. Using the cumulative daily oxygen consumption and, the daily feed ration, a daily average of the Oxygen Feed Ratio (Colt & Watten, 1988) was computed. These results opens the way toward smart oxygen controls, which can lead to optimize the diel oxygen supply in relation to water temperature, feed ration and other poorly known external forcings, i.e. water turbidity.

References

Colt, J., Watten, B., 1988. Applications of pure oxygen in fish culture. J. Aquac. Eng. Fish. Res. 7, 397–441.

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. Precision fish farming: A new framework to improve production in aquaculture. Biosystems engineering 173 (20 18): 176 -193.

Pastres, R., Ciavatta, S., Solidoro, C., 2003, The Extended Kalman Filter (EKF) as a tool for the assimilation of high frequency water quality data. Ecological Modelling, 170, 227-235.

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.

TESTING A REAL TIME WEIGHT MEASUREMENT SYSTEM IN A TROUT RACEWAY

Edouard Royer [(1)] Matteo Bolzonella [(1)*], Roberto Pastres [(1),(2)]

1) Università Ca'Foscari, Campus scientifico, Via Torino, 155, 30170 Mestre, Venezia (Italy)
2) Bluefarm, s.r.l., Venice, Italy
E-mail: edouard.royer@unive.it

Introduction
The Precision Fish Farming framework (Fore et al. 2018) was recently applied to the online estimation of DO demand in a trout raceway (Royer&al., 2020) Key to the implementation of this novel approach is the availability of real time or quasi real time data concerning the evolution of the external forcings, the environmental variables within the farming systems and the so called "animal variables", e.g. fish size,. In this study, a commercial real-time weight monitoring system, designed for salmon cages, was tested in a rainbow trout farm in Northern Italy, in order to monitor the weight evolution within a raceway.

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
The Biomass Daily (BD) system (https://vakiiceland.is/biomass-daily). is composed by an Infrared sensor, a sending box and antenna, which sends the signal to a computer: from here data are sent to the cloud and processed, in order to display relevant information on a dashboard . The latter allows one to visualize daily average parameters, i.e average weight, length, conversion factor, water temperature. Furthermore, the raw data can be downloaded and post- processed.

The system was deployed in a rainbow trout farm in Preore, Northern Italy and used to monitor three cohorts of fish, moving it from one raceway to another whenever necessary. Three different population were then monitored which average weights were around 70 g, 300g, and 1200 g.

The data were used to study the weight distribution of the population, and to compute daily mean and standard deviation, which were compared with the farmer estimates, based on direct sampling. A qqtest was run on population in order to assess its normal distribution, Furthermore, the daily pattern of the number of detections was investigated, in relation to that of fish activity within the raceway.

Results and discussion
Results from normality test show that gaussian distribution of weights can reasonably be confirmed, whenever measurements' number are above a minimum threshold: therefore, the daily mean and standard deviation (Figure 1) can adequately represent the dynamics of the weight distribution. A better knowledge of population distribution could lead to an improvement of feed ration estimation for a raceway, based not only on average weight and fish number but on weight distribution and fish number.

Furthermore, comparison of these statistical indicators with farmer's data shows that BD system succeed in estimating average fish weight. These indicators could then be used in farm management dynamic models based on real-time data acquisition.

Temporal distribution of measurements (Figure 2) show a clear daily pattern that can be correlated to light and temperature daily cycles and/or to feed activity. BD measurements temporal distribution could then be considered as a reliable proxy of fish activity within the raceway and a dynamic model could take advantage of such information when trying to describe daily fish metabolism.

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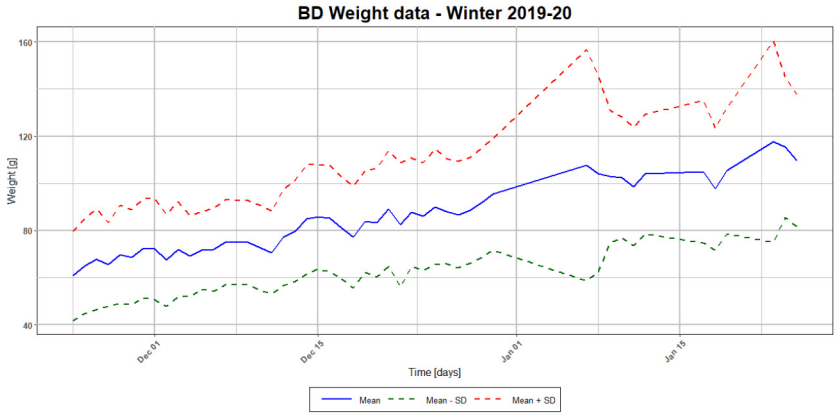

Figure 1: Daily mean and standard deviations of BD weight measurements

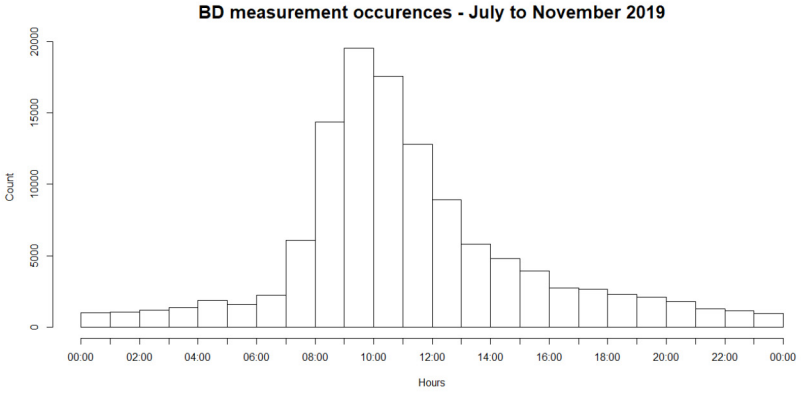

Figure 2: Histogram of measurement occurrences within a day

References

Bolliet, V., Aranda, A., Boujard, T., 2001. Demand-feeding rhythm in rainbow trout and European catfish synchronization by photoperiod and food availability. Physiol. Behav. 73, 625–633.

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. Precision fish farming: A new framework to improve production in aquaculture. Biosystems engineering 173 (20 18): 176 -193.

Heydarnejad, M.S., Purser, G.J., 2009. Agonistics acts as a possible indicator of food anticipatory activity (FAA) in rainbow trout (Oncorhynchus mykiss). Iran. J. Vet. Res. 10, 137–145.

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.

EFFECTS OF SEVERAL NEUROACTIVE COMPOUNDS ON THE INDUCTION OF THE METAMORPHOSIS PROCESS IN THE LARVAE OF THE MANILA CLAM, *Ruditapes philippinarum* (ADAMS AND REEVE, 1850)

José Luís Sánchez[1*], Antonio J. Pazos[1], Susana Nóvoa[2], Dorotea Martínez-Patiño[2], Justa Ojea[2] and M. Luz Pérez-Parallé[1]

[1] Departamento de Bioquímica y Biología Molecular. Instituto de Acuicultura. Universidade de Santiago de Compostela. Contantino Candeira s/n. Campus Vida. 15782-Santiago de Compostela. Spain
luz.perez-paralle@usc.es
[2] Centro de Investigacións Mariñas. Consellería do Mar. Xunta de Galicia. 27700 Ribadeo, Lugo, Spain

Introduction

Metamorphosis is one of the most critical points in the development of bivalve molluscs. The transition from a free-living larva to a sessile juvenile is modulated by chemical cues that bind to receptors and trigger a response that leads to morphological and behavioural changes of metamorphosis. Several reports have shown that different neuroactive compounds mimic the natural chemical cues such as L-DOPA in *Crassostrea gigas* and *Mytilus edulis* (Coon et al. 1985; Dobretsov and Qian, 2003; Vogeler et al. 2019), γ-aminobutyric acid (GABA) in clams, the mussel *Aulacomya maoriana* and the oyster *Ostrea edulis* (García-Lavandeira et al. 2005; Alfaro et al. 2011; Mesías-Gansbiller et al. 2008, 2013) and catecholamines in mussels, pectinids and oysters (Coon et al. 1985; García-Lavandeira et al. 2005; Mesías-Gansbiller et al. 2008, 2013; Vogeler et al 2019). In this study, we investigated the effect of highly effective inducers on the larval metamorphosis of the Manila clam *Ruditapes philippinarum* (Adams & Reeve 1850).

Materials and methods

Five experiments of induction of the metamorphosis of R. *philippinarum* larvae have been set in the laboratory following the protocol described in García-Lavandeira et al. (2005) and Mesías-Gansbiller et al. (2013). Competent larvae were supplied by the CIMA of Ribadeo. Larvae at a density of 4 larvae/mL were treated with several neuroactive compounds: GABA, L-DOPA, the catecholamines epinephrine and norepinephrine and IBMX for 72h. Three different concentrations were used: 10^{-4}M, 10^{-5}M and 10^{-6}M. Each experiment was performed in triplicate for each of the inducer concentrations and for the control with sea water and without potential inducer. Metamorphosis has been monitored with a Nikon SMZ-2T microscope. A larva was considered to have passed metamorphosis when it has lost its velum and crawled with its foot. The metamorphosis rate was calculated as 100 × (total number of larvae metamorphosed/total number larvae). Results were analysed with SPSS 20.0 statistical package and metamorphosis rates were analysed by ANOVA. Results were considered significantly different when $p \leq 0.05$.

Results and discussion

GABA, epinephrine and IBMX fail to increase the metamorphosis rate of Manila clam larvae comparing with control larvae after 72h of treatment. However, higher percentages of metamorphosis were induced by the concentration 10^{-4}M. These three compounds have not toxic effects on the larvae. In contrast, L-DOPA and norepinephrine have negative effects on the metamorphosis rates of *R. philippinarum* larvae, especially in the treatments with higher concentrations of these two inducers. In these cases, norepinephrine and L-DOPA have toxic effects, in fact, they significant increase the mortality rate of the larvae. García-Lavandeira et al. (2005) previously identified GABA and epinephrine as active inducers of metamorphosis of in two clam species *V. pullastra* and *R. philippinarum*, although the metamorphosis rates were very low compared to those obtained in the present study.

Other inducers such as acetylcholine and serotonin have previously been shown to be effective on Manila clam larvae (Urrutia et al. 2004). A great variability in the response of the larvae to different inducers both within the same species and between species is evident and it might be explained in the first case by the variability in the degree of larval development of individuals of the same cohort and in the second case by the different receptor pathways involved.

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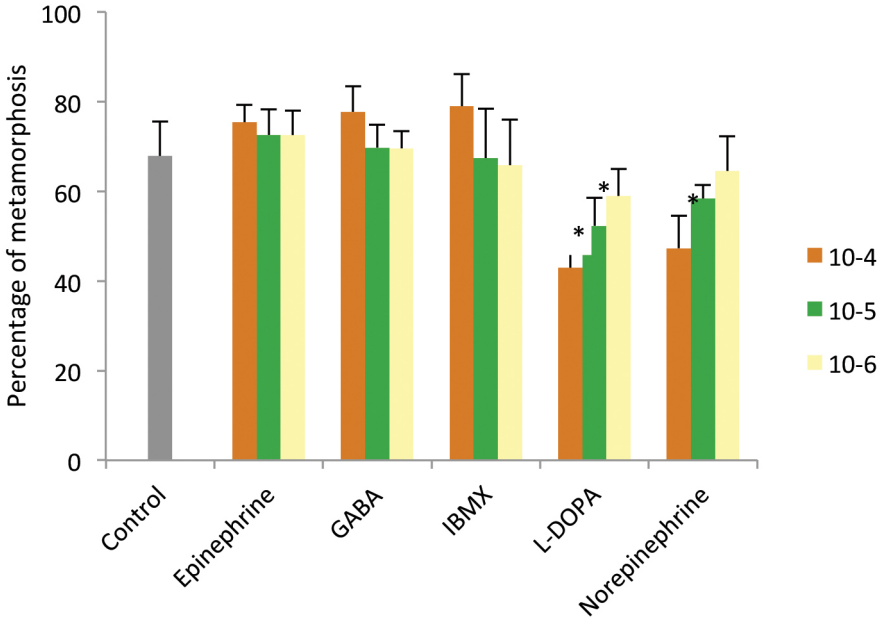

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Acknowledgement

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References

Alfaro, A.C., T. Young and A.M. Ganesan. 2011. Aquaculture 322-323, 158-168.

Coon, S.L., D.B. Bonar and R.M. Weiner. 1985. J. Exp. Mar. Biol.Ecol. 94, 211-221.

Dobretsov, S.V. and P-Y Qian. 2003. Biofouling 19, 57-63.

Estupinan, B. and J.H. Waite. 1988. J. Shellfish Res. 54, 189-190.

García-Lavandeira, M., A. Silva, M. Abad, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2005. J. Exp. Mar. Biol. Ecol. 316, 149-156.

Mesías-Gansbiller, C., E.A. Bendimerad, G. Román, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2008. J. Shellfish Res. 27, 261-264.

Mesías-Gansbiller, C., A. Silva, V. Maneiro, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2013. Aquaculture 376-379: 85-89.

Urrutia, P.M., Okamoto, K. and Fusetani, N. 2004. J. Shellfish Res. 23: 93-100.

Vogeler, S., Wikfors, G.H., Li, X., Veilleux, D., Miller-Ezzy, P. and Joyce, A. 2019. Aquaculture 511, 634173.

TOWARDS SEA BASS AND SEA BREAM LARVAE AND JUVENILE QUALITY MONITORING

Soraia IG Santos[1]*, Katerina Moutou[2], Giorgos Koumoundoros[3], Marco Galiotti[4], Deborah M Power[1,5]

[1]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal
[2]University of Thessaly, Biopolis, 41500 Larissa, Greece
[3]Biology Department, University of Crete, 70013 Heraklion, Crete, Greece
[4]Department of Agricultural, Food, Environmental and Animal Sciences (DI4A), University of Udine, 33100 Udine
[5]Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China
Email: dpower@ualg.pt

Introduction

European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*) are important aquaculture species in the Mediterranean (www.fao.org). The hatchery production stage of these species is of crucial importance as it supplies the juveniles that determine the productivity and quality of the commercialized fish. Despite major improvements in larval management since the 1990's, the survival, growth performance, incidence of deformities, and pathogens remain a challenge sector wide. PerformFISH is an industry driven European project that has as the objective of workpackage 2 to identify factors affecting hatchery key performance indicators (KPIs) through industry-wide monitoring and meta-analysis. The project is developing tools to improve and predict larval quality during the hatchery phase by using existing technical and biological knowledge to identify, optimize, and validate robust biomarkers of larval quality. To achieve industry-wide monitoring it is essential that standard operating procedures exist for the sampling, shipping, and storage of collected samples. Coupled to this is the need for standardized screening methods across collaborating analytical laboratories. The objective of the present study was a) to establish a standardized sampling strategy and establish a technical sampling manual and b) develop a standardized screening approach for the selection of potential morphological and molecular biomarkers of interest.

Methods

Development of the sampling strategy and methods was carried out in collaboration with industry to ensure that critical knowledge held by hatcheries was taken into consideration during the design of the sampling procedure. Specimen stage, sampling approach, sample number and preservation method were considered and validated in preliminary trials and through an iterative approach between scientists and hatcheries. Criteria considered during the design of the hatchery sampling procedures was a) ease of classifying stage, b) adequacy of sample size and representation of larvae, c) analytical procedures to be performed, d) the efficiency of the preservation methods, both short- and long-term e) health and safety issues related to methods, f) the limitations and logistics of transport and likely detrimental effects of duration of transport. To ensure the legacy of the implemented approaches a detailed technical sampling manual was elaborated and cost-effective and easy to implement procedures were given priority. The sampling methods proposed in the technical sampling manual were tested to validate them and confirm they favoured good sample stability and quality. Standardized procedures were established for morphological and molecular biomarker identification. In the case of quantitative PCR (qPCR) screening for candidate markers an optimized approach was developed and then screening methods for qPCR were standardized across the analytical laboratories.

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Results:

Results:

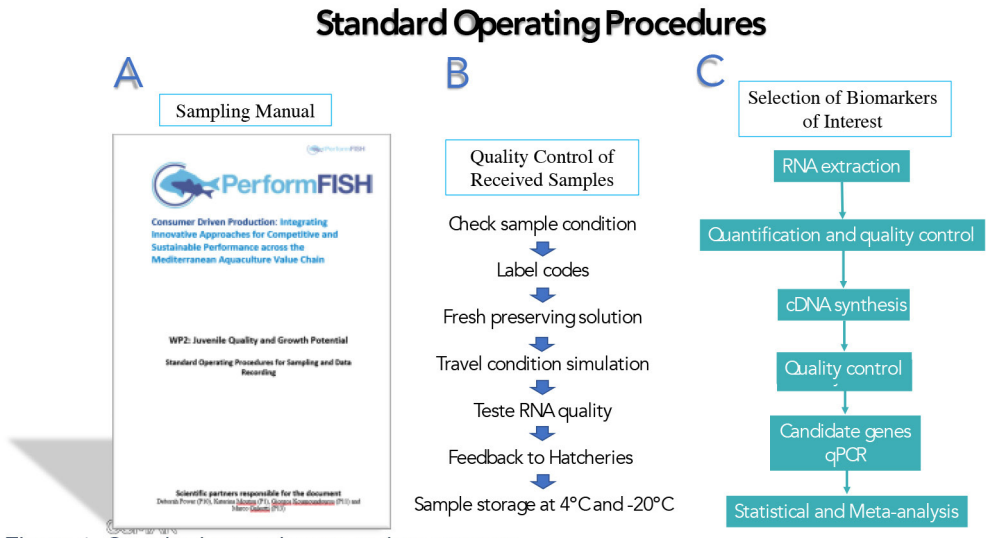

Figure 1- Standard operating procedure strategy

Discussion and conclusion

The manual for the sampling strategy was successfully implemented by the hatcheries after preliminary trials. The main failures were linked to inadequate ratios between the samples and solution for preservation and this was overcome through development of a sampling video. Another frequent failure was incorrect sample preparation prior to shipping and mislabelling or incorrect information about the samples, which was overcome by providing a demonstration video and developing excel sheets with labelling and cross-checks on receipt of the samples. A large biobank of hatchery-based samples was generated, and it consists of samples fixed in RNA later, methanol, Bouin and formamide fixative. All samples were screened on reception to remove samples demonstrating poor characteristics (e.g. fixation, fixative volume, evidence of breakdown of larvae) and all solutions were substituted prior to long-term storage. Several samples were randomly selected on their arrival at the RDI laboratories and RNA and genomic DNA extracted to confirm the quality of the samples. RNA was used to synthesise cDNA and adequate DNase treatment to remove genomic contamination was confirmed by amplifying 18S rRNA in all samples by RT-PCR. Molecular larval quality markers were developed using a candidate genes approach by screening "in-house" primers and primers reported in the literature on larval cDNA. Selection of candidate genes was based on analysis of their abundance in cDNA from pools of different larval stages and comparison of their abundance in cDNA pools from larvae classified as "good" or "bad" quality (based on survival, growth, malformation incidence etc). By screening approximately 60 candidate genes in sea bream and sea bass a core set were selected to differentiate "good" quality and "bad" quality larvae and being used in meta-analysis.

Acknowledgments

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SUCCESSFUL REARING OF THE COLD WATER SEA CUCUMBER *Parastichopus tremulus* JUVENILES

E. Schagerström*, K. S. Sundell

Department of Biological and Environmental Sciences and SWEMARC, University of Gothenburg, Göteborg, Sweden
ellen.schagerstrom@bioenv.gu.se

The red sea cucumber *Parastichopus tremulus* is an aspidochirotide cold water species, with a NE Atlantic distribution. The species is a potential candidate for integrated multi-trophic aquaculture (IMTA) in Scandinavia, and research on breeding and rearing this species has recently begun within the Swedish mariculture research centre SWEMARC. Previous studies (*in review*) showed that although *P. tremulus* is a cold water species, larval development was faster in 16 ^{0}C compared to 8 ^{0}C, the temperature for the natural habitat of the adults. This is combined with several observations in the Gullmar fjord on the Swedish west coast of an upwards migration of sexually mature *P. tremulus* during summer months, suggesting that warmer temperatures are favourable for reproduction. Mature animals were spawned in the laboratory during late July 2020 using heat shock treatment to induce gamete release. Eggs were fertilized and kept at 16 ^{0}C. Auricularia larvae were developed within 4 days and were fed commercial instant algal mix (Shellfish Diet 1800®, Reed Mariculture, Campbell, CA) at a calculated density of 10.000 – 20.000 cells/ml. After 37 days, larvae reached late auricularia stage, with well developed hyaline spheres. Doliolaria were discovered from 40 days post fertilization and developed into early pentactula after 1-2 days. Pentactula were fed naturally settled biofilm of benthic diatoms enriched with some instant algal mix. After 52 days the third podia had developed and the now 2 mm long juveniles began moving around. This is the first time juveniles of *P. tremulus* has been reared artificially. It is a vital step towards developing a rearing protocol and including this species in IMTA systems.

CLIMATE CHANGE RISKS AND ADAPTATION MEASURES FOR THE AQUACULTURE SECTOR OF EUROPEAN ISLANDS

Lena Schenke* and Tamás Bardócz, Kyra Hoevenaars, Nesrine Boulila

AquaBioTech Ltd, Central Complex, Naggar Street, Targa Gap, Mosta, Malta
Email: les@aquabt.com

According to the fifth Assessment Report of the Intergovernmental Panel on Climate Change, the warming of the climate system is unequivocal and continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe and irreversible environmental impacts, which can induce large socio-economic damage. New policies on mitigation and adaptation are needed. In the field of Climate Change adaptation, policy makers must have detailed and accurate information about likely impact chains and about the costs and benefits of possible resilience strategies corresponding to the potential decarbonisation pathways. EU islands are particularly vulnerable to Climate Change consequences.

The SOCLIMPACT project aims at modelling and assessing downscaled Climate Change impacts and their socioeconomic impacts in European islands for 2030-2100, complementing current available projections for Europe, and nourishing actual economic models with non-market assessment. The project is developing a thorough understanding on how Climate Change will impact the EU islands located in different regions and focuses on the Blue Growth sectors.

One of the fastest growing Blue Growth sectors is aquaculture. Climate change impacts and risks for the aquaculture sector were identified based on climate hazards models, vulnerability factors and exposure of EU islands' aquaculture.

Two main climate change risks for marine cage aquaculture were identified:

- Change in production due to temperature changes in seawater
- Increased fragility of the aquaculture activity due to extreme weather events

Impact chains for these risks were developed and operationalised to identify the major risks and enable comparison between risks and islands.

Based on these climate change risks, adaptation measures and risk management options for the aquaculture sector were designed. These were used to develop detailed integrated adaptation pathways for each island.

Acknowledgement
The SOCLIMPACT project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 77661.

BIOFILM AND BIOFOULING DEVELOPMENT ON NOVEL SENSING SURFACES IN A MARINE RECIRCULATED AQUACULTURE SYSTEM

Eirini Schoina *[1], Robert Daly[2], Pierre Lovera[2], Alan O'Riordan[2], Brendan O'Flynn[2] and George-John. Nychas[1]

[1] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
[2] Tyndall National Institute, University College Cork, Cork T12 R5CP, Ireland
E-mail: eschoina@aua.gr

Introduction

The control of the aquaculture systems is actualized with a variety of monitoring sensors that are operating 24/7 and providing measurements of water quality parameters. Once these sensors are introduced into the water, they serve as a new niche for microbial colonization and subsequent installation of macroorganisms. The development of biofouling on sensing surfaces and electrodes can affect their operation, leading to acquisition of inaccurate measurements.

The aim of the present study was to assess the biofilm and biofouling development on a new sensing surface developed by Tyndall, under real aquaculture conditions. The study was performed in a marine recirculated aquaculture system (RAS), so as to allow natural biofilm/biofouling development. The methods employed included microbiological analysis on the developed biofilms implemented with molecular techniques, as well as epi-fluorescent microscopy to monitor the biofouling installed on the surface of the sensing material.

Methods

Folded plastic nets containing coupons in triplicates of sensor's material and stainless steel as reference were placed into an aquarium tank, part of a marine RAS, where sea bass adults were reared.

Silicon chip sensor devices were fabricated using common microelectronic processes as described previously (Wahl et al., 2018). In brief, interdigitated gold microbands were patterned in resist by photolithography followed by metal evaporation (Ti/Au 5/50 nm) and standard lift-off. Similarly, photolithography, metal evaporation (Ti/Au 10/90), and lift-off procedures were then employed to overlay electrical interconnection tracks including peripheral probe pads. Macroscale gold and platinum counter and pseudo-reference electrodes, respectively, was also deposited during this process. Finally, a silicon nitride passivation layer (500 nm thick) – representing the majority of the surface of the sensors chip - was deposited to passivate the entire chip and windows selectively opened with a dry etch to allow exclusive contact between the working, reference and counter electrodes with the solution of interest.

At 14, 28 and 42 days after placement, microbiological analysis was conducted for planktonic bacteria in the water and biofilm cells. For the latter, sessile cells on coupons (in dublicates) were sampled following a bead vortexing method. Enumeration of viable biofilm and planktonic cells was performed on Marine Agar (MA) plates for marine heterotrophic bacteria and on TCBS agar plates for the detection of presumptive *Vibrio* species.

Temporal changes of the bacterial community composition of the water and biofilms' comparison were assessed by a PCR-DGGE method. Following a culture dependent technique, the total amount of bacterial colonies grown on MA plate of the minimum dilution was collected for both materials and for the water sample at each time point. Cells were lysed using a lysozyme based protocol to extract bacterial DNA. PCR-DGGE was performed as previously described (Schoina et al., 2019), targeting the hypervariable V3-V5 region of the 16S rRNA gene.

Biofilm/biofouling development monitoring was performed by fluorescent microscopy. At every sampling coupons were retrieved, biofouling was fixed with methanol and stained with Acridine Orange dye, to be observed under the microscope.

Results and Discussion

The population of planktonic marine heterotrophic bacteria throughout the experimental period ranged from 10^4 to 10^5 CFU ml^{-1}, in accordance with previous studies in similar marine RAS setups (Schoina et al. 2019). The presence of presumptive *Vibrio* species in the water samples was high, representing 32-55% of the population of cultured marine heterotrophs. The population of biofilm cells on both materials was comparable at each time point, being stable at 10^6 CFU cm^{-2}. Presumptive *Vibrio* species were also detected in the biofilms. Their participation in the biofilm increased through time, starting from 10^3 CFU cm^{-2} at day 14 and reaching 10^4 CFU cm^{-2} at days 28 and 42. This observation confirms that biofilms may act as a reservoir for potentially pathogenic bacteria in RAS setups, as previously reported (Bourne et al., 2006).

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The DGGE profiles revealed that the RAS water microbial association remained unchanged during the experimental period, in accordance with previous reports (Attramadal et al. 2012). Regarding the biofilm communities, DGGE fingerprinting showed high similarity of predominant operational taxonomic units (OTUs) sampled at the same time points, being independent of the type of material, as previously described when comparing stainless steel and glass (Schoina et al., 2020), while observing a dynamic succession of biofilm predominant OTUs. Similar findings of biofilm dynamics has been previously shown in other types of surface materials (Bourne et al., 2006).

Epi-fluorescent images revealed that after 14 days of immersion in the water, coupons were equally covered by biofouling, regardless of the material type. Accordingly, at days 28 and 42 biofouling was predominant on both types of surfaces. Even though the experiment was performed in a RAS where natural seawater is introduced after UV treatment, biofouling organisms were installed on the test surfaces.

Conventional microbiological analysis and molecular techniques, in combination with microscopy demonstrated the dynamics of biofilms/biofouling development on novel sensing surfaces in a Mediterranean RAS. The tested sensing surface was subjected to biofilm adherence with high contribution of *Vibrio* species, as well as to biofouling installation, with unknown effects on its function.

This work is part of a series of *in situ* studies for prolonged time periods, as an approach for better assessment of the biofilm and biofouling formation on novel sensing surfaces.

Acknowledgment
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References

Attramadal K.J.K., I. Salvesen, R. Xue, G. Øie, T.R. Størseth, O. Vadstein and Y. Olsen. 2012. Recirculation as a possible microbial control strategy in the production of marine larvae. Aquaculture Engineering 46: 27–39.

Bourne D.G., L. Høj, N.S. Webster, J. Swan and M.R. Hall. 2006. Biofilm development within a larval rearing tank of the tropical rock lobster, *Panulirus ornatus*. Aquaculture 260: 27–38.

Schoina E., E. Miliou . and G-J Nychas. 2019. Evaluation of the biofilm formation on stainless steel surfaces in a marine recirculated system. In: Aquaculture Europe 19 conference, Berlin, Germany, pp1389-1390.

Schoina E., I. Bru and G-J Nychas. 2020. Dynamics of Biofilm Formation in a Mediterranean RAS. In: ASM Microbe 2020 conference (online).

Wahl, A., Seymour, I., Moore, M., Lovera, P., O'Riordan, A., Rohan, J. Diffusion profile simulations and enhanced iron sensing in generator-collector mode at interdigitated nanowire electrode arrays, Electrochimica Acta, 277(2018) 235-43

EUROPEAN SEABASS (*Dicentrarchus labrax*) ALLERGENICITY AND FISH QUALITY AFTER CREATINE SUPPLEMENTATION

D. Schrama[1,2,*], C. Raposo de Magalhães[1,2], S. Engrola[2], P.M. Rodrigues[1,2]

[1]Universidade do Algarve, Campus de Gambelas, Faro, Portugal
[2]CCMAR, Universidade do Algarve, Faro, Portugal
E-mail: dschrama@ualg.pt

Introduction

European seabass (*Dicentrarchus labrax*) is a farmed marine fish of highly economic importance in the Mediterranean area [1]. Nonetheless, it is also a species which shows high allergenicity in fish allergic patients, resulting in an IgE-mediated allergic reaction [2]. The highly stable β-parvalbumin is the main protein responsible for this allergic reaction [2]. Its structure contains two helix-loop-helix motifs which bind to calcium or magnesium and are involved in the transport of these ions [3]. Previous studies in rat showed that the need for calcium buffering by parvalbumin decreased due to creatine supplementation [4]. With this knowledge the objective of this study was to lower European seabass allergenicity through the feeding of diets supplemented with creatine.

Methodology

Twenty European seabass juveniles, per triplicate tank, with an initial body weight (IBW) of 186 ± 0.83 g were reared in 500L conical tanks for 91 days. Four different experimental diets were tested namely, Control (CTRL, commercial diet without creatine supplementation), 2, 5 and 8% of creatine supplementation in the commercial diet, which will be referred as Creat2, Creat5 and Creat8, 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 proteomic analysis (2D-DIGE). Protein expression level was compared using the Samespots software and normalized data were used for statistical analysis using the R software (v3.5.3). Protein identification was performed using MALDI TOF/TOF and database search was performed on the Actinopterygii database.

Results

At the end of the trial, no significant differences in the final body weight (FBW) were shown between the four diets (Table1). Also, the feed conversion ratio (FCR) was not significantly affected by different supplementations.

Fish quality, muscle pH and *rigor mortis* index were measured during the first 72 hours *post-mortem*. Muscle pH decreased with time showing significant differences between the creatine diets at T4. Fish reached almost a full *rigor* index 24 hours *post-mortem* with no significant differences between treatments at any point.

Comparative proteomic analysis performed with 2D-DIGE showed a total of 485 spots, from which five spots showed significant difference in expression, like tropomyosin and myosin. The parvalbumin protein did not show a significant difference in expression.

Table1. Growth performance parameters of European seabass fed the experimental diets (CTRL, Creat2, Creat5, Creat8)

Diet	IBW (g fish $^{-1}$)	FBW (g fish $^{-1}$)	FCR
CTRL	186 ± 1	340 ± 12	1.63 ± 0.08
Creat2	183 ± 5	338 ± 13	1.73 ± 0.15
Creat5	186 ± 2	345 ± 10	1.67 ± 0.11
Creat8	189 ± 6	333 ± 14	1.83 ± 0.14

IBW – Initial body weight, FBW – Final body weight, FCR – feed conversion ratio. Data are represented by mean ± standard deviation.

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Conclusion

Dietary creatine supplementation preserved fish quality as edible food product. Proteomics showed that creatine supplementation in fish diets do not seem to influence critical metabolic pathways. Also, it was unable to modulate fish allergenicity. Even though the techniques used in this study, especially proteomics seem to be promising tools in studying protein expressions and effects of specific diets in aquaculture research.

Bibliography

1. Vandeputte, M.; Gagnaire, P.A.; Allal, F. The European sea bass: a key marine fish model in the wild and in aquaculture. *Anim. Genet.* **2019**, *50*, 195–206
2. 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
3. Carrera, M.; Cañas, B.; Piñeiro, C.; Vázquez, J.; Gallardo, J.M. Identification of commercial hake and grenadier species by proteomic analysis of the parvalbumin fraction. *Proteomics* **2006**, *6*, 5278–5287.
4. Gallo, M.; MacLean, I.; Tyreman, N.; Martins, K.J.B.; Syrotuik, D.; Gordon, T.; Putman, C.T. Adaptive responses to creatine loading and exercise in fast-twitch rat skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2008**, *294*, R1319--R1328

INTERACTION BETWEEN DIETARY LIPID LEVEL AND SEASONAL TEMPERATURE CHANGES IN GILTHEAD SEA BREAM *Sparus aurata*: EFFECTS ON PERFORMANCE, DIGESTIVE CONDITION AND GUT MICROBIOME

D. Scicchitano*[2], L. Parma[1], N. F. Pelusio[1], S. Busti[1], L. Mariani[1], L. Morsiani[1], F. Dondi[1], F. D'Amico[2], M. Candela[2], M. Yúfera[3], N. Gilannejad[3], F.J. Moyano[4], P.P Gatta[1], A. Bonaldo[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy
[2]Unit of Microbial Ecology of Health, Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[3] Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Apartado Oficial, 11519 Puerto Real, Cádiz, Spain
[4]Department of Biology and Geology, Universidad de Almería, La Cañada de San Urbano, 04120 Almería, Spain
Contact: daniel.scicchitano2@unibo.it

Introduction

The optimization of feeding strategy in relation to the environmental condition needs further investigation in order to maximise performance, fish health and fish quality of Mediterranean farmed species. Environmental temperature during seasonal changes may affects fish metabolism, digestive enzymes activity and gut bacterial community which may exert an effect on performance and tissue composition (Guerreiro et al., 2016). The effects of two dietary lipid levels during temperature changes were assessed on growth, feed efficiency, digestive enzyme activity and gut bacterial community of gilthead sea bream (*Sparus aurata*).

Material and Method

Two experimental diets with different lipid levels (16%, L16; 21%, L21) were tested in triplicated fish groups of 30 individuals (initial weight: 67.5g) and raised at two water temperatures (23 °C and 17°C) in the same recirculation system over 119 days. After 58 days fish were exposed to a switch in temperature (fish kept at 23°C were transferred to 17°C and the fish kept at 17°C were transferred to 23°C, HL and LH transition, respectively) while continued to receive the same diet in each group. Specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), somatometric indexes, nutritional indices, digestive enzyme activity, and gut microbiota (GM) by Next-generation sequencing were determined in the intermediate periods and at the end of the trial. Differences among treatments were considered significant at $P < 0.05$.

Results

At the end of the trial no significant diet effect on final body weight, SGR, FI and FCR were detected in fish exposed to HL transition (23/17) compared to those exposed LH transition (17/23) while gross lipid efficiency (GLE) and lipid efficiency ratio (LER) were higher in L16. After the temperature changes (days 58-119), FI and SGR were higher ($P < 0.05$) in L16 compared to L21 while mesenteric fat index was reduced. Before seasonal change, pepsin activity was higher in fish fed on diet L21 while for trypsin, a significant effect of rearing temperature and none of dietary lipid level was evidenced. After temperature changes, the combined effects of low lipid diet and low temperature conditions resulted in higher pepsin activity while trypsin, chymotrypsin and lipase were generally higher at high lipid content. For what concerns the gut microbiome, according to our findings in all groups there was a significant variation of the overall GM composition (assessed by PCoA; $P < 0.05$), except for fish fed with L16 diet and exposed to LH transition. In addition, only for fish fed with L16 diet and exposed to HL transition was highlighted a reduction of the internal ecosystem diversity (α-diversity; $P < 0.05$). Moreover, a diet impact was identified at warm temperature condition, where was highlighted a significant different GM layout between the two diets (assessed by PCoA; $P = 0.01$), with fish fed with L16 diet resulting in a higher load of *Lactobacillus*. Finally, the overall composition of the sea bream GM across groups was very similar. At phylum level the most abundant taxa were Firmicutes, Proteobacteria and Actinobacteria which represented about 88% of the whole gilthead seabream GM. At family level, we found that the gilthead seabream GM was dominated almost entirely by *Lactobacillaceae*, which represented around 60% of the whole ecosystem in all groups. While, with a focus on the genus level, specific compositional differences among groups were detectable, how showed in Fig. 1 (Wilcoxon rank-sum test $P < 0.05$).

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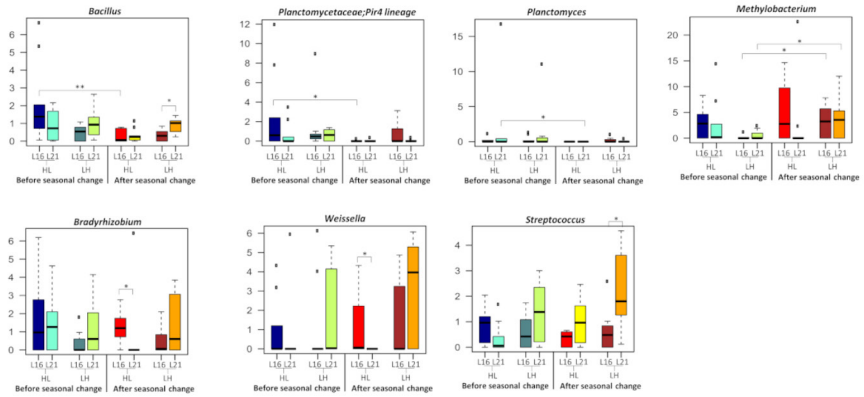

Figure 1. Distributions of relative abundance of genera that showed a significant variation between groups.

Discussion and conclusion

This study provided novel insight on the effects of dietary lipid level on growth, digestion condition and gut bacterial community of gilthead sea bream fed during seasonal temperature changes. More specific, high dietary lipid levels 21% did not improve growth and feed efficiency for both temperature transition in comparison to low dietary lipid levels 16%. On the other hand, L16 diet improved FI, growth, lipid efficiency and reduced perivisceral fat after temperature changes, especially in fish exposed to LH transition (17/23). After temperature changes, the combined effects of low lipid diet and low temperature condition resulted in higher pepsin activity while trypsin, chymotrypsin and lipase were generally higher at high lipid content. GM composition was similar among all groups with the dominance of beneficial taxa (such as *Lactobacillus*) representative of a healthy ecosystem in this species. However, after the temperature reduction fish fed with L16 diet was characterized by a higher abundance of the potential beneficial taxa *Weisella spp*, while after the increase of temperature, L21 diet supports the growth of the potential pathogens *Streptococcus spp*. According to the results, the utilization of 16% dietary lipid levels in gilthead sea bream should be preferred during seasonal temperature changes in order to optimize feed utilization and gut health.

Acknowledgements

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Rerefences

Guerreiro, I., Enes, P., Rodiles, A., Merrifield, D., Oliva-Teles, A., 2016. Effects of rearing temperature and dietary short-chain fructooligosaccharides supplementation on allochthonous gut microbiota, digestive enzymes activities and intestine health of turbot (*Scophthalmus maximus* L.). Aquaculture Nutrition, 22, 631-642.

DATAMINING FOR AQUACULTURE PRODUCTION ANALYSIS – APPLICATION TO AN OYSTER NURSERY AND A SEAWEED FARM

R. Serradeiro*, G. Zarifis, D. Komninos, H. Abreu, F. Hubert, J. Leitão, S. Cardoso, I. Martins, A. Nobre

RIASEARCH, Murtosa, Portugal
renataserradeiro@riasearch.pt

Aquaculture operations generate a large number of variables and data. Data science methodologies can support informed decision making. However, given the emergence of this field, farmers are still not using these techniques to improve their decisions and knowledge about their production. The objective of this presentation is to illustrate the application of data analytic methodologies in an oyster nursery and in a seaweed farm in the context of the Valormar project (24517 supported by Compete2020, Lisboa2020, CRESC Algarve2020, PT2020 and the EU through FEDER/ERDF). Before carrying out the relevant data analytics the company should define specific objectives and questions to be analysed. Also, a data flow for the analysis is needed, which requires a data management system for data gathering and assimilation. Datamining is an on-going task, during which are identified new questions, variables to be collected and further analysed.

The first step to make available advance analytics to production managers is to jointly explore the data with visual datamining, in order to give visual tools for identification of drivers of days in production, growth, mortality, among other. As an example of the data exploration carried out at the oyster nursery, Figure 1 shows the influence of the initial stocking month in the number of days to reach the target size.

With datamining tools, machine learning algorithms can be applied to create statistical models from historical data for prediction of key performance indicators (KPI) based on selected variables (predictors). Figure 2 illustrates the application of a Random Forest model to calculate the seaweed net harvest density of a given tank based on solar radiation, number of light hours, days in production and seeding density.

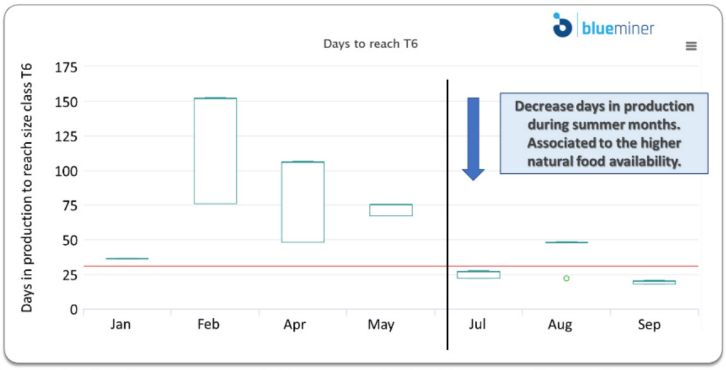

Figure 1. Oyster nursery: Influence of stocking month on the number of days to reach size T6 (~ 6 mm seeds, sell size).

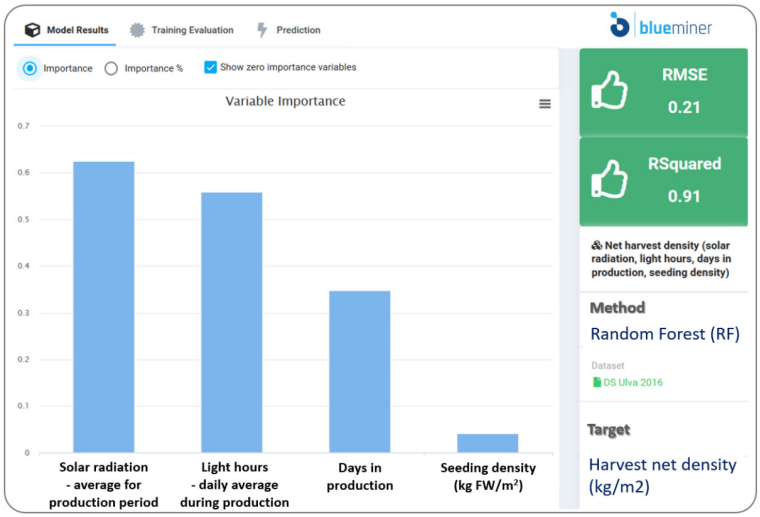

Figure 2. Variable importance for prediction of harvest net density at the seaweed farm.

SYSTEMATIC HYDRODYNAMIC MEASUREMENTS AND NUMERICAL MODELLING OF AQUACULTURE STRUCTURES

Jorrit-Jan Serraris[1], Linda Kemp[1], Robbert Jak[2], Stefan Matthes[3]

(1) Maritime Research Institute Netherlands (MARIN). P.O. Box 28, 6700 AA, Wageningen (Netherlands)
Email: j.w.serraris@marin.nl
(2) Wageningen University and Research (WUR), (3) GICON Grossmann Ingenieur Consult GmbH

Introduction

Aquaculture systems at open sea will experience high hydrodynamic loads from current and waves. Experimental knowledge and numerical modelling techniques of these hydrodynamic loads are vital for the design of robust and cost efficient designs of aquaculture systems.

Within the Horizon 2020 research program Space@Sea (https://spaceatsea-project.eu) various aquaculture activities have been studied. The studies include economical, ecological and technical assessment of fish farming, mussel cultivation, seaweed farming and microalgae production. The aquaculture concepts and their viability are described by (Jak, Poelman, Schram, Matthes, & Fagerland, 2019). The present paper focusses on the technical aspects of these activities. Results of systematic physical model tests on mussel lines, fish nets and microalgae tubes are described. Secondly it is outlined how these results are used in a numerical model to simulate the complex loading and motion behaviour of the aquaculture constructions at sea.

Systematic hydrodynamic measurements

Current and wave loads on mussel lines, fish nets and microalgae tubes were measured in MARINs Concept Basin. The setups consists of:

- Mussels on single drop lines. A line consist of a 1 cm diameter rope of 1.5 m length with the mussels attached to it in sockets. Each line with mussels has an average diameter of 10 cm. The lines are vertically suspended in two frames, positioned 1.2 m apart from each other, to measure undisturbed flow as well as shielding effects. One frame is equipped with five lines, the other with two lines of 1.2 m length. The overall forces on both frames are measured.

- Microalgae silicon-based double-wall hoses prepared and provided by GICON. Each hose sample has a diameter of 6 cm and length of 2 m. The hoses are natural buoyant and float horizontally. The hoses are suspended in the basin by horizontal wires at which the hydrodynamic loads are measured. Loads are measured on a single hose as well as on a set of three hoses. The hoses are positioned in parallel and crossed to the current and waves.

- Fish nets with a solidity of 22%. The setup is similar to the mussel setup and consists of a large (1.41 x 1.455m) and small vertical frame (0.66 x 0.705m). The distances between the frames and orientations of the frames are varied to measure the effects of shielding and oblique inflow.

The focus of the remainder of the publication is on the fish nets. The measurement are performed and analysed as follows:

- Current loads are measured by towing the aquaculture structures through the basin. The current loads are measured at for three towing velocities: 0.5, 1.0 and 1.2 m/s. Current drag load coefficients are derived as follows:

$$C \qquad \_\_\_\_\_ \tag{1}$$

Where F_d is the measured force in x-direction, ϱ is the known density of water (1025 kg/m^3), V is the imposed towing velocity, and A is the area within the frames.

- Harmonic forced oscillation tests are performed to measure hydrodynamic added mass and damping. The added mass is in-phase with the acceleration and the drag load inphase with the relative velocity and can be isolated and defined as coefficients as follow:

$$\boldsymbol{f} \quad \boldsymbol{f} \quad \boldsymbol{f} \quad F\cos\omega t \qquad \varepsilon \qquad F\cos\omega t\cos\varepsilon\, F\sin\omega t\sin\varepsilon \tag{2}$$

$$C \qquad a \qquad \overline{\rho V\dot{V}} \qquad C\,1 \qquad\qquad 1\_\_\_\_\_1$$

(3)

$$\frac{f_d}{\frac{1}{2}\rho AV^2} = \frac{-F_1\sin\varepsilon_1}{\rho AV^2}$$

$$C \tag{4}$$

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- Where *C* is the added mass coefficient, *C* the linear damping coefficients, F_1 the amplitude of the measured force in x-direction and ε_1 the phase between the imposed motion and measured force.

- Wave loads are measured in regular waves with 0.1, 0.2 and 0.3m amplitude (Ha) and 1.5, 2.5 and 4s wave period (T). From the measurements in regular waves the Response Amplitude Operators (RAO) and phase are derived by harmonic analysis. Since the aquaculture structures are slender and open structures the wave load consists only of the Froude-Krilov force and no diffraction contribution.

For the fish net the average value of the drag coefficient based on the three towing velocities is -0.293.

Time-domain calculations compared to forced oscillation model tests show that the forces on a unit net panel is drag dominated. The drag coefficients found in forces oscillation tests for Keulenger-Carpenter number (KC) between 7.7 and 77.4 show an average of 0.332. The added mass coefficients strongly variates between 0.8 and 2.4, with an average of 1.622.

Differences between towing tests and forced oscillations tests are in the disturbance of the flow due to net. In towing tests, a uniform inflow velocity is exposed to the fish net while the forced oscillations will be carried out in a disturbed flow due to previous oscillations. Differences of about 10% for drag coefficients are assumed to be related to these disturbances in the flow field.

Numerical modelling

The fish net is represented in a time domain simulation model in order to extend the model to realistic fish farm constructions. MARINs existing time domain simulation model aNySIM*xmf* is used (MARIN, 2019). This model allows to determine the motion response of multiple coupled bodies under the influence of current, waves and wind. A flexible fish net or fish farm construction can be represented by a large number of coupled bodies. A unit net panel is modelled as a so-called Morison plate element. The hydrodynamic force contributions on the Morison element consist of the Froude-Krilov force, added mass and drag force contribution and act on the center of the element. The added mass and drag coefficients C_a and C_d as derived from the model tests are specified as input parameters.

To obtain the most accurate and realistic behavior of a flexible structure the number of elements should be large. However a large number of elements reduces the calculation time of the simulation. For a simulation with approx. 400 elements the calculation time is approx. 50 time real time.

Conclusions

For the fish nets drag loads are the dominant contribution to the hydrodynamic loads in forced oscillation tests. For nets with 22% porosity drag coefficients between 0.30 and 0.35 can be applied to Morison structures in time-domain calculations. Added mass coefficients for nets are suggested to choose between 1.5 and 2.0, where the added mass loads are rather small compared to the drag loads on a net.

Measurements on mussel lines and microalgae hoses have been performed within the research project as well. Results of these measurements will be presented in alternative publications.

Recommendations

Based on the results of the present research the following is recommended for further research:

- To continue systematic model testing of (subcomponents of) aquaculture structures in order to determine empirical hydrodynamic coefficients for current and wave loads.
- Further studying of model scaling effects on nets for fish farming.
- Further development of the numerical code to represent the hydro-elastic behaviour of flexible aquaculture structures accurately and time efficiently.

References

Jak, R., Poelman, M., Schram, E., Matthes, S., & Fagerland, K. (2019). *Outline of concepts for aquaculture on floating modular islands (D8.1).* Space@Sea Deliverable.

MARIN. (2019). *aNySIM Theory Documentation.* Wageningen.

https://spaceatsea-project.eu

DIETARY PROTEIN REQUIREMENT OF WHITELEG SHRIMP (*Penaeus vannamei*) POSTLARVAE

Andreia Silva[1*], Wilson Pinto[1], André Barreto[2], Bruna Silva[2], Renata Serradeiro[2] and Luís Conceição[1]

[1]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[2]Riasearch Unipessoal Lda, Cais da Ribeira de Pardelhas N21, Murtosa (Portugal)

*E-mail: andreiasophiass@gmail.com

Introduction

Whiteleg shrimp (*Penaeus vannamei*) is one of the most important farming shrimp species, representing 52.9 % of all crustaceans production and reaching a production of 4.9 million tonnes in 2018 (FAO, 2020). In later life-stages, studies on protein requirements have allowed optimizing dietary protein levels to maximize shrimp growth performance, (Hu et al., 2008; Kureshy and Davis, 2002; Lee and Lee, 2018). Yet, it is extremely important to understand dietary protein requirements at initial developmental stages, being crucial for the development of a suitable microdiet to improve shrimp quality and maximize growth and survival under intensive rearing conditions. This study aimed at determining the optimal dietary protein requirement of whiteleg shrimp postlarvae. To this end, a three-week trial was conducted to assess the effect of six experimental microdiets with graded protein levels (34 to 63 %) in shrimp post-larvae growth performance, survival and feed conversion ratio.

Materials and Methods

Six experimental diets were formulated to contain 34, 44, 49, 54, 58 and 63 % crude protein (wet matter basis) levels. These diets were produced by cold-extrusion and fed in triplicate to whiteleg shrimp postlarvae (3.2 mg mean initial wet weight; PL18) randomly distributed by 18 tanks of 47 L. Diets were provided by an automatic feeder, which provided meals in 8 daily cycles (2 hour feeding and 1 hour stop). Shrimp were reared for 21 days at 28ºC and a density of 892 shrimp m^{-2} in a RAS system. At the end of the trial, 6 pools with a total of 90 postlarvae from each tank were randomly sampled for wet weight and growth analysis. Postlarvae survival was also determined at the end of the trial. A non-linear regression was performed on postlarvae weight gain using a broken line model to estimate the optimal dietary protein level.

Results

At the end of the trial, significant differences were found in the growth performance, survival and feed conversion ratio of shrimp postlarvae fed the different diets tested. These differences (ANOVA) occurred only between a 34 % dietary protein level and the remaining diets tested (higher than 44 % protein), with no significant differences being obtained between remaining treatments. A dietary protein level of 44 % or above significantly increased shrimp wet weight (45 to 68 mg), mean weight gain (42 to 64 mg), relative growth rate (13.5 to 15.5 % day^{-1}) and survival (between 81 to 87%), whilst decreasing feed conversion ratio (1.4 to 1.0). Values achieved for a 34 % dietary protein level were as follows: wet weight (27 mg), mean weight gain (24 mg), relative growth rate (10.8 % day^{-1}), survival (75%), feed conversion ratio (2.1). Based on the broken-line regression analysis on weight gain obtained, a dietary protein level of 47.1 % was estimated for maximum weight gain of shrimp postlarvae under this trial's conditions (Fig. 1).

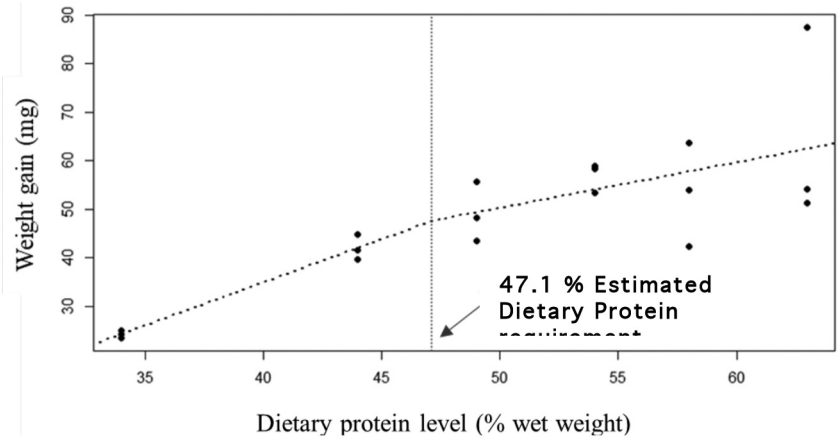

Fig. 1. Broken line model – dietary protein level (%) vs mean weight gain (mg) – and estimated dietary protein level for whiteleg shrimp postlarvae.

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Discussion

A high shrimp postlarvae survival was obtained for all treatments at the end of the trial, reflecting good zootechnical conditions and high quality of the microdiets here tested. Survival results were very similar to those reported by Hu et al. (2008) and Lee and Lee (2018), which ranged from 85 to 93 % in both studies. Results from the current study also showed no significant differences in growth performance, survival and feed conversion ratio for shrimp postlarvae fed dietary protein levels of 44 % or above. In addition, a dietary protein requirement of 47.1% was established for shrimp postlarvae (3.2 g initial wet weight). Previous findings suggest that optimal dietary protein levels may vary in shrimp according to their size. Namely, Velasco et al. (2000) estimated an optimal dietary protein level for smaller shrimp postlarvae (1.0 mg wet weight) to range around 20 and 22 %. However, these results may be underestimated, as the maximum dietary protein level tested was 25 %. In shrimp juveniles, a dietary protein requirement between 32 and 36 % was established by Kureshy and Davis (2002) and Lee and Lee (2018). Considering these results with those obtained in the current study, it may be inferred that shrimp may require higher dietary protein levels at early life-stages, when growth is also exponential, with protein requirement decreasing along with development. The estimation of an optimal dietary protein requirement for shrimp post-larvae empowers the formulation of an adequate diet for maximum growth and survival of this species. It also allows to reduce nitrogenous end-products of protein metabolism in post-larval rearing tanks where shrimp are intensively cultured, thereby improving water quality.

References

FAO, (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

Hu, Y., Tan, B., Mai, K., Ai, Q., Zheng, S., & Cheng, K. (2008). Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquaculture Nutrition, 14(6), 499–506.

Kureshy, N., & Allen Davis, D. (2002). Protein requirement for maintenance and maximum weight gain for the pacific white shrimp, *litopenaeus vannamei*. Aquaculture, 204(1–2), 125–143.

Lee, C., & Lee, K. J. (2018). Dietary protein requirement of Pacific white shrimp *Litopenaeus vannamei* in three different growth stages. *Fisheries and Aquatic Sciences*, *21*(1), 1–6.

Velasco, M., Lawrence, A. L., Castille, F. L., & Obaldo, L. G. (2000). Dietary protein requirement for *Litopenaeus vannamei*. Avances En Nutrición Acuícola V. Memorias Del V Simposium Internacional de Nutrición Acuícola. Merida, Yucatan, Mexico, 181–192.

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SIDE STREAM PRODUCTS FROM WHITE FISH CATCH FOR SUSTAINABLE AQUACULTURE GROWTH

Hanne Jorun Sixten*, Raja Mansingh Rathore, Jon Vestengen, Marie Hillestad, Ivar Rønnestad, Katerina Kousoulaki

*BioMar AS, R&D Nutrition requirement Department, Havnegata 9, N-7010 Trondheim
Email: hansi@biomar.com

Marine ingredients account for a declining share in feed for fish farming, as catches of wild fish are stable while we see a large growth in the aquaculture industry. Marine raw materials have a high nutritional value and provide safe and healthy growth in fish. Compared to plant raw materials, they contain a significant proportion of nitrogenous water-soluble compounds. Wild fish side stream products provide efficient use of largely unutilized marine raw materials, increased sustainability and potentially 50% increased growth in the aquaculture industry.

Changing the composition of raw materials in fish feed requires good knowledge of food safety, nutritional value and technical characteristics. In this study we have used side stream products from whitefish that were processed into various feed ingredients in pilot scale. Raw material was separated into head (h), bone (b) and viscera (v) and processed into two fish meal (FM); FM-hbv, FM-hb, and two hydrolysates; Hydr-hb and Silage-v. The raw materials were characterized and were found to have different nutritional value and level of several bioactive components.

Six Atlantic salmon diets were produced to study performance, digestibility, appetite and food intake; Medium FM (MFM), Low FM (LFM), and 4 diets where half of the FM was replaced with test raw material, one in each diet. The four test diets were optimized to the same level of marine protein as MFM.

A salmon feeding trial was run for 8 weeks in 1 m^3 tanks at Nofima Sunndalsøra, Norway, and the fish grew on average from 112 g to 249 g. Differences in food intake, performance, digestibility and morphology were found between the different treatments. Neuropeptides and hormones involved in appetite control based on the gut-brain axis were also studied.

Some of the raw materials from the side stream product of whitefish gave similar growth performance as commercial fish meal (control) and others showed improvements in terms of FCR, nutrient apparent digestibility and slaughter yield. This provides interesting opportunities for the aquaculture industry, as the use of these types of products contributes to an environmentally friendly and sustainable growth in the industry.

NUTRIENT-BASED MODELS AS BENCHMARKING, MONITORING, AND FORECASTING TOOLS

Filipe Soares*[1], Ana Nobre[1], Ricardo Severino[2], João Cruz[2], Luís Conceição[1], Tomé Silva[1]

[1]SPAROS Lda., Olhão (Portugal)
[2]Piscicultura Vale da Lama Lda., Odiáxere (Portugal)

Email: filipesoares@sparos.pt

Introduction

Nutrient-based mathematical models can be used to support informed decisions in aquaculture, for instance, to perform *in silico* evaluation of different feeding strategies and the prediction of the ongoing production. Given the high level of expertise required to implement this type of models, most fish farming companies end up using simpler models (e.g., SGR, TGC). Although the use of simpler models presents some advantages, such as the need for less computational power and ease of calibration for specific conditions, they are limited in their nature and often fail to provide accurate predictions of fish growth when considering different sets of farming conditions (e.g., temperature, feed quality, feeding rates; Reid et al., 2020). In turn, more complex models such as nutrient-based models, despite presenting some technical challenges related to their implementation, when properly calibrated allow an accurate prediction of fish growth, as well as other indicators (e.g, body composition, waste production), for a wide range of conditions. This is possible due to the fact that these types of models have into account a broader set of factors that affect fish growth and composition (i.e., temperature, feed composition, and feeding amounts). As some of these factors are manageable and can be controlled during the farming operations, nutrient-based models can be used for benchmarking, monitoring and forecasting, to support a knowledge-based decision-making that leads to more incisive actions and, ultimately, to more efficient production.

The main objective of this work is to illustrate how, a nutrient-based model has been used in a commercial farm (Piscicultura Vale da Lama, Portugal) in two distinct but complementary ways: (i) as a benchmarking tool, to test and evaluate the performance of fish when subjected to different feeding strategies, and (ii) as a monitoring and forecasting tool, to provide on a regular basis the status of the ongoing production.

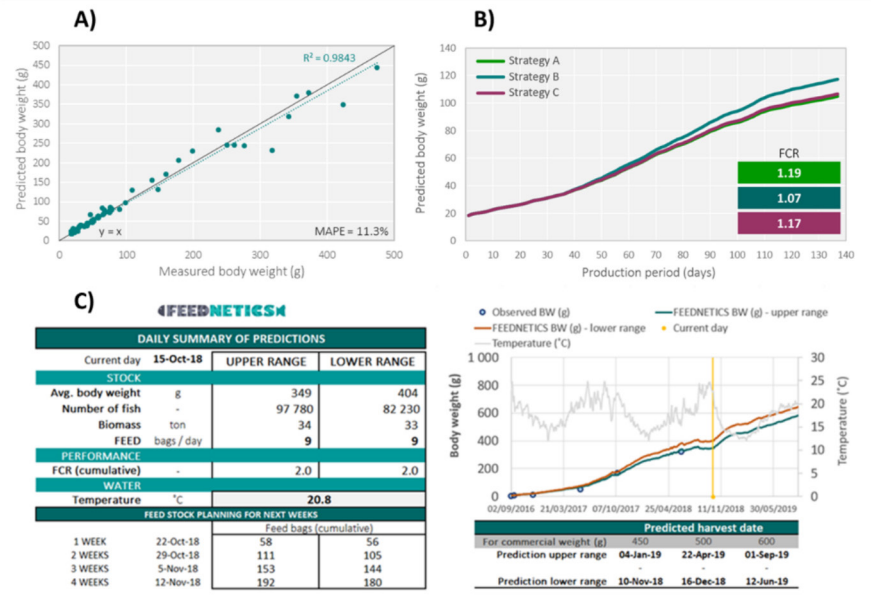

Figure 1 – FEEDNETICS™ model performance evaluation (A), feeding strategy benchmarking (B), and dashboard for monitoring and forecasting (C).

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Methods

In this work the FEEDNETICS™ dynamic nutrient-based model was used to run predictions for gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). In a first phase, the performance of the model was evaluated by comparing the model results against historical data sets of the commercial farm. Then, in a second phase, and after having confidence in the model robustness, it was used in two different ways: (i) as a benchmarking tool, where the performance of fish fed under different feeding strategies was compared in terms of growth, feed conversion, economic conversion, total N waste and total solids waste; and (ii) used as a monitoring and forecasting tool, where a prototype tool was developed in order to update on a regular basis the status of the ongoing production, based on the farm data records and the farming strategies planned for the future.

Results

The evaluation of the model performance (Figure 1A) indicates that the model is able to accurately predict the growth of both seabream and seabass, with an overall mean absolute percentage error (MAPE) of about 11.3%. Figure 1B shows the results of FEEDNETICS™ as a benchmarking tool, where three different feeding strategies were evaluated. For the farming and environmental conditions considered, the model results suggest that following feeding strategy B leads to a higher performance compared to the others feeding strategies that were evaluated. Figure 1C shows a prototype dashboard that was developed to use FEEDNETICS™ as a monitoring and forecasting tool, where the status of the ongoing production is presented (e.g., fish weight, FCR, feed consumption, harvest dates).

Final remarks

In this era of digitalization, where data is increasingly abundant, it is important to develop tools that easily translate data into insights. Solutions towards precision fish farming practices are booming worldwide. Nutrient-based mathematical models are part of the available portfolio of new tools and approaches that contribute to more efficient production. As illustrated in this application nutrient-based mathematical models can be used to evaluate different feeding strategies under specific farming conditions, and to monitor and forecast the ongoing production, thus providing insights that contribute to more efficient production.

Acknowledgments

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References

Reid, G. K., Lefebvre, S., Filgueira, R., Robinson, S. M., Broch, O. J., Dumas, A., & Chopin, T. B. (2020). Performance measures and models for open-water integrated multi-trophic aquaculture. Reviews in Aquaculture, 12(1), 47-75.

HOST DEFENCE AND INFLAMMATORY SIGNALLING IS DRIVEN BY GUT MICROBIOTA IN GILTHEAD SEA BREAM FED FISH MEAL-FREE DIETS

P. Solé-Jiménez[1], F. Naya-Català[2], M.C. Piazzon[3], I. Estensoro[3], J.A. Calduch-Giner[2], A. Sitjà-Bobadilla[3], D. Van Mullem[1], J. Pérez-Sánchez[2]

[1]LSAqua (Lambers-Seghers NV), Hooglede, Belgium. [2]Nutrigenomics and Fish Growth Endocrinology Group and [3]Fish Pathology Group, Institute of Aquaculture Torre de la Sal, CSIC, Spain
E-mail: paula.sole-jimenez@lsaqua.be

Introduction
The sustainability and physiological effects of high inclusion levels of new feed ingredients is often questioned, and their potential application requires the use of conventional methodologies, but also cutting-edge tools, for unravelling the close talk between diets, host metabolism and gut microbiota (Fontinha et al., 2020). In this challenging scenario, the aim of the present study was to evaluate, in a high valuable farmed fish model (gilthead sea bream), the effects on growth performance and gut microbiota of partial (50%) and total (100%) replacement of fish meal (FM) by a combination of processed animal proteins and single cell proteins.

Methods
The feeding trial (8 weeks, May-July) with control (CTRL) and experimental diets (50LSAqua, 1000LSAqua) was conducted in triplicate 500 L tanks under natural photoperiod and temperature conditions in a flow through system with well aerated sea water (O_2 concentration > 5.5 ppm). Fish with an initial body weight of 23 g were fed near to visual satiety one time per day, six days per week. Nine fish per group were sacrificed, and the adherent bacteria from the anterior intestinal portion were collected and immediately used for DNA extraction. 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.

Results
All fish grew efficiently, with feed conversion ratios between 1.09 and 1.19 (no statistical differences). Specific growth rates did not vary significantly between CTRL and 50LSAqua fish, though a slight decrease of growth rate (5%) was found with the total FM replacement (100LSAqua). Organosomatic weight indexes of liver, mesenteric fat and intestine were not altered by dietary treatment, but intestine length was shortened by the strategy of FM replacement. In addition, the concentration of lactic acid, was increased in stripped faeces of fish fed LSAqua diets. When fish of fish fed LS-Aqua diets were put together (50/100LSAqua), the bacterial richness was similar in both fish groups, but a remarkably lower diversity was found in CTRL fish. At the phylum level, *Proteobacteria* was the most abundant phylum, constituting ≥ 55% of the total resident bacteria in the anterior intestine. *Firmicutes* was the second most abundant phylum in the CTRL group (~20%) with a significant decrease (10.3%) in the 50/100LSAqua group. Conversely, *Actinobacteria* raised from 11.9% in CTRL fish to 23.2% in 50/100LSAqua fish, whereas the abundance of *Bacteroidetes* remained almost constant (~4.5%) in both fish groups. PLS-DA analyses showed significant differences in the microbial composition among dietary groups. For these discriminant bacteria, a first type of response was mediated by 27 OTUs overrepresented in fish fed 50/100LSAqua diets. In this group, it was remarkable the presence of *Verrucomicrobia* and *Chlamydiae* phyla, the class *Betaproteobacteria* and the genera *Paracoccus, Omithinimicrobium, Tetrasphaera, Rubellimicrobium* and *Butivibrio*. A second type of response grouped 20 OTUs with a significantly higher proportion in CTRL fish. This group included *Bacteroidetes* and *Cyanobacteria* phyla, *Chromatiales, Bacillales* and *Methylococcales* orders and *Aggregatibacter, Clostridium sensu stricto, Acinetobacter, Rhodolyulum, Albimonas, Propioniclava* and *Psychrobacter* genera. Inferred metagenome analysis showed that 27 pathways could be significantly changing in the fish fed 50/100LSAqua diets compared to CTRL ones. Pathways related with IL-17 signalling pathway, Th17 cell differentiation, and antigen processing and presentation were underrepresented in 50/100LSAqua fish, whereas quorum sensing, flavone and flavonoid biosynthesis, isoflavonoid biosynthesis, Fc gamma R-mediated phagocytosis, and antimicrobial production were markedly overrepresented (Fig. 1).

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Conclusions

These results point to a reduced activity of IL-17 pathways in fish fed LSAqua diets, which, among their functions, is to limit proliferation of resident bacteria (Douzanez-Mobarrez et al., 2019). This anti-inflammatory response would be reinforced by the over-representation in the gut microbiota of OTUs involved in flavones and flavonoids biosynthetic processes. At the same time, host defence and inflammation processes would be counter-regulated by the overrepresentation of mucosal microbes involved in the natural production of antimicrobials, which would contribute to control proliferation of specific bacteria in our model of carnivorous fish fed with FM-free diets.

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References

Fontinha et al. 2021. Effect of dietary poultry meal and oil on growth. digestive capacity. and gut microbiota of gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture*. 530:735879.

Douzanez-Mobarrez et al. 2019. Gut Microbiota and IL-17A: Physiological and Pathological Responses. *Probiotics & Antimicrobial Proteins*. 11:1-10.

FUNCTIONAL FEEDS TO TACKLE MEAGRE (*Argyrosomus regius*) STRESS: PHYSIOLOGICAL RESPONSES UNDER CHRONIC AND ACUTE STRESSFUL CONDITIONS

Sousa, Carla*[1]; Monteiro, Marta[1,2]; Castro, Carolina[2]; Coutinho, Filipe[2]; Guerreiro, Inês[2]; Diaz-Rosales, P.: Fontinha, F; Pousão-Ferreira, Pedro[3]; Matos, Elisabete[4]; Oliva-Teles, Aires[1,2]; Enes, Paula[1,2]; Couto, Ana[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, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal
[3] IPIMAR/CRIPSul Av.5 de Outubro S/N, Olhão, Portugal
[4] SORGAL, Sociedade de Óleos e Rações, S.A., Estrada Nacional 109 Lugar da Pardala, 3880-728 S. João de Ovar, Portugal
carladaniela.sousa@gmail.com

Chronic and acute stressors such as temperature fluctuation, crowding, water quality, handling, transportation or confinement are a major problem in aquaculture. They cause changes in metabolic, hematological, cellular, endocrine, antioxidant and immune functions that make fish more vulnerable to diseases, affecting production yield by reducing growth and reproduction, which leads to economic losses. To mitigate such effects, fish antioxidant response to stressors can be modulated nutritionally, since nutritional factors were reported to differently affect fish oxidative status. This study aimed to alleviate negative stress effects through the use of functional feeds. Algal extracts from *N. gaditana* and *F. vesiculosus*, with reported antioxidant potential, were incorporated into four experimental diets - a control diet (N0F0), a *F. vesiculosus* supplemented diet (N0F1), a *N. gaditana* supplemented diet (N1F0) and a mix diet (N1F1). These diets were fed to two groups of fish, one subjected to a chronic stressor (temperature fluctuation) and other to an acute stressor (handling/ confinement). It was observed an overall positive effect of the extracts inclusion in the mitigation of the effects felt by stress. Both extracts demonstrated to detain anti-inflammatory action and the ability to reduce plasma metabolites. The use of *F. vesiculosus* also showed improvements on feed efficiency, as well as enhanced ability to fight pathogens, which offers fish fed with this inclusion short-term protection. This extract showed an increase of muscle glutathione reductase activity and reduced lipid peroxidation. On another hand *N. gaditana* supplementation led to an overall reduction of hepatic antioxidant enzymes activity and glutathione. These results show that algal extracts in aquafeeds reduce physiological imbalance resulting from chronic and acute stresses, being able to improve aquaculture fish's health and welfare.

PATHOLOGIES OF MACROALGAE: RISK TO TAKE INTO A NEW FARM MANAGEMENT VISION

Damiano Spagnuolo[1*], Antonio Manghisi[1], Marina Morabito[1], Javier Cremades[2], Giuseppa Genovese[1]

[1]University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Salita Sperone, 31, 98166 Messina, Italy
[2]University of A Coruña, Centro de Investigaciones Cientificas Avanzadas (CICA), Campus de Zapateira s/n, 15071 A Coruña, Spain
E-mail: dspagnuolo@unime.it

The boom of commercial macroalgae in Europe in the last ten years has been constantly expanding. In general in the world, the attention on macroalgae, especially as food, has increased with the awareness of a person's good health; for example in Korea in 2020 there was an increase in the consumption of nori equal to 60%.

Several species are exploited for food and dietary supplements, animal feed, chemicals, and much more. The volume of the biomass cultivated for commercial purpose is fast-growing, also through integrated multi trophic aquaculture (IMTA).

Considering economical and social benefit, the farms have to face the emergence of algal diseases. It is a situation not to be underestimated indeed, the control and prevention of diseases is estimated at about 35% of total costs. The infectious disease in algae involves a transmissible infectious agent (bacteria, fungi, virus, etc.) while the non-infectious is induced by physiogenic factors such as extremes of temperature, salinity, light intensity or pollution; for example "blisters disease" in *Laminaria* spp. (Fig. 2).

In macroalgae the concept of pathogen-disease is not always appropriate, because frequently is the synergy of both (infectious and non-infectious factors) to reach the state of pathology. It is therefore important to clarify all the different kinds of pathologies and, were possible, prevent and treat the diseases, also in accordance with the new economic policies and European directives. Some diseases are better characterized than others and this is because some cultivation systems are better established than others.

For example, considering "nori", there are more than ten different known diseases that affect *Porphyra*/*Pyropia* farming: oomycetes (Fig. 1), such as species of the genera *Pythium* and *Olpidiopsis*, known as "red rot disease" and "*Olpidiopsis* disease", respectively, or the *Pyro V1* virus responsible of "green-spot disease".

In the Gigartinales order, *Eucheuma* spp. and *Kappaphycus* spp., it is very common to diagnose the "ice-ice" disease, which is a "pathology" defined as a symptom and has recently been treated as such. For these carragenophytes, another important problem is the "goose bump" disease, an infestation caused by an endophytic filamentous alga (EFA), mainly of the genus *Neosiphonia*. The infestation of EFA is a serious trouble of farmed *Saccharina*, often caused by species of *Laminariocolax* and *Laminarionema*.

Symptoms are often caused by synergy of different pathologies and their identification can be difficult or misleading. In this regard, the use of molecular analyses could be very useful to achieve an accurate identification and, therefore, for testing suitable treatment. It is noteworthy that some pathogens can carry out a "cross-species transmission" often resulting in symptoms which are more severe and more incisive in the new host than in the previous one.

Examples are the EFAs from *Sargassum* infecting *Kappaphycus* and species of the genus *Pythium* from *Porphyra* that could infect *Ulva* spp. Macroalgal pathologies are often an underestimated and undervalued field. However, in the near future, due to the growing increase in algal farms, it will be necessary to outline appropriate protocols to quickly achieve a correct identification of the pathologies and to apply an adequate treatment to the algae.

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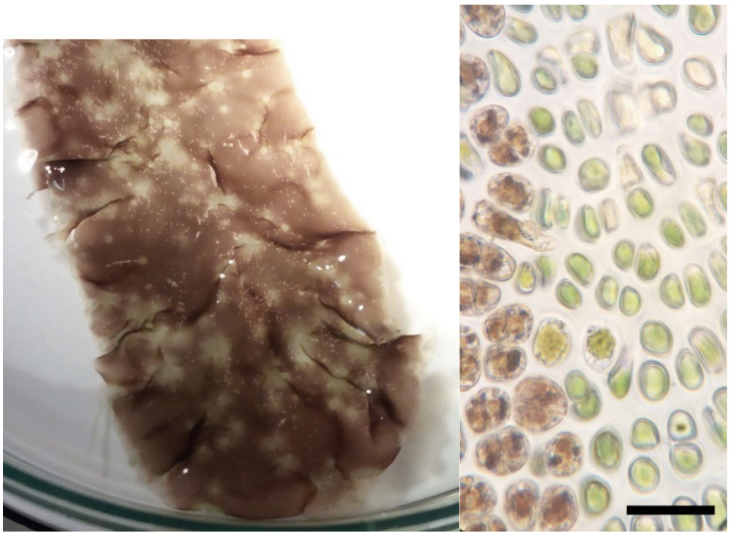

Fig. 1. Suspected infection of *Olpidiopsis* sp. on *Porphyra dioica*. Scale bar = 20µm

Fig. 2. Blisters disease on *Laminaria* sp. Scale bar = 5cm (photo by D.Mayes)

COMPINED CULTIVATION OF *Oreochromis mossambicus* AND *Eruca vesicaria* IN AN AQUAPONIC SYSTEM

P. Stathopoulou[1], E. Tsoumalakou[2], T. Vanikiotis[3], E. Levizou[2] and P. Berillis[1*]

[1] Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, 38446 Fytoko Volos, Magnesia, Greece.
[2] Department of Agriculture Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, 38446 Fytoko Volos, Magnesia, Greece.
[3] Department of Biological Applications and Technology, University of Ioannina, 45110, Ioannina, Greece.

E-mail: pveril@uth.gr

Introduction

Aquaponics combines the culture of aquatic animals and the cultivation of plants in recirculating systems, integrating aquaculture and hydroponics in a soil-less system (Racocy et al., 2004). Aquaponic systems use less than 10% of the water consumed in conventional farming (Somerville et al., 2014). More than 150 species of vegetables, herbs, flowers, and small trees have been successfully tested in aquaponics systems. Many of them show great potential for cultivation in the Mediterranean region where aquaponics are at their first steps, yet the challenge of coping with adverse climatic conditions, notably in summer, is considerable. In the present study, a typical Mediterranean plant species, arugula, was co-cultivated with red tilapias and the modifications of nutrient inputs targeted at delineating the system's weak points as they may be depicted in both species productivity.

Materials and Methods

A laboratory-scale combined cultivation of red tilapias (*Oreochromis mossambicus*) and arugula (*Eruca vesicaria*) was performed for 30 days. Nine autonomous aquaponics systems (fish tank, hydroponic unit filled with clay, mechanical filter, biological filter, sump filter) were used. Each one had 625L total water capacity and 5100cm^3/min water flow. The nutrient input modifications resulted in three treatments (three replicate systems each): i) control, no nutrient addition, ii) Fe, where only Fe-DTPA was added (target value 5mg/L) and iii) Fe+K, in the form of Fe-DTPA and K_2SO_4 (target values 5mg/L and 120mg/L or Fe and K respectively). 270 Nile tilapias of 8.73 ± 0.152g mean body weight were totally reared (30 in each aquaponic systems). Fish were fed ad libitum, two times per day a pelleted diet. 12 arugula plants were placed in each hydroponic bed. Artificial light was supplied by a 600 W HPS lamp placed 65 cm above each growing area and accompanied by a timer for accurate control of the photoperiod (10 h light: 14 h dark).

Results

Weight gain (WG), food conversion rate (FCR) and specific growth rate (SGR) were calculated for fish (Table 1), while leaf fresh weight (LFW), total produced biomass (TPB) and root biomass to aerial biomass ratio (R/A) were calculated for plants (Table 2).

Table 1: Red tilapia growth performance. Means ± S.E.M (n = 90 for each treatment).

	Control	Fe	Fe+K
WG (g)	15.02±0,660^a	13.73±0.624^a	14.32±0.699^a
FCR	0.94±0,054^a	0.97±0.058^a	0.98±0.063^a
SGR (%/day)	3.25±0,067^a	3.07±0.076^a	3.13±0.069^a

Means in a row followed by the same superscript are not significantly different ($p > 0.05$).

Table 2: Arugula growth performance. Means ± S.E.M (n = 36 for each treatment).

	Control	Fe	Fe+K
LFW (g)	78.48±8.597^b	116.07±10.794^a	98.08±8.977^{ab}
TPB (g/m^2)	941.75±321.98^a	1386.94±442.09^a	1177.01±368.63^a
R/A	0.08±0.004^a	0.10±0.008^a	0.08±0.004^a

Means in a row followed by the same superscript are not significantly different ($p > 0.05$).

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Discussion

The addition of fertilizers (Fe and K) did not affect the survival of tilapia fish, showing 100% survival in all treatments. The fish growth parameters (weight gain, FCR, SGR) did not differ between treatments, proving that the addition of fertilizers to aquaculture systems, at specific concentrations, did not affect growth. Few studies have examined the effect of fertilizers on tilapia growth.. Rafiee et al., (2019) performed an experiment with tilapia of initial size 5.62 ± 3.75 g and reported fish survival of 73% in the treatment where additional nutrients input took place for a period of 110 days. They recorded statistically higher SGR and lower FCR at zero nutrient input (1.84 ± 0.12% / day and 0.88 ± 0.07 respectively) relative to additional nutrients treatment (1.61 ± 0.20% / day and 1.25 ± 0.15 respectively). In our study fish had higher SGR and lower FCR in all treatments. However, the main conclusion of our work is that the addition of nutrients did not have a negative effect on fish growth.

Arugula plants treated with additional Fe outweigh all other treatments in productivity reaching 1386 g/m^2; the final product biomass was 40% higher than control plants and 10% higher compared to Fe+K treatment. Nicoletto et al. (2018) cultivated arugula in an aquaponics system but the production hardly reached 500 g/m^2. Fe deficiency in aquaponics is considered one of the major weak points of the system as relative experiments with leafy vegetables have documented. Nozzi et al. (2018) reported that weekly additions of iron, phosphorus and potassium significantly promoted lettuce production in aquaponics.

Acknowledgment
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References

Racocy, J., et al. (2004) Design and operation of the UVI aquaponic system. University of the Virgin Islands. Agricultural Experiment Station. St. Croix, U.S. Virgin Islands.

Somerville, C., et al., 2014. Small-scale aquaponic food production: integrated fish and plant farming. FAO Fisheries and Aquaculture Technical Paper, (589), p.I.

Rafiee, G. R., et al. (2019). Effects of supplementary nutrient in an aquaponic system for production of ornamental red tilapia (Oreochromis Sp.) and lettuce (*Lactuca sativa* var longifolia). Survey in Fisheries Sciences, 5(2), 65-75.

Nicoletto, C., et al., (2018). Extension of aquaponic water use for NFT baby-leaf production: mizuna and rocket salad. Agronomy, 8(5), 75.

Nozzi, V., et al. (2018). Nutrient management in aquaponics: comparison of three approaches for cultivating lettuce, mint and mushroom herb. Agronomy, 8(3), 27.

THERMAL TOLERANCE AND BIOLOGICAL PERFORMANCE OF EUROPEAN SEABASS *Dicentrarchus labrax* REARED UNDER HIGH TEMPERATURES

Orestis Stavrakidis-Zachou[1,2]*, Konstadia Lika[2], Pavlidis Michail[2], Asaad H. Mohamed[3] and Nikos Papandroulakis[1]

[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
[3]Beacon Development, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia
e-mail: ostavrak@hcmr.gr

Introduction

European seabass (*Dicentrarchus labrax*) is one of the main farmed fish in the Mediterranean with the annual production exceeding 200,000 tons (FEAP, 2019). The species is eurythermal, and its biological responses to temperatures typically exhibited in its natural environment have been well documented (Claireaux *et al*., 2006; Person-Le Ruyet *et al*., 2004) while lethal limits are thought to surpass 30°C (Dulger *et al*. 2012). Although recent research has set out to pinpoint critical tolerance thresholds (Islam *et al*., 2020) the biological responses of E. seabass towards the upper edge of its temperature tolerance range are not fully understood. The present study investigates the thermal tolerance and the biological performance of E. seabass in the 24-34°C temperature range.

Materials and methods

Juvenile E. seabass (135g) were reared under three temperature regimes (24, 28 and 33±1°C) in triplicate tanks (2m^3) in a marine RAS for a period of three months. Feeding was provided to satiation twice a day with commercial feeds. Weight and total length were individually measured on a monthly basis and 15 fish per treatment were sacrificed for collection of blood and tissues and subsequent determination of hematological, biochemical and hormonal parameters. Intermittent-flow respirometry was used to determine the standard (SMR) and maximum (MMR) metabolic rates and calculate the aerobic scope.

Results

Husbandry findings suggest that E. seabass performs similarly between 24 and 28°C in terms of growth (figure 1) and FCR but growth diminishes at the highest temperatures. In addition, substantial time (one month) is required for complete acclimation to new temperature regimes as indicated by slow growth and elevated cortisol concentrations during the first month. The poor overall performance at the highest temperature was also corroborated by high mortality rates, elevated cortisol and lactate concentrations, and low levels of hematological (hematocrit and hemoglobin) and biochemical (glucose and triglycerides) parameters. Finally, SMR increased linearly with temperature but this was not the case for MMR, resulting in the highest temperature treatment exhibiting the lowest aerobic scope.

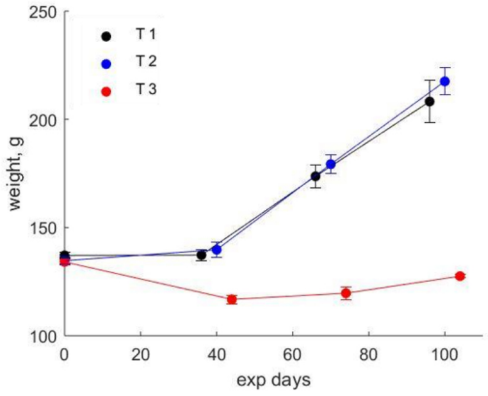

Fig. 1. Evolution of weight (g) during the trial under the three temperature treatments (T1: 24°C, T2: 28°C, T3: 33±1°C). Points denote mean weights at samplings and vertical bars express the standard deviation.

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Conclusion

Overall, the husbandry and physiological findings of the thermal trial suggest that E. seabass performs similarly between 24ºC and 28ºC, indicating the thermal optimum, while temperatures of 32-34ºC are sharply close to the upper end of the species temperature tolerance range. This is supported by high levels of stress indicators (cortisol and lactate) in combination with high energetic demands and reduced capacity for aerobic metabolism.

References

Claireaux G., C. Couturier, and A.L. Groison. 2006. Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax)*. *The Journal of Experimental Biology,* 209, 3420-3428.

Dülger N., M. Kumlu, S. Türkmen, A. Ölçülü, O. Tufan Eroldoĝan, H. Asuman Yilmaz, and N. Öçal. 2012. Thermal tolerance of European Sea Bass (*Dicentrarchus labrax*) juveniles acclimated to three temperature levels. *Journal of Thermal Biology*, 37, 79–82.

FEAP. (2019). European Aquaculture Production Report 2014-2019. Federation of European Aquaculture Producers. Prepared by the FEAP Secretariat. Accessed January 2021.

Islam, M.J., M.J. Slater, and M. Bögner. 2020. Extreme ambient temperature effects in European seabass, *Dicentrarchus labrax*: Growth performance and hemato-biochemical parameters. *Aquaculture*, 522, 735093.

Person-Le Ruyet J., K. Mahé, N. Le Bayon, and H. Le Delliou, H. 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. *Aquaculture*, 237, 269–280.

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AN INNOVATIVE FREHWATER MULTITROPHIC AQUACULTURE SYSTEM WITH MINIMAL WATER ABSTRACTION, UTILISING DUCKWEED FOR BIOREMEDIATION

V. Stejskal[1,2]*, S. Paolaci[1], M.A.K. Jansen[1], D. Toner[3]

[1]University College Cork, School of Biological, Earth and Environmental Sciences & Environmental Research Institute, Cork, Ireland
[2]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
[3]Bord Iascaigh Mhara (BIM), Crofton Road, Dun Laoghaire, Ireland

E-mail: vlastimil.stejskal@ucc.ie

Introduction

Aquaculture continue to be the fastest growing food production sector in the world. On the other hand, the industry is increasingly limited by the lack of suitable water resources and sites. An initiative towards developing a sustainable food production system incorporating elements of the circular economy including utilising renewable energy was initiated by Bord Iascaigh Mhara (BIM), University College Cork (UCC), Bord na Móna (BnM), Keywater Fisheries and Goatsbridge Rainbow Trout Ltd on cutaway peatlands in Ireland. The cessation of traditional peat harvesting in the midlands of Ireland due to licensing restrictions and environmental protections has led to increased diversification projects. The development of pond-based aquaculture systems is suited to the topography given the presence of glacial till and water resources. The system was developed with the aim of investigating the productivity and the impact of a new and sustainable technology that combines aquaculture, phytoremediation and wind energy. The duckweed biomass produced in the remediation process must be periodically harvested. This biomass has a high protein content, and it can potentially be used to replace unsustainable protein sources in livestock feed. The concept embraces the circular economy whereby there is a balance between the extractive capability of the duckweed and algae community, and the waste production of fish.

Materials and methods

The OASIS multitrophic pond system is located at Mount Lucas (County Offaly, Ireland) within an area that has been harvested for peat. Water is sourced from existing peat drainage. The entire aquaculture system consists of 4 adapted split ponds. Each pond has two fish holding D-end compartments of 200 m^3 (usable total water volume for fish is 1600 m^3). Fish holding ponds are connected via channels with a duckweed-based remediation system (DBS). The entire farm is powered by energy from a nearby wind turbine. The farm is designed to discharge excess water only during heavy rainfall. Eurasian perch *Perca fluviatilis* and rainbow trout *Onchorhynchus mykiis* are kept at a density that does not exceed the organic farming standard (< 20 $kg.m^{-3}$). Technical details of farm setup were presented in Stejskal et al., 2019 and O'Neill et al., 2020. The fish growth, health and plant growth (drone DJI Phantom 4 Pro V2.0) were monitored for two years. The protein content, and parameters related to digestibility and nutritional value were also analysed in the plant biomass produced. The DBS was planted with the duckweed *Lemna minor* and *Lemna gibba* and its cover is stabilised with shelters to prevent the negative impact of wind. Cyanobacteria, chlorophyll and turbidity were monitored on daily basis using the AlgaeTorch (bbe moldanke GmbH). Total ammonia, nitrite, nitrate and orthophosphate concentration were measured every 3 days using a HACH DR3900 spectrophotometer and LCK kits.

Results

Fish biomass, feed load duckweed cover and water quality results for years 2019 and 2020 are presented in Figure 1. Duckweed cover was close to 100% of treatment area in the second year of study. Values for all critical water parameter were within suitable values recommended for perch and trout, except a high peak for total ammonia (NH4) in Winter 2019. Several smaller ammonia peaks were observed especially before the duckweed mat establishment or in periods with low algae level (measured as chlorophyll). Moreover, oxygen levels varied from 75 to 145 % dependent on chlorophyll level and day phase. Another, factor showing high levels was the cyanobacteria level in 2020 as levels reached up to 210 μg/l. Chlorophyll level fluctuated in different pattern over 2019 and 2020. The second year of study was also characterized by an elevated pH level in the period from April to June. It was calculated that the potential annual duckweed biomass production at the Mount Lucas farm is around 40 tonnes of dry matter per year. The biomass contained between 20 and 35% of protein, depending on concentration of N in the water. The developed system abstracts minimal amounts of water (<0.001%day-1) and discharge is minimalised.

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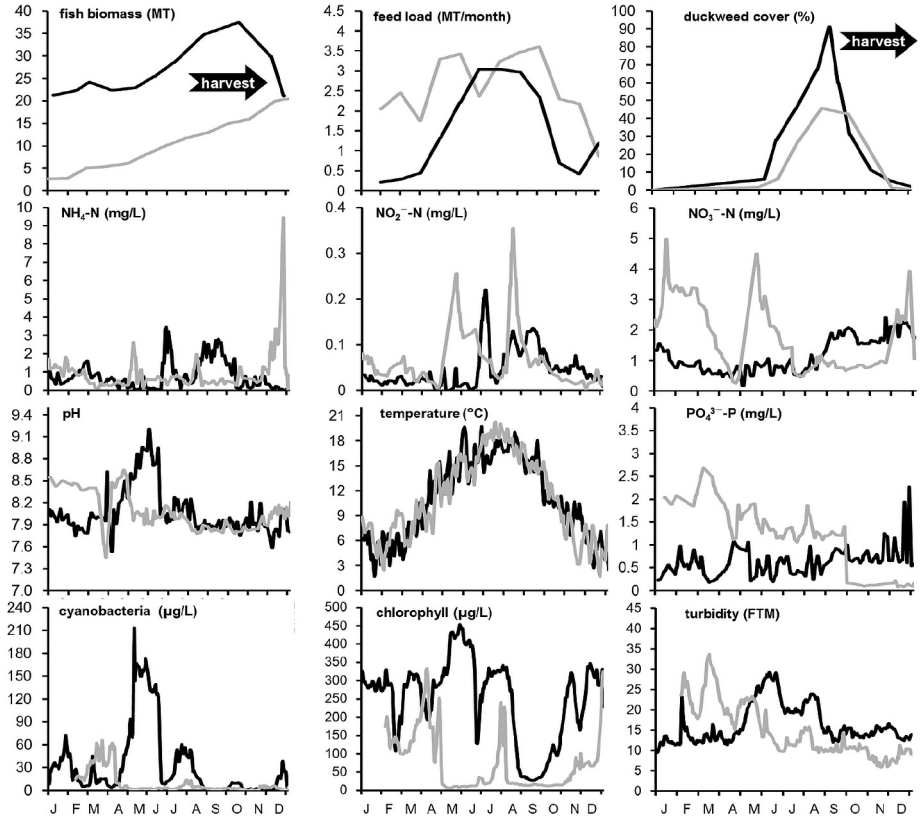

Fig. 1. Development of fish biomass (perch and rainbow trout), feed load, duckweed cover, total ammonia, nitrite, nitrate, pH, temperature, phosphate, cyanobacteria, chlorophyll, and turbidity level levels during two-years study of OASIS multitrophic system. Grey and black line represent year 2019 and 2020, respectively.

Conclusion

The system developed was extremely successful and productive. Fish were produced sustainably, water quality was maintained, and a significant biomass of duckweed produced, which has the potential to generate an extra income.

Acknowledgements

The study was supported by Bord Iascaigh Mhara (co-funded by the Irish Government and European Union).

References

O'Neill, E., Clifford, E., Stejskal, V., Rowan, NJ. 2020. Potential use of Irish peatlands as future locations for the sustainable intensification of aquaculture production processes for farmed fish – a case study characterising water quality. Science of the Total Environment, 706, 136044

Stejskal V., Paolaci S., Jansen M.A.K., Toner D. 2019. A new concept in multitrophic aquaculture farm cultured Eurasian perch (*Perca fluviatilis*), rainbow trout (*Onchorhynchus mykiis*), common duckweed (*Lemna minor*) and gibbous duckweed (*Lemna gibba*). In: Book of abstracts. Aquaculture Europe 2019, October 7–10th, Berlin, Germany, pp. 1458–1459

A CONCEPT FOR INTERACTIVE COMMUNITY WORKSHOPS IN THE MULTI-USE OFFSHORE SECTOR

E. Strothotte*, M. Jaeger

R&D Centre Kiel University of Applied Sciences GmbH, Schwentinestr. 24, D-24149 Kiel
Email: eva.strothotte@fh-kiel-gmbh.de

Introduction

The past year has taught us, to reconsider our general understanding of imparting knowledge in pursuing new approaches to stay connected and informed while maintaining the opportunity for lively debates and exchange of ideas. A key objective within the co-funded Horizon 2020 UNITED project is the design and implementation of an interdisciplinary and transferable training concept addressing risk management, safety and efficiency for multi-use offshore operation. The project seeks to examine the technical, regulatory, economic, social and environmental viability of ocean multi-use (MU) through the development of demonstration pilots in five different offshore locations. As it is essential to widen the understanding for offshore aquaculture cooperation in Europe, the workshop series has a clear focus on knowledge transfer, supporting the qualification of stakeholders from different backgrounds (industry, science, engineering, administrative authorities). The aim is to actively engage sectors involved in future MU offshore operations with properly educated staff, sufficiently knowledgeable in disciplines of most ongoing and future combined offshore operations. Furthermore, extensive briefing and information material (project deliverables, summaries, policy briefs, expert interviews), supplementary to the workshop topics, are provided to encourage an even greater collaboration and exchange between industry, science and administration. The newly designed training and teaching concept serves two purposes: i) the certification of trainees for work on MU systems; (b) a pandemic conform education concept, imparting operational skills to meet major challenges and new adaptations in the MU sector.

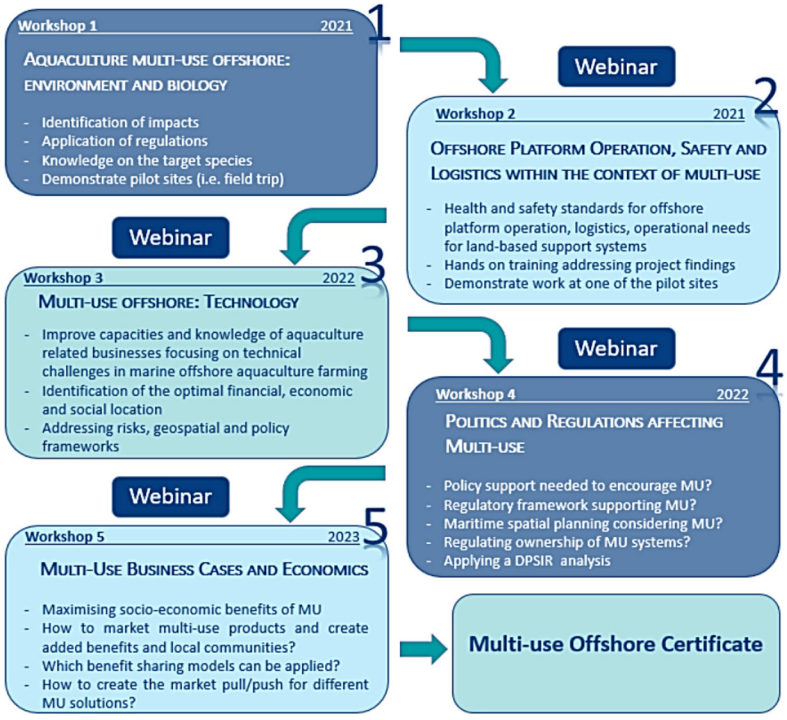

Figure 1: Schematic presentation of the interactive UNITED community workshop series.

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Materials and Methods

The workshops (Figure 1) will focus on knowledge transfer from the five pilots and exchange of ideas between stakeholders. The proposed topics reflect also knowledge gaps and needs identified in the MUSES project, and across UNITED pilots. Input and results gained during the workshops will be included in the course of the project, while experts in their respected fields will share insightful information, new developments as well as hands on experience reflecting MU. The five workshops are supposed to raise public awareness with a focus on regulations and legal rules applying to general aspects of:

a) Offshore platform operation (e.g. health and safety standards)

b) Environmental regulations related to handling and disposal of wastes in an appropriate manner (incorporating legal requirements of various regions)

c) Basic knowledge on the biology of target species employed and offshore aquaculture d) Basic knowledge on aquaculture technology employed in marine offshore farming

e) Handling procedures and management issues of aquaculture offshore systems (e.g. maintenance, stocking, harvesting)

f) Basic knowledge on operational needs for land-based support systems (spare parts logistics, perhaps data processing, further handling of harvest, storage and distribution)

g) Basic knowledge on legislation and operation for special support boats (including courses on navigation, safety at sea and other regulatory requirements).

Results

Within this multi-level (international, EU, national, local) and multi-sector (different marine and maritime industries) stakeholder engagement process, an educational program was developed, promoting capacity building of personnel, incorporating elements from hands-on experience and learning modules (offshore platform operation, environmental regulations, biology of target species, aquaculture issues, operational needs for land-based support systems, legislation; support boat operations). During the first interactive webinar (June 2020), over 90 participants informed themselves about the project and shared concerns, and opinions during a live poll session. Particularly, the creation of new and local jobs as well as a sustainable exploitation of the marine environment were powerful arguments for the MU development, favoured by many attendees. Also, distinct apprehensions were expressed regarding competition with local fisheries. Resolving the potential opposition of local fishers and coastal communities by offering innovative employment possibilities is a major topic of UNITED.

Conclusion

One recommendation of the MUSES workshop is to research how MU activities could contribute to more jobs and to improve the dissemination of existing knowledge. This educational program will address these recommendations and will develop new specialized job profiles in the fishery sector. A second effect should be the by stakeholders demanded empowerment of the fishermen by having direct involvement in the co-management of MPA, in terms of environmental monitoring and surveillance.

This Project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no 862915.

References

Lukic, I., 2018. Notes from the MUSES Workshop

MULTI-USE PLATFORMS IN MARINE SPACE – A VIABLE APPROACH FOR THE EUROPEAN MARITIME INDUSTRY AND LOCAL ECOSYSTEMS?

E. Strothotte*, M. Jaeger, Ivana Lukiç Ioanna Drigkopoulou, Roderik Hoekstra, Manuel Lago, Olga Mashkina, Luc van Hoof, Steven Draeger, Eef Brouwers, Nancy Nevejan, Hans Christian Søerensen, Alex Ziemba, Elias de Korte, Rieke Santjer, Ghada 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 (e.g. combination of coastal tourism and aquaculture) has been cited as a potential solution to both, space scarcity and viability of offshore operations (e.g. via combination with other offshore industry such as offshore wind energy production). Many of the past research projects have focused on the topic of ocean multi-use, involving aquaculture (e.g. TROPOS, MERMAID, MARIBE, MUSES). The Ocean Multi-Use Action Plan produced under the MUSES project has influenced many of the planning processes EU-wide and beyond. Several countries now either have integrated the concept of multi-use in their maritime spatial plans or they consider doing so. However, the actual implementation of the multi-use concept is still limited, mainly due to the lack of impact on the local environment and economy. While aquaculture is one of the key Blue Growth sectors, the marine space close to shore may be limited for its expansion or may face conflicts with existing activities such as tourism. On the other hand, aquaculture development in far offshore exposed areas also remains difficult from the operational, technological and, thus economic perspective. The UNITED project aims to implement five multi-use pilots in the real environment to assess socio-economic and environmental impacts and advice policy and research agendas.

Materials and Methods

Based on five demonstration pilots, the economic and ecological output of combining activities for offshore renewable energy (wind or solar) and aquaculture, wind energy and environmental restoration (oyster beds restoration), offshore aquaculture and tourism services and offshore wind and tourism, in the same marine space, is examined. The pilots are located in the North Sea, the Baltic Sea and the Mediterranean Sea, of which four pilots address the combination of offshore wind farms with tourism or aquaculture. The German pilot (North Sea) evaluates the feasibility of an offshore wind research platform, in close proximity to three wind farms, combined with seaweed and blue mussel cultivation. The Dutch pilot (North Sea) investigates the up-scaling potential of seaweed cultivation, floating solar and offshore wind energy production. Integrating native flat oyster cultivation and restoration as well as seaweed production in wind farms is assessed by the Belgian pilot (North Sea), while the Danish pilot (Baltic Sea) considers multi-use of tourism and offshore wind farms. The Greek pilot investigates the potential of fish aquaculture combined with diving tourist expeditions. Through close monitoring, dividing and reducing offshore operation expenses, applying technological innovations, expanding and enhancing developed business models, technology readiness levels of optimal MU solutions will be enhanced to "demonstration in an operational state" (TRL7). Presenting suitable solutions to all five pillars (technology, economy, legal/government, society, and environment) shall encourage key industrial actors in the sectors of renewable energy, aquaculture, and tourism to become involved in future MU concepts.

Results

Within the course of the project, business plans and risks assessments will be generated, emphasizing possible trade-offs for various sectors (tourism, energy and food production, nature restoration), taking into account geospatial, cultural and environmental differences of pilot site locations. Moreover, optimal MU solutions for economic revenues, addressing bottlenecks and uncertainties of technical, regulatory, financial, environmental, and socio-economic characteristics will be presented. As indicated in Figure 1, a variety of tools will be created and used in the project, which contribute to the development of co-location concepts as well as boost the blue economy of the European marine realm. In order to facilitate up-scaling of MU concepts, as applied in the UNITED pilots, a generic roadmap, blueprints for installation, operation and maintenance, a decision support system, stakeholder training concepts as well as environmental impact assessment analyses will be published.

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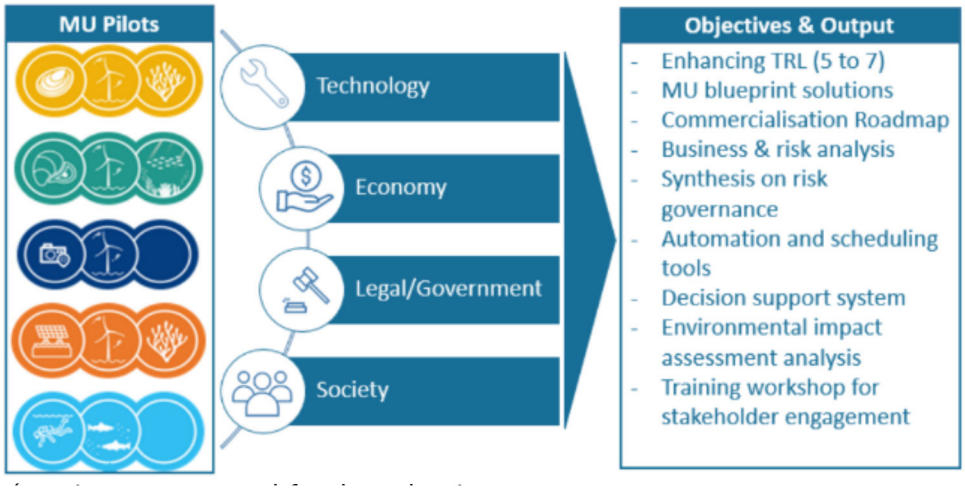
Results

Within the course of the project, business plans and risks assessments will be generated, emphasizing possible trade-offs for various sectors (tourism, energy and food production,



Conclusion

The competition for marine space is a well-recognized challenge, with the increasing need for renewable offshore energy production, food and resource security, as well as for the sustainable tourism. Establishing MU offshore platforms is complex, bearing unprecedented and manifold obstacles, as different levels of authorities, economic operators, stakeholders and diverse industrial sectors are involved. However, under the right circumstances, MU may contribute to a more efficient and sustainable exploitation of ocean space, encouraging innovative economic concepts and sustainable ecological processes that allow for win-win solutions. When the project outcomes prove to be ecologically, legally, socially and economically feasible, the way for future European implementation of multi-use systems on a broader scale is paved.

This Project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no 862915.

BACKYARD TILAPIA IN THE UK – LESSONS FROM A 'POSITIVE DEEP ADAPTATION' APPROACH TO AQUACULTURE *Oreochromis niloticus* IN A TIME OF CRISIS

James R. F. Sutton*, BSc PhD.

Rest-o-Tank Ltd, Bristol
jamessutton600613@gmail.com

AIM :
to investigate the possibilities of small-scale low budget aquaculture of *Oreochromis niloticus* (silver and red) in a home garden/greenhouse system in Southern England, UK, with regards to the problems associated with society decline such as during a pandemic or post-apocalyptic scenario as predicted by 'PDA' advocates, and co-incidentally experienced.

METHODS:
July 2020: Static aquaculture, mixed-sex fry, off-grid: aeration , mini-pump, fans. *Artemia* (decapsulated eggs) , *D. pulex*, *Panagrellus*, porridge, larval *Nematocera* and *Chironomidae*, algae, *Lemnoideae sp (2mm),* mixed blended vegetable slurry incl gingko leaf). Compost-decay under-tank heating-silo/pit, greenhouse, thermal bricks (retain daytime heat), woodburner, cooling by shading, fans, fountains and water changes. Salt monitored TDS meter/refractometry. Dechlorination chemicals, airstones, sand ,ceramic rings, home-made/proprietary charcoals. Insulated lids / loft-lagging, open / shaded, effluent waters *Lycopersicum* (non-hydroponic). O_2 meter; pH meter/ascorbic acid, temp outdoors healthy day/night; Greenhouse shielded from sun by dustsheets/wood, cold by wood/foil sheeting; logger-thermometer.

September, indoors, heaters / mains aeration, 27-35C. Anti-parasitic: incr NaCl conc./ flubendazole / praziquantel. Anti-bacterial, anti-fungal: methylthioninium chloride. Immune-stimulating vegetable extracts. +LEDs,, plants, bogwood, stones, clay pot,

RESULTS:
We raised 22 fry (<1cm) to up to about 9cm; ~80% survived ~130-150 days from. Causes of death: sudden pH fluctuations by ascorbic acid (from ~8 to <6), ammonia poisoning due to overfeeding, aggressor killing, de-oxygenation-related effects, filter-upsuction, bacterial bloom at 35C or failure to eat. Highs of 37C, lows 16C, accidental chlorination, kribensis and angelfish co-culture aggression (angel removed). Dominant male, paired with 2 females, all out-competed by single, smaller *Pulvichromis* (2/3 territory of a 190L); during recovery (accidental chlorine poisoning), the (male) *Pulvichromis* exhibited nursing behaviour. Parasites?: fluke-scratching behaviour, easily eliminated by in-tank medications. No bacterial infections in tilapia; white spot or fungus on corydoras (briefly co-cultured) medicated tank several times, no ill effect. Ammonia never detected , oxygen always >7, usually >12, temp dependent. Max weight: 15g breeding behaviour=> decline in feeding behaviour. No breeding observed, females, slower growth-rate at first did catch up (sexing based on size differences and lack of breeding colouration), only females grazed algae, often, did not sexually mature. Forms of fighting (male only) included forward/reverse 'fencing' (with the beak), death spirals (into rocks below), and males chasing into rocks/corners/filters and walls both male and females. Pseudo-female ('gay') behaviour was observed. Gaping at surface due to cessation of aeration mistaken for duckweed browsing. Uneaten sunken microworm/porridge meal observed forming massive networks on the base of one tank. Some nest building behaviour observed, some female hierarchy apparent, females: red tilapia- mildler behaviour, may have been hawaian golds. aggressive attacks aside (caused deaths of 5 fish both male and female, may been due co-culture of sexes and low-density culturing),

CONCLUSION:
Our study led to useful insights into how to better keep *Oreochromis* away from professional aquaculture setting. Most problems: caused by attempts to perfect water : sudden acid/base modifications in the 40L tanks in the greenhouse which were very difficult to control, probably insufficient buffering capacity, overfeeding (ammonia), and pump-related deaths (one tiny female was sucked into the pond filter which was only used on about 2 occasions; then high powered pond pump combined with very warm temperatures and uneaten food is believed to have caused a sudden death of the final two females in the 190L due to bacterial bloom/deoxygenation – sole surviver, male, died of unknown causes, a suspected broken heart. These tilapia did not eat this *lemnoideae* in this study, which is odd, maybe due to size of leaf/root. Compost-decay/ solar heating/solar pumps could probably enable an annual harvest for these tropical fish in a temperate climate in the backyard (e.g. in England or Wales), especially if combined with recognised dietary regimes, NMT fry, and non-runt fry,

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and non-stunting conditions (e.g. climate moderation thermostatic controls), notably an indoor overwinter rearing of fry prior to a final 3-5 months under glass. The biggest problem outdoors : high temperatures, shading prudent, prior to cold onset. Solar panel aeration was sufficient (airstones/mini pumps) in 40L(/64L plastic box tanks, avoids leaks), we found necessary use small low-cost solars with inbuilt rechargeable batteries / lowest-power option ensures aeration through the night. SolarPanels need be turned 3x/day (labour intensive). No de-oxygenation-related effects observed in summer greenhouse; to save electricity: combined a no filter/no aeration approach with O_2 monitoring – 3 tanks (60L,90L,190L) – 2/2 fish died in the 60L tank, erroneously assumed to be due to overfeeding as O_2 meter normal before and after; when fish then died in 90L tank, realised more likely a water volume:fish ratio issue, or biofilter fail. Yet NH3 (by pH) measured normal in the hours before and after death, which was overnight. In conclusion we still have a lot to learn, but enjoyed the experience and what it taught us about *Oreochromis* aquaculture, and 'PDA' in sensitive times, with the help of these marvellously tough yet at the same time also extremely delicate and beautiful creatures. Tank temperatures were mostly influenced by insulated lids, and compost (grass cuttings) compacted . High temp was ***not*** conducive to optimum tomato growth, leading to a slower plant growth and reduced fruit-setting- closed doors/windows fewer pollinators . We becoming an aquaculture farm using cyclic economy approach, local production, decreased industry spoilage marketing approaches, enabling aquaculture technology by home/farm/restaurateur supplement protein diet needs, by introducing aquaculture and/or hydroponics to the average permaculturist/gardening enthusiast/restaurateur.

DEEP NEURAL NETWORK ANALYSIS - A PARADIGM SHIFT FOR HISTO-LOGICAL EXAMINATION OF HEALTH AND WELFARE OF FARMED FISH

Lene Sveen*, Gerrit Timmerhaus, Lill-Heidi Johansen, Elisabeth Ytteborg

Nofima, Muninbakken 9, 9019 Tromsø (Norway)
E-mail: lene_sveen@hotmail.com

Introduction

To secure sustainable growth in the Aquaculture sector, optimal health, and welfare of farmed animals are crucial. Skin, together with the gill and the gut, are the primary mucosal barriers in fish. Due to its importance in protecting the fish from its external environment, histological analysis of Atlantic salmon skin is part of routine work for the assessment of fish welfare (Roberts, 2012), as well as a current field of intense research (Karlsen et al., 2018, Mota et al., 2019, Sveen et al., 2020).

The present work address two sub-goals related to exploitation of a commercially available convolutional neural network (CNN) to evaluate fish health. We sought to develop an AI-model on the Aiforia® platform, which produces reliable spatial measurements of the successive skin layers of Atlantic salmon. Second, we verified the AI-model on two independent sample sets: Samples collected from fish reared under controlled conditions in a research facility and samples from production fish collected from a commercial fish farm.

Materials and methods

The AI-model was trained on scanned tissue sections as described by Penttinen et al., 2018. For the skin AI-model, the main segment layer identified the epidermal and the dermal layer (Fig. 1). This layer was further subdivided to identify tissue and cell types within the epidermal (mucous cells) and dermal layer (scales, loose connective tissue, dense connective tissue, dark pigment). For the verification of the AI-model the neural network was run on two manually drawn regions of interests (ROI), one large ROI (L_ROI) covering the entire length of the tissue section (approximately1.5 cm), and one small ROI (2- 4 mm in length). The neural network was run in both regions and the data was exported on to a local hard drive. Manual assessment measurements of the skin samples were done independently by two researchers. Second, we applied the AI-model on two independent sample sets to investigate spatial and temporal changes in skin: 1) Samples collected from eight different body positions from fish reared under controlled conditions in a research facility, and 2) Samples from production fish collected from a commercial fish farm following specific time-point linked to major operational events.

Results

After repeated trainings on samples from our skin database, the AI-model managed to separate well between the two major skin compartments (epidermis and dermis), the mucous cell area, blue and pink mucous cells, scales and dark pigment (Fig. 1). These anatomic structures are distinct in terms of shape and color. The deep neural network had some difficulty in distinguishing between the two major dermal compartments, the stratum compactum and the stratum laxum. However, this was improved with training. The correlation between the AI-model and the human observers were also high for epidermal

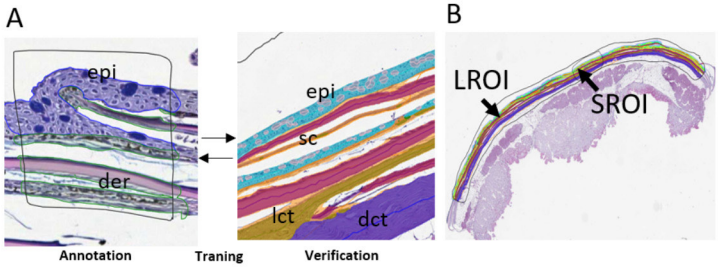

Figure 1. A. Repeated annotations, trainings, and verification for training of the deep neural network. B. Verification of AI-model to human observer was done on small regions of interests (ROIs), and results correlated to large (L)ROI covering the tissue section.

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thickness ($R \geq 0.95$, $p < 0.001$), total mucous cell number ($R \geq 0.99+$, $p < 0.001$), and dense connective tissue thickness ($R \geq 0.89$, $p < 0.001$). We further investigated the correlation between the small and the large region of interest (S_ROI and L_ROI) for the multiple skin tissues, the correlation coefficient was strong with most values being close to 0.8. For in depth validation of the algorithm and to characterization of the skin, the AI-model was deployed on skin samples from six different body positions. The area of epidermis, mucous cells sand scales followed the same trend, decreasing in anterior-posterior direction, while dense connective tissue was most abundant in the tail region. Results confirmed that skin properties change according to body site.

To further test the relevance of the AI-model, we collected samples from commercially produced Atlantic salmon (four net-pens) in Troms municipality, Norway. During the production cycle the fish were sampled prior to sea-water transfer, and at five time points during 16 months of the production time in sea. In general, the health of the production fish was characterized as good between September to May by the local fish health service. From June to November the general fish health was also classified as good, however there was an increase in mortality after fish transportation to new net-pens in June, and after mechanical delousing with Hydrolicer in August. Nearly every event, being sea-water transfer, growth, handling, and sexual maturation resulted in changes in one or more of the successive skin tissues. The transportation event had the overall largest negative impact on the skin morphology, whereas sexual maturation led to the greatest structural changes.

Conclusion and remarks

The AI-model showed correlations with normal histological features of the skin, enabling us to follow the development of the skin of Atlantic salmon at a new and more informative level compared to traditional histological evaluations. The main advantage running an AI-model is the generation of large and reproducible data sets which can be compared with other production parameters to discover significant biological changes. However, before developing or implementing an AI-model, we recognize that some considerations should be made. As the AI-model learns from the input data, a good quality training set and well considered annotations, are crucial for success. Moreover, the AI-model is flexible and will change as more data is incorporated into the model, continuous updating and validation of the AI-model is required. Quality control of the data processing require collaboration between data analysts and histologists to ensure correct interpretation of output data with biological significance. A main disadvantage with the model is that pathologies are not included in the training of the model and will therefore not be recognized. This may lead to loss of information or misinterpretation of results. Manual verification of random samples and knowledge of the samples being analyzed may reduce this risk. In the long run, AI-based models for evaluation of salmon health may represent a paradigm shift in how information from histological samples are used and how health of the farmed animals is evaluated.

References

Karlsen C, Ytteborg E, Timmerhaus G, Høst V, Handeland S, Jørgensen SM, Krasnov A (2018) Atlantic salmon skin barrier functions gradually enhance after seawater transfer. *Scientific Reports,* **8**, 9510.

Mota VC, Nilsen TO, Gerwins J, Gallo M, Ytteborg E, Baeverfjord G, Kolarevic J, Summerfelt ST, Terjesen BF (2019) The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. *Aquaculture,* **498**, 578-586.

Roberts RJ (2012) *Fish pathology,* John Wiley & Sons.

Sveen L, Karlsen C, Ytteborg E (2020) Mechanical induced wounds in fish – a review on models and healing mechanisms. *Reviews in Aquaculture,* **12**.

BENEFICIAL EFFECT OF THE NUTRITIONAL EMULSIFIER, EXCENTIAL ENERGY PLUS, ON GROWTH PERFORMANCE AND HEALTH STATUS OF NILE TILAPIA (*Oreochromis niloticus*)

M.A.C. Swinkels*, E. Wangkahart, A. Chantiratikul, B. Bruneel

Orffa Additives B.V., Vierlinghstraat 51, 4251 LC Werkendam (The Netherlands)
E-mail: swinkels@orffa.com

Introduction

Lipids supply energy, act as a structural component of cell membranes, play a key role in the immune defense system and may spare protein in diets for aquatic animals (Ghanawi et al., 2011; Li et al., 2019; Sun et al. 2011). In the absence of an emulsifier, lipids are poorly absorbed in the aqueous environment of the gastro-intestinal tract. Commercially, nutritional emulsifiers, such as lecithin and lysolecithin are included in diets to improve lipid digestibility (Li et al., 2019). However, these products are positioned lower on the hydrophilic lipophilic balance (HLB) scale compared to the GPGR (glycerol polyethylene glycol ricinolate) based emulsifier, Excential Energy Plus. An emulsifier with a high HLB value allows fatty acids to form into micelles in an aqueous environment more efficiently, enhancing lipid metabolism, nutrient digestibility, and growth performance of animals (Tocher et al., 2008). GPGR is widely used in livestock and poultry feed. The present study was conducted to evaluate whether GPGR can decrease the lipid requirements of Nile tilapia.

Material and methods

The Division of Fisheries of the Mahasarakham University in Thailand conducted a trial with 300 Nile tilapia with an initial weight of 8.06 ± 0.15 gram. Fish were adapted to laboratory conditions in circular fiberglass tanks and fed a commercial diet for 2 weeks. Ten treatments were formulated in triplicate. The basal diets contained 4.2, 5.7, 7.2, 8.7 or 10.7% crude lipid, with soya oil as lipid source. In each diet 35% crude protein (fishmeal, soybean meal, corn gluten) was included. Each of the basal diets, was tested with and without supplementation of the nutritional emulsifier Excential Energy Plus (0.35 g kg^{-1}, Orffa Additives BV, Werkendam, the Netherlands). Fish were fed 5.0% of their body weight twice a day. After 8 weeks of feeding, growth performance was evaluated and blood samples were taken.

Results

As shown in Figure 1, the fillet yield of fish significantly increased when fed the dietary treatments supplemented with GPGR. The emulsifier improved growth performance as it significantly enhanced the weight gain, specific growth ratio (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) ($P < 0.001$). The serum biochemical parameters showed that the innate immune response was improved by GPGR through the increased level of glutathione peroxidase (GPx) (Figure 2). In addition, activities of catalase (CAT) and superoxide dismutase (SOD) significantly increased ($P < 0.001$), whereas the stress marker, malondialdehyde (MDA), was significantly reduced ($P < 0.01$) due to the nutritional emulsifier. It was observed that the lipase activity in the intestine significantly increased ($P < 0.001$) with supplementation of Excential Energy Plus compared to the control groups.

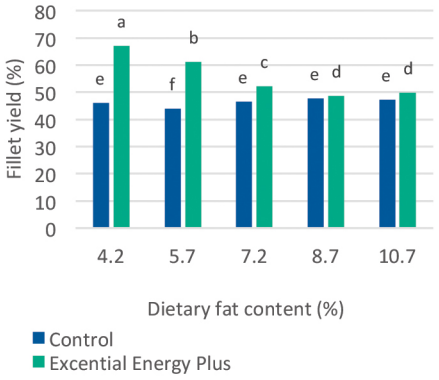

Figure 1. *The effect on fillet content of Nile tilapia through inclusion of Excential Energy Plus in diets varying in lipid content. The different letters on top of the bars are significantly different at P < 0.01.*

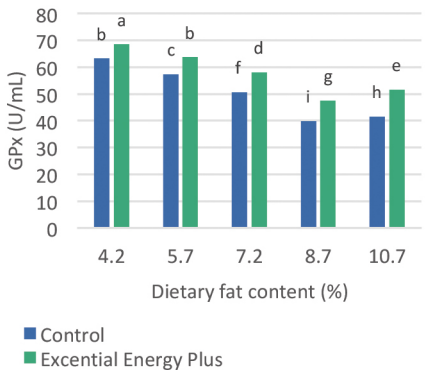

Figure 2. *The effect on antioxidant status of fish, indicated by the glutathione peroxidase (GPx) level, through supplementation of Excential Energy Plus in diets varying in lipid content. The different letters are significantly different at P < 0.001.*

(Continued on next page)

Conclusion

Fat level in the diet is a major cost in the fish feed industry. Therefore, improving lipid digestibility is related to cost savings (Tocher et al., 2008). Supplementation of GPGR enhanced lipid emulsification in Nile tilapia which resulted in an improved growth performance, enhanced innate immune system, improved antioxidant status and increased enzyme activity compared to the control group. Similar observations were shown in earlier studies about nutritional emulsifiers in juvenile turbot (Li et al., 2019) and yellow croaker (Ding et al., 2020). In the current study, the best results were achieved by fish fed diets containing the lowest fat content, 4.2 to 5.7% with inclusion of GPGR. In conclusion, Excential Energy Plus, as a nutritional emulsifier, is able to improve the performance of Nile tilapia.

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.

Ghanawi, J., Roy, L., Davis, D. A., & Saoud, I. P. (2011). Effects of dietary lipid levels on growth performance of marbled spinefoot rabbitfish Siganus rivulatus. *Aquaculture*, *310*(3-4), 395-400.

Li, B., Li, Z., Sun, Y., Wang, S., Huang, B., & Wang, J. (2019). Effects of dietary lysolecithin (LPC) on growth, apparent digestibility of nutrient and lipid metabolism in juvenile turbot Scophthalmus maximus L. *Aquaculture and Fisheries*, *4*(2), 61-66.

Sun, S., Ye, J., Chen, J., Wang, Y., & Chen, L. (2011). Effect of dietary fish oil replacement by rapeseed oil on the growth, fatty acid composition and serum non-specific immunity response of fingerling black carp, Mylopharyngodon piceus. *Aquaculture Nutrition*, *17*(4), 441-450.

Tocher, D. R., Bendiksen, E. Å., Campbell, P. J., & Bell, J. G. (2008). The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, *280*(1-4), 21-34.

ECOAQUA PROJECT: SUPPORTING THE SUSTAINABLE DEVELOPMENT OF THE IRISH FRESHWATER AQUACULTURE INDUSTRY

Alexandre Tahar*, Alan Kennedy, Ronan Cooney, Eoghan Clifford

School of Engineering, National University of Ireland, Galway, Ireland, H91 HX31
Ryan Institute, National University of Ireland, Galway, Ireland, H91 TK33
alexandre.tahar@nuigalway.ie

Introduction

Irish freshwater aquaculture involves mainly traditional flow-through salmonids production (rainbow trout and smolt), generally performed without or with limited water treatment and with high flow rates to ensure oxygen replacement and good water quality. Various EU and national policies envisage a significant increase in the production of sustainably produced fish based food products. However, due to more and more stringent regulations, the expected production increase must be through a sustainable intensification of aquaculture. EcoAqua was BIM funded project (2017-2019) with the aims to implement a water quality and energy monitoring program on 4 Irish freshwater fish farms. A graphical abstract of the project approach and objectives is presented below (Figure 1):

This particular study had a number of major objectives (i) ascertain the impacts of each site in terms of water quality, energy emissions and other water framework directive (WFD) biodiversity related impacts (ii) leverage this analysis to inform targeted decision making relating to capital investment and operation/control on each site and thus increase the efficiency on site, (iii) reduce the levels of nutrient discharge of selected farms to meet WFD criteria by implementation of appropriate technologies to treat the wastewater and facilitate re-use of the treated water, thereby reducing both the volumes of extracted and discharged waters and (iv) help position the industry as a sustainable food producing sector with evidence based research.

Materials and methods

The facilities chosen have various configurations (i.e. pond/tank based, trout/salmon smolt/perch production) that are highly representative of the whole freshwater aquaculture industry and was first benchmarked during year-1 of the program. This involved extensive and regular water quality monitoring – influent and effluent as well as locations downstream the facilities– of the 4 aquaculture sites to assess the performance of each and subsequent identification/benchmarking of their performance and impacts on receiving water quality. Monitoring was performed weekly throughout a whole year using 24 hour sampling.

Results and discussion

The main results of the EcoAqua project are summarized below:

- Impact of the farms observed directly downstream (water quality, biodiversity) but recovery observed within 1 km
- Identification of other nutrient inputs impacting river quality upstream and downstream from studied fish farms
- LCA demonstrated efficient feed usage as key to increasing sustainable fish production to the farm gate
- Potential energy savings identified if sludge was to be used in anaerobic digestion process

Conclusion

In conclusion, flow-through aquaculture can provide a low impact, high value food production with positive impacts in rural communities with increased policy/investment support. However, and as was also observed in other EU countries, the current Irish licensing system still limits the annual production levels and standing stocks for each farm while also forcing stringent limits on influent/effluents differential concentrations for a range of parameters. Thus, if national aquaculture production was to increase, a change in the licensing criteria will have to take place. On this aspect, licencing of fish farms by regulating emissions only could provide a pathway for further investment and expansion of the industry while protecting the environment

GROWTH PERFORMANCE OF GILTHEAD SEABREAM (*Sparus aurata*) FED LOW FISHMEAL DIETS WITH INNOVATIVE INGREDIENTS

A. Tampou[1]*, S. Andreopoulou[1], I. Nengas[2], K. Kousoulaki[3], A. Vasilaki[2], E. Mente[1]

[1]School of Agricultural Science, Department of Ichthyology and Aquatic Environment, University of Thessaly, Fytoko Street, GR-38446, Volos, Greece
[2] Institute of Marine Biology, Biotechnology & Aquaculture, Hellenic Center for Marine Research, Anavisos, 16777, Athens, Greece
[3] Nofima AS, Feed Technology Centre, Kjerreidviken 16, N-5141 Fyllingsdalen, Norway
Email: tampou@uth.gr

Introduction

The constant demand of sea products forces the global fisheries and aquaculture to produce more. Aquaculture of carnivorous species rely on marine protein and oil, but during 2018, 18 million tons of wild fish have used for the production fishmeal and fish oil (FAO ,2020). This, nowadays, has led most of the research to focus on the replacement of fishmeal and fish oil with sustainable sources of protein and lipids. This study aims to evaluate the effect of dietary fishmeal replacement with alternative ingredients such as algae meal, insect meal and tunicate meal on growth performance of gilthead sea bream (*Sparus aurata*).

Material and methods

The experimental trial was conducted at the Department of Ichthyology and Aquatic Environment, University of Thessaly, in Volos, Greece. Briefly, 720 individuals *S. aurata* (initial mean weight 6.57±0.04g) were distributed randomly to twelve 250l tanks. Four experimental diets were formulated to be isonitrogenous and isolipid, with diet 1 (control, FM) consisted of 26.55% fishmeal, diet 2 (0%FM) consisted of total replacement of fishmeal with algae meal (*Schizochytrium limacinum Phaeodactylum tricornutum*), insect meal (*Hermetia illucens*) and tunicate meal (*Ciona intestinalis*). Diet 3 (IM) consisted of 68.09% replacement of fishmeal with insect meal (*H. illucens*) and diet 4 (TM) consisted of 45.91% replacement of replacement of fishmeal with tunicate meal (*C. intestinalis*). Each diet was assigned to triplicate groups of 60 fish per group. The trial lasted 65 days, after 15 days of acclimatization. The fish were fed three times per day *ad libidum*. Fish were weighted individually at the beginning and end of the experimental trial under anesthesia. Feed consumption was recorded daily in order to be able to evaluate accurately values for feed utilization.

Results

The results showed that weight gain, feed consumption, specific growth rate, feed conversion ratio and protein efficiency ratio did not have statistically significant differences between the control diet (FM) and the total replacement of fishmeal with algae meal, insect meal and tunicate meal (0%FM). Fish fed with 68% replacement of fishmeal with insect meal diet (IM) had statistically significant lower feed consumption, weight gain and SGR values compared with diets FM and 0%FM. However, FCR and PER values for diet IM were not statistically significant different compared to diets FM and 0%FM. In addition, fish fed with tunicate meal diet (TM) had statistically significantly the lowest growth, feed consumption, FCR and PER ($p<0.05$).

Discussion

Karapanagiotidis et al. 2014 found that 30% fishmeal dietary replacement by *H. illucens* meal in *S. aurata* diet did not significantly affect SGR, FCR and PER, but the weight gain and feed consumption were reduced compare to control diet. In addition, 45% replacement of fish meal by insect meal (*H. illucens*) on *Dicentrarchus labrax* diet had not adverse effect on growth and feed utilization in contrast with protein utilization which was lower compare to control diet (Magalhães et al. 2017). Fishmeal replacement with the microalgae *P. tricornutum* in started diets of *S. aurata* showed that SGR was not affected ($p>0.05$) but had an adverse effect on survival (Atalah et al. 2007). Moreover, the inclusion of 2.5% *P. tricornutum* in finishing diets of gilthead sea bream did not significantly change growth and feed utilization parameters ($p>0.05$) (Ribeiro et al. 2017). Furthermore, the 2.5% inclusion of *Schizochytrium* sp. as lipid source and especially DHA source in microdiets of *S. aurata* had no negative effect on larvae, nevertheless the total replacement of fish oil reduced the growth and survival (Ganuza et al. 2008). The results of this study showed that total replacement of fishmeal with algae meal, insect meal and tunicate meal did not affect sea bream growth performance compared with the 68% replacement of fishmeal with insect meal and 45% replacement of fishmeal with tunicate meal.

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Table 1: Growth performance parameters of gilthead seabream fed the experimental diets

	FM	0%FM	IM	TM
Final Weight (g)	28.41±0.43 ^{a}	28.86±0.47 ^{a}	26.4±0.47 ^{b}	19.83±0.37 ^{c}
Weight gain (g)	21.87±0.43 ^{a}	22.19±0.47 ^{a}	19.84±0.47 ^{b}	13.3±0.37 ^{c}
Feed consumption (g)	17.95±0.42 ^{a,b}	19.36±0.75 ^{a}	16.56±0.43 ^{b}	13.96±0.53 ^{c}
Specific growth rate (SGR, %/day)	2.25±0.02^{a}	2.21±0.02 ^{a}	2.09±0.02 ^{b}	1.65±0.03 ^{c}
Feed conversion ratio (FCR)	0.89±0.02^{a}	0.95±0.02 ^{a}	0.93±0.3^{a}	1.51±0.23 ^{b}
Protein efficiency ratio (PER)	2.62±0.05^{a}	2.62±0.05 ^{a}	2.73±0.06 ^{a}	2.19±0.06 ^{b}
Survival (%)	91.71±2.88 ^{a}	97.82±0.58^{a}	95.86±1.44^{a}	96.13±1.09^{a}

Values are presented as means±standard error). Means sharing the same superscript are not significantly different from each other (P<0.05)

In terms of sustainable feed formulations, recent advances prove, among other low trophic level organisms the concept of the nutritional (Kousoulaki et al., 2017) feasibility of substituting fish oil by heterotrophically produced microalgae in salmon feeds. This study confirms that a mixture of innovative ingredients used for aquafeeds that can be grown on byproducts and waste of other agricultural industrial practices open new horizons for fishmeal replacement in fish feeds.

Acknowledgement

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References

Atalah, E., Cruz, C. M. H., Izquierdo, M. S., Rosenlund, G., Caballero, M. J., Valencia, A., & Robaina, L. (2007). Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for seabream (*Sparus aurata*). *Aquaculture*, *270*(1–4), 178–185.

FAO (2020). The state of world fisheries and aquaculture 2020. Sustainability in action. Rome.

Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R., & Izquierdo, M. S. (2008). *Crypthecodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets. *Aquaculture*, *277*(1–2), 109–116.

Karapanagiotidis, I.T., Daskalopoulou, E., Vogiatzis, I., Rumbos, C. I., Mente, E., & Athanassiou, C.G. (2014). Substitution of fishmeal by fly *Hermetia illucens* prepupae meal in the diet of gilthead seabream (*Sparus aurata*). Hydromedit, November13-15, Volos, Greece.

Kousoulaki, K., Carlehög, M., Mørkøre, T., Ytrestøyl, T., Ruyter, B., Berge, G.M. (2017): Long term supplementation of Heterotrophic microalgae in Atlantic salmon diets. Aquaculture Europe 2017, Dubrovnic, Croatia, 17-20 October, 2017.

Magalhães, R., Sánchez-lópez, A., Silva, R., Martínez-llorens, S., Oliva-teles, A., & Peres, H. (2017). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). *Aquaculture*, *476*, 79–85.

Ribeiro, A. R., Gonçalves, A., Barbeiro, M., Bandarra, N., Nunes, M. L., Carvalho, M. L., Silva, J., Navalho, J., Dinis, M. T., Silva, T., & Dias, J. (2017). *Phaeodactylum tricornutum* in finishing diets for gilthead seabream: effects on skin

IMPAQT – AN INTELLEGIENT MANAGEMENT SYSTEM FOR INTEGRATED MULTI-TROPHIC AQUACULTURE- A CASE STUDY: CAMLI PILOT SITE

Seyma Tarkan* [1] and Kemal Can Bizsel[2]

[1] Camli Yem ve Besicilik San.ve.Tic. A.S.Yunus Emre Mah., 7402/1 sok Pinarbasi, Bornova İzmir Turkey
[2] Dokuz Eylül University Institute of Marine Sciences and Technology
Email: seyma.tarkan@camli.com.tr

Introduction

As aquaculture evolving through industrial scales by exploiting advance technology, a world wide interest in both land-based and nearshore aquaculture systems for combining as an integrated systems comprising fed aquaculture species (e.g. finfish), inorganic extractive aquaculture species (e.g. seaweeds) and organic extractive species (e.g. suspension- and deposit-feeders) has become more concrete (Chopin et al, 2008; Bird et al., 2009; Silva et al. 2012). Integrated Multi-Trophic Aquaculture (IMTA) describes the arrangement whereby species are co-cultured for mutual benefit. IMTA allows the by-products, including waste, from one aquatic species to be the input (fertilizer, food) for another (STFC, 2013). Currently, such systems, which has been referring as "Integrated Multi-Trophic Aquaculture (IMTA)", has also been perceived as the most prominent progress towards the sustainable of aquaculture, by considering on its potential economic, societal and environmental benefits without any substantial contradictions since it is based on the principle of exploiting waste nutrients from higher trophic-level species for producing lower trophic-level species as added commercial value within a single production system (Troel *et al, 2009*). Such an integrated system not only mitigates considerably emission of production related wastes and thus reduces the nutrient load in the water (FAO,2018), but also increase capability for managerial efficiency in terms of cost reduction and improved product quality The IMPAQT project is an ambitious challenge to design and demonstrate an IMTA system supported by and Intelligent Management System (IMS). The project's demonstration has been planned to be carried out at six different pilot sites around Europe and one in China, all which has their production and product properties comprising different trophic species such as seaweeds, mussels, scallops, lobster and fish. The pilot in Camli (Cesme, Turkey) has been installed within the boundaries of the offshore production sites of a commercial enterprise at industrial scale which mainly produces seabream, seabass and meagre. It has been designed to carry out an integrated aquaculture production for seabass, blue mussel and sea lettuce. The development of such an offshore IMTA system requires the identification and analysis of environmental, economic and operational costs and benefits in order to be able to compare to a traditional monoculturing offshore system with a similar scale . The comparatie results of such investigations will enabel to assess the actual feasibility for anIMTA system for the future development of offshore aquaculture.

IMPAQT Camli Pilot in Cesme-Turkey.

The IMPAQT project (https://impaqtproject.eu/) has an overall objective for developing and validating in-situ a multi-purpose (inland, coastal and offshore productions), multi-sensing (heterogeneous sensors and new/emerging technologies) and multi-functional (advanced monitoring, modelling, data analytics and decision making) management platform for sustainable IMTA production. Its ambition is to drive a paradigm shift in the European Industry by paving the way to both a more environmentally friendly and more efficient/higher yielding European Aquaculture Industry.

IMPAQT's approach is to synchronize 4 main target objectives: ***i***) To design and implement new/emerging efficient and cost-effective technologies in monitoring and management systems for IMTA production, ***ii***) To validate the IMPAQT systems and IMTA model in-situ and the fish/seafood product in laboratory, ***iii***) To demonstrate an optimal sustainable IMTA development in a holistic perspective based on ecosystem services and circular economy principles, and ***iv***) To promote an effective transfer of knowledge derived by IMPAQT activities to the EU aquaculture stakeholders.

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The Camli Pilot site is appended to the only commercial offshore site at industrial scale with a production capacity about ..... tonnes of marine fishes. A system composed of three offshore fish cages, a floating pipeline for mussels and another one for seaweeds has been installed within the allocated marine area of the company Camli AS. As a pilot capable to carry out commercial scale production, the experiences that are obtained through the planned activities of IMPAQT project contains a series of information and and knowledge of interest for the aquaculture society. The aim of this presentation is to share those experiences with emphasizing the pros and cons so that the future issues to be implemented or challenged can have their place into the agenda of the European Aquaculture Society. The presentation will provide information on the level of contribution of IMPAQT IMTA system and discuss them in terms of a series of aquaculture aspects such as;

- monitoring growth and feed consumption for the optimization of stocking, feeding and harvesting,
- monitoring and preventing health related problems
- monitoring environmental footprint, and.
- monitoring welfare of cultured organisms.

References

Bird, C. J., McLachlan, J.& E. C. Oliveira (2009) Traditional vs. Integrated Multi-Trophic Aquaculture of Gracilaria chilensis Productivity and physiological performance. Aquaculture 293 (2009) 211–220

Chopin.T, Robinson. S M C, Troel, M., Neori.A, Bushman, A.H.,Fang, J. (2008) Multitrophic Integration for Sustainable Marine Aquaculture. Encyclopedia of Ecology, Volumes1-5.Elsevier.

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.

https://www.researchgate.net/publication/269996200_Multitrophic_Integration_for_Sustainable_Marine_Aquaculture.

Scientific, Technical and Economic Committee for Fisheries (STECF) – Summary of the 2013 Economic Performance Report on the EU Aquaculture sector (STECF-13-30). 2013. Rasmus Nielsen and Arina Motova (Eds), Publications Office of the European Union, Luxembourg, EUR 26368 EN, JRC 86673, 56 pp.

Silva C., Yáñez E., M.L. Martín-Díaz, M. L., and T.A. DelValls (2012) Assessing a bioremediation strategy in a shallow coastal system affected by a fish farm culture – Application of GIS and shellfish dynamic models

Troell, M.,Joyce, A., Chopin, T., Neori,A., Buschmann, A.H.,Fangh, J.(2009) Ecological engineering in aquaculture — Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture,* Volume 297, Issues 1–4, 1 December 2009, Pages 1-9. https://www.sciencedirect.com/science/article/abs/pii/S0044848609007856

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EUROPEAN PERCID FISH CULTURE (EPFC): BRINGING TOGETHER THE PERCID COMMUNITY

S. Teerlinck

Inagro, Practical Research Aquaculture Center, Ieperseweg 87 8800 Rumbeke- Beitem (Belgium)
E-mail: stefan.teerlinck@inagro.be

The European percid fish culture (EPFC) is a thematic group within EAS (European Aquaculture Society), active since 2012. The goal of this group is to disseminate and gather information on the culture of pikeperch, perch, and other species of the family percidae for human consumption, stocking and conservation.

In 2018 the EPFC-CG, a closed group with commercial and scientific stakeholders, was created. The aim of this group is to identify and solve current bottlenecks that prevent the further growth and success of the percid sector in Europe. The main issues are translated into relevant scientific and technical research questions.

In this presentation, an overview about the vision, challenges and progress of the EPFC will be presented.

CARRYING CAPACITY & FUTURE OPPORTUNITIES FOR EXPANSION IN UK

Trevor Telfer*

Environment Research Group, Institute of Aquaculture, University of Stirling, UK
*Email: t.c.telfer@stir.ac.uk

The overall concept of carrying capacity when applied to aquaculture development can be divided into four categories: physical capacity, production capacity, ecological capacity and social capacity (Ross, et al 2013). All these aspects are instrumental in ensuring environmental and social sustainability of new aquaculture developments. The four-stage carrying capacity approach is a key contributor to the Ecosystem Approach to Aquaculture as promoted by the FAO, by taking a holistic overview. More recently all aspects of carrying capacity been incorporated into regulation and governance of the aquaculture sector as it intensifies and expands into new areas of operation (Telfer et al, 2018). It is also seen as having major implications in overcoming the bottlenecks in licensing of new and existing locations for both inshore and offshore aquaculture.

The presentation will explain the importance of the four-stage approach to implementing carrying capacity use, summarize how it can be incorporated into regulation and licencing of UK aquaculture, highlight what are the major challenges are, and why it is an important process in modelling the suitability for inland, coastal and offshore production sites. This will be discussed in terms of existing aquaculture and potential for the future expansion in the UK.

Telfer, T., Falconer, L., Kaas, H., Jackson, D. 2018. TAPAS – Tools for Assessment and Planning of Aquaculture Sustainability – Horizon 2020. Impact, 8: 9-11. http://doi.org/10.21820/23987073.2018.8.9.

Ross, L.G., Telfer, T.C., Falconer, L., Soto, D. & Aguilar-Manjarrez, J., eds. (2013). *Site selection and carrying capacities for inland and coastal aquaculture.* FAO/Institute of Aquaculture, University of Stirling, Expert Workshop, 6–8 December 2010. Stirling, the United Kingdom of Great Britain and Northern Ireland. FAO Fisheries and Aquaculture. Proceedings No. 21. Rome, FAO. 46 pp. + 282 pp.

A NOVEL APPROACH TO QUANTIFY TOTAL AMINO ACID LEACHING FROM MICROPARTICULATE FEEDS USING TOC/TN ANALYSIS

Anıl Axel Tellbüscher*[1,2], Annette Simon[1], Bernd Ueberschär[2]

[1]Humboldt-University of Berlin, Albrecht Daniel Thaer-Institute, Invalidenstr. 42, 10115 Berlin, Germany
[2]Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum, Germany
E-Mail: anil.axel.tellbuescher@student.hu-berlin.de

Introduction

Free amino acids (AA) act as feed attractants (Kolkovski, 2013) and improve growth performance of fish larvae (Holt et al., 2011). Their diffusion from fish feeds into the adjacent water is thus desirable. However, diffusion losses of water-soluble AA and other nutrients might significantly lower the nutritive value of feeds, especially microdiets, before being ingested (Yúfera et al., 2002). Consequently, the balance between nutrient diffusion from and retention in the feed particle has to be considered when developing feeds. As alternative to existing protocols, an approach using Total Organic Carbon (TOC) and simultaneous Total Nitrogen (TN) determination was developed to exemplarily compare total AA diffusion from an experimental microencapsulated diet (EMED) with Gemma Micro 300 (GM), a microbound diet. A lower diffusion loss was expected for the EMED.

Material and Methods

The crude protein content of the diets was assessed by Kjeldahl analysis prior to the experiment (GM: 59% of DM, EMED: 63% of DM). Both diets had particle sizes of 300 μm. Prior to analysis, a calibration for both TOC and TN was conducted using a combined stock solution and the relative standard deviation of the procedure (v_{x0}) was calculated. For the feed samples, a glass beaker was filled with deionized H_2O (500 mL), placed on a magnetic stirrer (100 rpm) and 1 g of weighed feed sample was added. Water samples (20 mL) were taken with a 25 mL plastic syringe after 1, 3, 5, 15, 30 and 60 min. Samples were then immediately filtered using a vacuum filtration unit and a fiberglass filter (Whatman 934-AH, d = 47 mm, Merck, Darmstadt, Germany) to remove the feed particles. The water was discarded. The filtrate was transferred into a vial (40 mL) and the filtration unit rinsed with 50 mL deionized H_2O after each sample. A TOC analyzer (TOC-L, Shimadzu, Hamburg, Germany) equipped with an additional TN analyzer module (TNM-L, Shimadzu) and an autosampler (ASI-L, Shimadzu) was used for measurements. The device was set to simultaneous non-purgeable organic carbon/total nitrogen (NPOC/TN) detection mode with an injection volume of 50 μL, purging time of 1.5 min, a gas flow of 40 ml min^{-1} and an integration time of 4.5 min. Each sample was measured in triplicate. Measured C:N ratios were compared with the average C:N ratio 2.96 : 1 in proteinogenic AA to validate plausibility of the results. Relative AA diffusion losses were calculated according to Equation 1,

$$x = \frac{\gamma_N f_k V}{CPm} \qquad (1)$$

with γ_N being the TN mass concentration, the Kjeldahl factor f_k = 6.25, the volume V of water in which the feed sample with crude protein content CP and the mass m was immersed.

Results and Discussion

High linearity of calibration curves (R^2 = 0.99) and a low relative analysis error v_{x0} was found with 1.20% and 1.72% for TOC and TN analysis, respectively. During the experiment, all measured C:N ratios were >2.96 : 1. The excess C can be explained by diffusion of carbohydrates and results can thus be considered as plausible. The only feed ingredients containing N beside AA are vitamins that are present in amounts three orders of magnitude lower than AA. Measured C and N can therefore be accounted to AA diffusion into the water. Results for AA diffusion are presented in Figure 1. For both feed samples, a plateau was reached latest after 15 min. Therefore, the observed decrease of diffusion velocities occurred within the first minutes. Moreover, it clearly revealed that diffusion was slowed down considerably when feed particles are microencapsulated, which corresponds to results of earlier publications (López-Alvarado et al., 1994).

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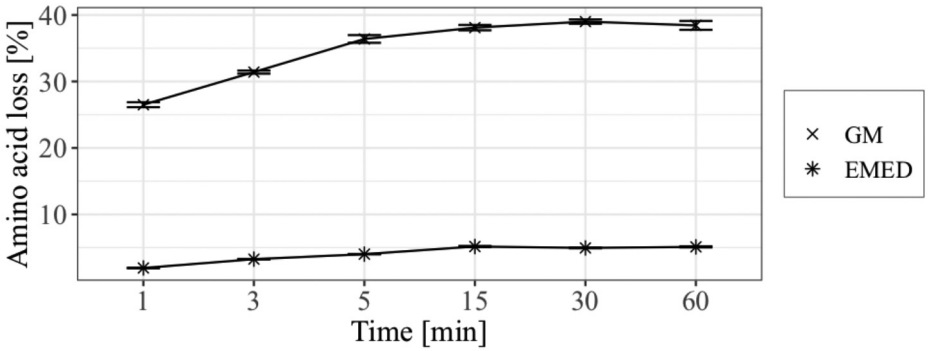

Figure 1: Time course of total amino acid loss [%] from microbound (GM) and microencapsulated (EMED) diets after immersion in water. Measurement in triplicate (means ± sd, $n = 1$).

Conclusion

Based on the low analysis error, the proposed method could serve as new way to characterize microdiets with respect to AA diffusion. However, validation needs to be done and accuracy and robustness have to be demonstrated with a sample size that allows statistical testing.

References

Holt, G. J., Webb, K. A., & Rust, M. B. (2011). Microparticulate Diets: Testing and Evaluating Success. In G. J. Holt (Ed.), *Larval Fish Nutrition*. pp. 353–372. Wiley-Blackwell. https://doi.org/10.1002/9780470959862.ch13

Kolkovski, S. (2013). Microdiets as alternatives to live feeds for fish larvae in aquaculture: Improving the efficiency of feed particle utilization. In G. Allan & G. Burnell (Eds.), *Advances in Aquaculture Hatchery Technology*. pp. 203–222. Woodhead Publishing. https://doi.org/10.1533/9780857097460.1.203

López-Alvarado, J., Langdon, C. J., Teshima, S. I., & Kanazawa, A. (1994). Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture*, *122*(4), 335–346. https://doi.org/10.1016/0044-8486(94)90342-5

Yúfera, M., Kolkovski, S., Fernández-Díaz, C., & Dabrowski, K. (2002). Free amino acid leaching from a protein-walled microencapsulated diet for fish larvae. *Aquaculture*, *214*(1–4), 273–287. https://doi.org/10.1016/S0044-8486(01)00902-4

DO DIETARY FORMULATIONS INFLUENCE THE METABOLIC FATE OF AMINO ACIDS IN GILTHEAD SEABREAM?

R. Teodósio[1,2]*, C. Aragão[1,2], L.E.C. Conceição[3], J. Dias[3], S. Engrola[1]

[1] Centre of Marine Sciences (CCMAR), 8005-139 Faro, Portugal
[2] Universidade do Algarve, 8005-139 Faro, Portugal
[3] SPAROS Lda., 8700-221 Olhão, Portugal

*Presenting author: rteodosio@ualg.pt

Introduction

The sustainability of the Aquaculture sector is largely dependent on optimised diets that promote nitrogen retention and maximise fish growth performance. Fish nutritional and health status, as well as diverse environmental and rearing conditions may require different dietary formulations. Therefore, the aquafeed industry have been focused on the optimisation of diets that meet fish nutritional requirements in various conditions. Despite the advances achieved over the years, there is still room for improvement.

Growth is essentially protein deposition and optimised growth requires the knowledge on the ideal dietary amino acid profile. To maximise fish growth, amino acids are required to be available in tissues at an optimum ratio, as imbalances will compromise protein synthesis and lead to an increased catabolism.

Amino acids may be catabolised in multiple pathways and be classified into three different categories according to their metabolic fate: ketogenic, glucogenic, and ketogenic plus glucogenic. Ketogenic amino acids, such as lysine, are catabolised only to produce acetyl-CoA, a precursor of ketone bodies or long-chain fatty acids. On the other hand, glucogenic amino acids (*e.g.* methionine) can be converted into glucose through gluconeogenesis. Additionally, some amino acids such as tryptophan, can be both ketogenic and glucogenic.

The aim of this study was to assess how different dietary formulations could affect the metabolic fate and consequently the bioavailability of selected indispensable amino acids: a ketogenic (lysine), a glucogenic (methionine) and an amino acid that is both ketogenic and glucogenic (tryptophan). Gilthead seabream (*Sparus aurata*) juveniles were fed practical diets with different protein and/or lipid levels. Metabolic flux assays were performed by tube-feeding ^{14}C-labelled diets to estimate evacuation, catabolism and retention of the selected amino acids.

Materials and Methods

Four experimental diets were formulated with two levels of crude protein (44 and 40 % CP) and two levels of crude lipids (21 and 18 % CL) using fishmeal (21 – 27 %) and plant (50 – 60 %) ingredients as protein sources. Fish oil to rapeseed oil ratio was kept at approximately 1.5 to 1.0. Diets were designated 44P:21L, 44P:18L, 40P:21L and 40P:18L according to their protein and lipid contents.

Gilthead seabream juveniles (*Sparus aurata*; body weight ± 9.5 g) were acquired from a commercial aquaculture and transported to the CCMAR facilities (Faro, Portugal), where they were maintained at 20 °C in 40 L cylinder-conical tanks in a recirculating aquaculture system (seawater at 32 ppt). Fish were assigned one of the four experimental diets and fed by hand, twice a day at a ration of 3 % body weight day^{-1}.

After one month of feeding the experimental diets, random fish from each dietary treatment were tube-fed with the respective experimental diet labelled with one of the following tracers: ^{14}C-lysine, ^{14}C-tryptophan or ^{14}C-methionine. The metabolic fate of the selected indispensable amino acids (n = 6 fish for each diet and tracer) was determined as a function of the amino acids' ketogenic and/or glucogenic nature, and of the dietary treatment.

^{14}C-amino acids' utilisation in seabream juveniles was determined based on the percentage of nutrient evacuated, catabolised or retained in the gut, liver and muscle samples. Differences among amino acids metabolic fate as a function of their nature and of the diet were identified by two-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test at $P < 0.05$ level of significance.

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Results and Discussion

In all dietary treatments, tryptophan was significantly more evacuated (± 60 %) compared to lysine or methionine, which presented similar evacuation values of approximately 26 and 32 %, respectively. Therefore, tryptophan bioavailability will be very low in fish tissues, compared to methionine and lysine.

Lysine was significantly more catabolised than the other selected amino acids, suggesting that amino acid catabolism in gilthead seabream juveniles is mainly ketogenic.

Amino acids retention in the gut was solely affected by the nature of the amino acid with lysine being the most retained amino acid in this tissue, although only significantly higher than methionine. Retention of the selected amino acids in the liver was influenced by the amino acid nature and also by the diet formulation. Fish fed the higher protein diets (44P:21L and 44P:18L) presented lower amino acid retention in this tissue, but only significantly different from fish fed the 40P:18L diet. Concerning the nature of the amino acids, lysine was more retained in the liver than tryptophan, while methionine presented intermediate values. Methionine was found to be preferentially retained in the muscle (± 37 %). Additionally, the retention of methionine in the muscle was significantly higher than lysine and tryptophan that were retained only 26 % and 8 %, respectively.

The assessment of ^{14}C-labelled amino acid utilisation in seabream juveniles fed experimental diets with distinctive dietary protein and/or lipid content showed that the present dietary treatments (44P:21L, 44P:18L, 40P:21L and 40P:18L) had no influence on evacuation, catabolism, and gut and muscle retention of lysine, tryptophan, and methionine. Liver retention was found to be affected by both nature of amino acids and the dietary formulations. While amino acid evacuation may be related to their physical or chemical properties (*e.g.* affinity to intestinal transporters), catabolism and muscle retention are strongly dependent on the amino acid ketogenic and/or glucogenic nature. The *in vivo* approach is a valuable tool that allows a fine-tunning of diet formulation. Optimisation of diets considering the amino acids bioavailability will maximise protein retention in fish and is a viable solution to develop cost-effective fish diets.

Acknowledgments

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EFFECTS OF FULL REPLACEMENT OF DIETARY FISHMEAL WITH INSECT MEAL FROM *Tenebrio molitor* ON RAINBOW TROUT GUT AND SKIN MICROBIOTA

G. Terova[1]*, L. Gasco[2], F. Moroni[1], M. Antonini[1], M. Saroglia, and S. Rimoldi[1]

1 Department of Biotechnology and Life Sciences, University of Insubria, Via J.H. Dunant, 3, 21100 Varese, Italy
[2] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo P. Braccini 2- 10095 Grugliasco, Torino, Italy
E-mail: genciana.terova@uninsubria.it

Introduction

Aquafeeds have largely been relied on fishmeal (FM), which is an optimal protein source to ensure fast growth and good health of farmed fish, but scarcely sustainable. In this regard, insects can represent a new world of sustainable and protein-rich ingredients for farmed fish feeds. Insects are excellent organic waste recyclers and their breeding has low environmental footprint (Bosh et al, 2019). In this view, the yellow mealworm, *Tenebrio molitor* (TM), is a great match because it is very efficient at bio converting organic waste and the percentage of edible biomass in larval and pupal stages is only slightly less than 100% (Ghaly et al. 2009). Larval and pupal stages of TM are rich in protein and lipids whose levels range from 47% to 60%, respectively. Furthermore, in terms of protein quality, meal from TM larvae has a well-balanced amino acid profile and the content of some indispensable amino acid is higher (as % of protein) than in land plants and slightly lower than in FM (Li et al. 2013). Accordingly, the present research aimed at investigating the effects of full replacement of FM with TM larvae meal in the diet of rainbow trout (*Oncorhynchus mykiss*) on fish growth performance, and microbiota of gut and skin.

Materials and methods

Two diets were formulated by SPAROS LDA (Olhão, Portugal) and **Ÿnsect** (Evry, France): one control diet with 0% of TM and one experimental diet with 100% of TM as substitutes for FM.

A 22 weeks feeding trial was performed on rainbow trout of 78.3 ± 6.24 g initial mean weight. Fish were randomly distributed into six 400-L tanks (3 tanks/diet, 21 fish/tank) and supplied with artesian well water at 13 ± 1 °C in a flow-through open system. Feed intake was monitored at each administration.

At the end of the trial, six fish/diet were sampled and the whole intestine was aseptically dissected out. The skin mucus microbiota was obtained by gentle scraping of fish body with a sterile cotton swab, whereas the gut autochthonous microbiota was obtained by scraping the mucosa of the entire intestine (excluding pyloric caeca).

The bacterial DNA was extracted from four aliquots from each feed, six samples of skin mucus, and six samples of intestinal mucosa per each dietary fish group.

Raw sequencing data were processed by QIIME 2 (2018.8). All data were checked for normality and homoscedasticity by Shapiro-Wilk's and Levene's test, respectively. Depending if normality of the data was satisfied or not, differences between groups were analysed by t-test or by nonparametric Mann-Whitney test using Past4 software version 4.02. Statistical significance was set at $P<0.05$.

Results and Discussion

At the end of the feeding trial, all fish tripled their mean body weight, but there were no significant differences between the dietary groups for any of the considered growth performance indexes ($P > 0.05$).

Thirty-two microbiome profiles (from 8 feeds, 12 skin mucus, and 12 gut mucosa samples) were successfully obtained by high throughput sequencing of 16S rRNA gene amplicons on Illumina MiSeq platform. Regarding the overall feed microbial community, at phylum level, Firmicutes and Proteobacteria constituted together approximately 99% of bacteria population.

By taking into account the gut microbial community, for both the experimental diets, the most abundant phylum was Tenericutes, followed by Proteobacteria and Firmicutes in descending order of abundance. Among them, relative amount of Proteobacteria, mainly represented by Beta- and Gammaproteobacteria, was significantly influenced by diet ($P = 0.047$) resulting higher in control group (3-fold increase).

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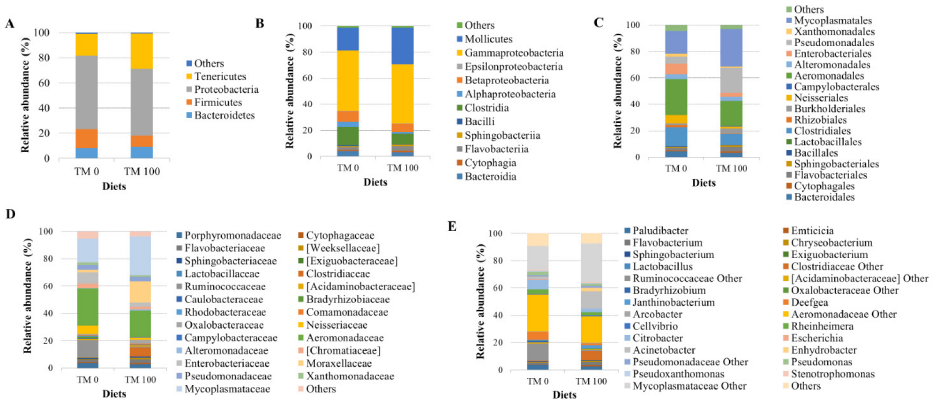

Figure 1. Relative abundance (%) of the overall most prevalent bacterial phyla (a), classes (b), orders (c), families (d), and genera (e) in Skin mucosa microbial communities (SMMC).

The skin microbial community was mainly consisted of 4 phyla, 11 classes, 17 orders, 25 families, and 20 genera (Fig. 1). Regardless of the diet, the skin microbiome of trout was dominated by four phyla: Proteobacteria, Firmicutes, Tenericutes, and Bacteroidetes. At order level, the only difference between two groups was for Neisseriales, mainly represented by Neisseriaceae family, that were significantly higher (2-fold increase, P=0.013) in fish fed control diet. At family level, Clostridiaceae resulted enriched (4- fold increase, P=0.013) in skin microbiota of trout fed with insect-based diet TM 100. Only genus Deefgea resulted significantly affected by diet (P= 0.017), being two fold increased in control feeding group TM 0.

In summary, the data demonstrated that yellow mealworm (TM) larvae meal is a valid alternative animal protein to replace FM in the aquafeeds. The totally replacement of FM with TM did not cause negative effects on rainbow trout gut and skin microbial communities. Last, but not least, the mapping of the trout skin microbiota represents a novel contribution of the present study since fish skin microbiota is still scarcely investigated. Indeed, in contrast to the increasing knowledge on gut microbiota, the skin microbiota of major farmed fish species remains largely unmapped but it deserves thorough consideration.

Acknowledgements

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References

Bosch G, van Zanten HHE, Zamprogna A, Veenenbos M, Meijer NP, van der Fels-Klerx HJ, et al. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. J Clean Prod. 2019; 222:355–63.

Ghaly AE, Alkoaik FN. The yellow mealworm as a novel source of protein. Am J Agric Biol Sci. 2009;4:319–31.

Li L, Zhao Z, Liu H. Feasibility of feeding yellow mealworm (*Tenebrio molitor* L.) in bioregenerative life support systems as a source of animal protein for humans. Acta Astronaut. 2013;92:103–9.

EARLY DRY FEED INTRODUCTION IN GILTHEAD SEABREAM *Sparus aurata* LARVAE: INFLUENCES ON ROTIFER CONSUMPTIONS, LARVAL GROWTH AND PERFORMANCE

J. Teske*, T. De Wolf, G. Franchi, V. Carbone, P. Joos, S. Lenzi and S. Debono

INVE Technologies, Hoogveld 93, 9200, Dendermonde (Belgium)
E-mail: j.teske@inveaquaculture.com

Introduction

Larval rearing is still considered one of the most delicate periods in marine finfish aquaculture. The high dependency on rotifers and variable enrichment and microbial quality are correlated with inconsistent survival rates, larval quality and performance. Although some hatcheries have mastered the art of rotifer production, the possibility to substitute this live food with a microdiet could significantly simplify production and improve larval development and quality.

The development of microdiets, capable of fulfilling the nutritional requirements during early stages, and rotifers without any negative effect on survival quality and performance remains very challenging. For a successful feeding and larval development, the microdiet needs to: 1. have a proper particle size to be ingested; 2.be easily digested and assimilated by the undeveloped digestive system of the larvae, and 3. be efficiently converted into energy for proper growth and assuring a high survival rate.

In the present study, seabream development (larval stage) was evaluated to determine the effects of reduced rotifer quantities in the feeding regime, and the introduction of a tailor-made microdiet as soon as 4 dph (days post hatch).

Material and Methods

The trial was conducted at INVE Aquaculture Research Center, Italy. Seabream larvae were cultured for 56 days in 390 L tanks, under two different feeding protocols (in triplicate):1. LFC- Live food control: exclusive use of rotifer as a first feed, and 2. RS- Rotifer substitution protocol: reduction of rotifers during the first 23 dph and introduction of microdiet at 4 dph.

The larval rearing tanks were maintained under optimal conditions for seabream, and parameters such as T (°C), salinity, DO (mg/L) were measured daily. The pH, and nitrogen compounds were measured weekly.

Total length and individual wet weight were measured weekly, from 7 dph, to determine larval growth performance. Dry feed intake was evaluated under the microscope every two days from 4 to 14 dph. The bottoms of the tanks were siphoned daily after 6 dph to estimate the mortality rate.

Results

Results have shown no differences in individual wet weight and total length between the treatments. However, seabream reared under protocol 2 (RS) had a survival of 32%, whereas in protocol 1 (LFC), survival was 25%.

Conclusion

This study indicates that partial reduction of rotifers in early stage combined with an early introduction of high-quality dry feed on 4 dph is feasible for seabream larvae, without compromising larval development and survival.

LOW-TROPHIC AQUACULTURE POLICY AND GOVERNANCE AROUND THE ATLANTIC BASIN: WHAT THE REGULATIONS ARE, WHAT PRODUCERS WANT

Paul Tett*[1], Rosa Chapela[2], Kåre Nolde Nielsen[3], Sofia C. Franco[1], Björn Suckow[4], Eric Arthur Bastos Routledge[5], Lucas Simon Torati[5], Felipe Vieira Nascimento[6] , Gonçalo Silva Marinho[7,8], Itziar Burgues[7], Isabel Sousa-Pinto[7,8], G. Courtois de Viçose[9] , Peter Britz[10]

corresponding author: paul.tett@sams.ac.uk
[1] Scottish Association for Marine Science, Oban, Scotland PA37 1QA, UK
[2] CETMAR, Rua de Eduardo Canello, Vigo 36208, Spain
[3] Universitetet i Tromsoe, Tromsoe 9019, Norway
[4] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany
[5] Brazilian Agricultural Corporation, Caixa Postal Nº 90, Palmas, TO, 77008-900, Brazil
[6] Federal University of Santa Catarina, Servidão dos Coroas, 503, Florianopolis, 88061-600, Brazil
[7] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros de Leixões, 4450-208 Matosinhos, Portugal
[8] Department of Biology, Faculty of Sciences of the University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[9] Research Group of Aquaculture, University of Las Palmas de Gran Canaria, Spain
[10] Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa

Introduction
The H2020 AquaVitae project (no. 818173, commenced June 2019) aims to sustainably increase aquaculture production in and around the Atlantic Ocean by developing new and emerging low trophic species and by optimising production in existing aquaculture (low and high trophic) value chains under the principles of circular economy. As part of this project, we have investigated public policy frameworks, and producer perceptions of these frameworks, for Low-Trophic Aquaculture (LTA) and Integrated Multi-Trophic Aquaculture (IMTA) in European countries, Brazil and South Africa.

Public policy frameworks
Information was obtained from published documents and, where possible, by interviews with policy makers. We focussed on seaweed cultivation and IMTA in three groups of countries: (i) those with well-developed aquaculture industries (for salmonids in Norway and UK and shellfish in Spain); (ii) those with growing production (Portugal, Brazil, South Africa); and (iii) those with limited growth (Germany and Sweden). The legal analysis addressed four building blocks: 1) the access to natural resources, seawater and coastal space through licensing procedures for aquaculture; 2) the site selection within Marine, and Town & Country, Spatial Planning; 3) the rules for aquaculture operation, and 4) the environmental, food security, and animal health, protection.

We found that comprehensive legislation is in place for aquaculture, especially for the farming of finfish and bivalve molluscs in most countries. Seaweed regulation exists in some countries, or regions within countries (Portugal, Spain, Norway, and Brazil) whereas there is little regulation that is specific for IMTA. Laws in Most countries have flexibility to cope with innovation and technological developments, opening the door to the diversification of species and culture system. Such flexibility can be, however, a double-edged sword when it is interpreted restrictively by the management authorities. Policy and societal priorities are reflected in the legal frameworks. In countries where environmental concerns dominate the public agenda (e.g. Germany, Sweden and Brazil), regulations tend to limit the activity; in countries or regions where aquaculture is a mature economic sector (UK, Spain and Norway), the provisions that enable finfish and shellfish aquaculture seem not so well suited for seaweed and IMTA.

Producer perceptions of current policy frameworks
Six workshops were held to gather insights from aquaculture producers and other stakeholders on what has promoted or constrained the development of LTA. The discussions focused on issues relating to public policy and regulation, licensing, food safety, and support for research, development and start-up, market aspects and industry organization. The workshops addressed macroalgae cultivation in northern Europe (Norway and Scotland, UK) and in Southern Europe (Portugal), Integrated Multi-Trophic Aquaculture (IMTA) in Brazil and South Africa, and the case of offshore aquaculture (of seaweed and shellfish) in a Nordic and a global context.

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A time consuming and complicated application process was identified as an impediment to the development of LTA in all cases and regions. In South Africa and in Brazil, the further development of IMTA was noted to be impeded by regulatory deficiencies specific for this novel type of aquaculture. Similarly, regulatory uncertainty was thought to result in investment risks that might constrain development of offshore aquaculture. In most cases, workshop participants were concerned that novel LTA production forms may be hampered by inadequacies in food safety procedures for these forms and resulting products. The importance of financial support for R&D, innovation and start-up was highlighted in nearly all cases. Low trophic aquaculture has not developed yet to a mature and competitive industry segment and its further development will depend on financial support and adequate administrative processes and legislation. Finally, public support was noted to be important for the development of LTA, and thus it will be desirable to help public discourse to distinguish between LTA and less sustainable forms of aquaculture.

Conclusions

There is a clear need for development of public planning and regulatory policies that can aid the growth of LTA and IMTA. AquaVitae will organise virtual meetings with high-level policymakers and industry representatives to discuss what might be done, drawing on experience from case studies carried out during the project.

Acknowledgements

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Links

AquaVitae website: https://aquavitaeproject.eu

BIOFLOC MEAL AS A DIETARY INGREDIENT FOR GENETICALLY IMPROVED FARMED TILAPIA IN INLAND SALINE WATER AND THEIR INCLUSION LEVEL

Thejaswini*, Gopal Krishna, Chadha. N. K., Chandrakant. M. H., Babitha Rani. A. M,

ICAR - Central Institute of Fisheries Education, Mumbai 400061, India
Email: thejaswini.0501@gmail.com

Introduction

Globally, around 20% of cultivated areas and nearly 50% of irrigated land are affected by the secondary salinisation of soil. These areas are unsuitable for agriculture due to an increase in salinization day by day. Fortunately, aquaculture is the most suitable and potential measure for the utilization of these inland saline land and water resources named Inland Saline ground water (ISGW). ISGW has proven the potential for sustainable fish production of euryhaline and marine species (Allan *et al*., 2009). Evaluation of the digestibility of the feed is a critical aspect while selecting/developing feed for fish. In the long run, highly digestible feed improves the growth performance of fish as well as reduce the production of waste. Noticeably, Biofloc meal is important and represent as an alternative ingredient for the development of cost-effective feed for fishes as well as reduces the dependence on fish meal for the fish feed and environmental impact (Prabu *et al*., 2018). Now Biofloc meal has been gaining the focus of intensive research in fish nutrition as a protein source in compounded feeds which can be produced by ex-situ biofloc technology (bioreactor) and addition of dried microbial floc could be novel approach of alternative feed (Kuhn *et al*., 2009) Biofloc meal is a potential viable alternative ingredient that can be used as feed at 20% Inclusion level for GIFT Tilapia for better growth performance (Prabu *et al*., 2018). Addition to that, the bioflocs harvested from a suspended growth biological reactor could be used in shrimp diets and biofloc (10, 20, and 30% biofloc inclusion) replaced soyabean and (10 and 20% biofloc inclusion) replaced fishmeal (Kuhn *et al*., 2016).

Materials and methods

Experiment was conducted for 60 days from 12th March-12th May 2020 at the Central Institute of Fisheries Education, CIFE Centre, Rohtak, Haryana by following completely randomized design (CRD) with varied inclusion level of biofloc meal. Four treatments in triplicates were setup based on the percentage of biofloc meal inclusion (0 or control, 10%, 20%, 30%) to follow CRD. This experiment was performed in inland saline ground water having 10ppt salinity. The control (C-without biofloc) and 3 diets (T1-10%, T2-20%, T3-30% of biofloc meal) were prepared using formulated levels of ingredient. The formulated experimental diets were iso-nitrogenous and iso-caloric in nature. The experimental tanks were stocked with 10 numbers of fishes per 250 L of water. The average initial weight of fishes during stocking was 3.43±0.15 g. Feeding was done @ 5% of bodyweight throughout the experiment and were fed at 10.00 hrs and 18:00 hrs. Water Quality parameters such as temperature, pH, salinity and dissolved oxygen were measured daily. Total alkalinity, total hardness, calcium and magnesium, total ammonia nitrogen (TAN), nitrite-N, nitrate-N, were measured weekly. Growth sampling was done every 10 days interval by taking total weight of fishes from each treatment units.

Results

The result of this study showed that there was significant difference ($p<0.05$) among the treatments in terms of weight gain, percentage weight gain (PWG), specific growth rate (SGR), daily increment (DI). There was no significant difference ($p>0.05$) among the treatments for feed conversion ratio (FCR), feed efficiency ratio (FER), hepatosomatic index (HIS) and gastrosomatic index (GSI). Good growth performance of GIFT tilapia was observed in the diet with 20% of biofloc meal in terms of PWG (81.38±1.22%), DI (0.23±0.02gday^{-1}), SGR (2.81±0.11), feed conversion ratio (1.27±0.03), and feed efficiency ratio (0.79±0.02).

Discussion and Conclusion

In the present study, survival rate in all the treatments was were 100% which supports Ekasari *et al*. (2019). High growth performance of GIFT tilapia in terms of weight gain, percentage weight gain, daily increment, specific growth rate, feed efficiency ratio was obtained in T2 followed by C, T1, T3. However, there was no significance between the control and T2, which supports the finding of Prabu *et al*. (2018). The highest growth was observed in T2 than control. This confirms previous reports on African catfish juvenile (Ekasari *et al*., 2019). This result might be explained by three possibilities that are first by higher protein, lipid, phosphorous digestibilities in T2 (20% inclusion level of biofloc meal) than control (Ekasari *et al*., 2018), second by the higher feed digestibility and higher bioavailability of nutrients, and third by the enhancement of the fish health status through the contribution of bioactive compounds (Ekasari *et al*., 2019). The reduced

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growth performance in the T3 (30% inclusion level of biofloc meal) might be explained by the reduction of feed palatability and digestibility with higher levels of microbial supplementation (Kiessling and Askbrandt, 1993). In the present study, the higher values of growth performances of GIFT tilapia were observed in T2 (20% inclusion level of biofloc meal). The results of the current study recommend that 20% biofloc meal in the diet is optimum for the growth of GIFT tilapia in inland saline ground water.

References:

Allan, G.L., Fielder, D.S., Fitzsimmons, K.M., Applebaum, S.L. and Raizada, S., 2009. Inland saline aquaculture. In New technologies in aquaculture, pp. 1119-1147.

Ekasari, J., Pasha, H.K. and Priyoutomo, N.B., 2018. Utilization of biofloc meal as a feed ingredient for Nile tilapia and common carp. Jurnal Akuakultur Indonesia, 17: 9-15.

Ekasari, J., Setiawati, R., Ritonga, F.R., Setiawati, M. and Suprayudi, M.A., 2019. Growth and health performance of African catfish Clarias gariepinus (Burchell 1822) juvenile fed with graded levels of biofloc meal. Aquaculture Research, 50: 1802-1811.

Kiessling, A. and Askbrandt, S. 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (Oncorhynchus mykiss). Aquaculture, 109: 119-130.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L. and Flick Jr, G.J., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture, 296: 51-57.

Kuhn, D.D., Lawrence, A.L., Crockett, J. and Taylor, D., 2016. Evaluation of bioflocs derived from confectionary food effluent water as a replacement feed ingredient for fishmeal or soy meal for shrimp. Aquaculture, 454: 66-71.

Prabu, E., Rajagopalsamy, C.B.T., Ahilan, B., Jeevagan, J.M.A. and Renuhadevi, M., 2018. Effect of dietary supplementation of biofloc meal on growth and survival of GIFT tilapia. Indian Journal of Fisheries, 65: 65-70

MICROPLASTIC EXPOSURE NOT DETECTED IN *Ostrea edulis* L. FROM BROODSTOCK CAGES FOR RESTOCKING EFFORTS

C. J. Thiele*[1], M. D. Hudson[1], A. E. Russell[2] and G. Sidaoui-Haddad[1]

[1] Centre for Environmental Science, Faculty of Environmental and Life Sciences
[2] 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—solid particles of synthetic or semi-synthetic origin and ≤ 5 mm in size—are contaminants of emerging concern (Arthur et al., 2009). They are present throughout the marine realm including in biota. The European flat oyster *Ostrea edulis* has been in decline throughout its range and restocking efforts are underway to increase the presence of this species (Helmer et al., 2019)often to the point of functional extinction. However, other negatively interacting factors attributing to this catastrophic decline include disease, invasive species and pollution. In addition, a relatively complex life history characterized by sporadic spawning renders O. edulis biologically vulnerable to overexploitation. As a viviparous species, successful reproduction in O. edulis populations is density dependent to a greater degree than broadcast spawning oviparous species such as the Pacific oyster Crassostrea (Magallana. The effects of microplastic ingestion on *O. edulis* is largely unknown, but may range from negligible to reduced offspring survival as demonstrated in *Crassostrea* (*Magallana*) *gigas* (Green, 2016; Sussarellu et al., 2016). The aim of this study was to assess microplastic concentrations in specimens from an actively on-going restocking project and seabed locations nearby in the Solent, UK.

Materials and methods

Sampling of *O. edulis* (n = 30; 60% caged, 40% subtidal seabed) was conducted from March to November 2017 from waters adjacent to the Solent, UK. Particles were extracted by digesting oysters in 10% potassium hydroxide for 48 hours at 40°C and filtering digestates onto 1.2 μm filter papers (Thiele et al., 2019)varying target particle sizes can hinder result comparison between studies. Human health concerns warrant recovery of small microplastics. We compared existing techniques using hydrogen peroxide, Proteinase-K, Trypsin and potassium hydroxide to digest bivalve tissue. Filterability, digestion efficacy, recoverability of microplastics and subsequent polymer identification using Raman spectroscopy and a matching software were assessed. Only KOH allowed filtration at ≤25 µm. When adding a neutralisation step prior to filtration, KOH digestates were filterable using 1.2-µm filters. Digestion efficacies were >95.0% for oysters, but lower for clams. KOH destroyed rayon at 60 °C but not at 40 °C. Acrylic fibre identification was affected due to changes in Raman spectra peaks. Despite those effects, we recommend KOH as the most viable extraction method for exposure risk studies, due to microplastics recovery from bivalve tissues of single-digit micrometre size.","author":[{"dropping-particle":"","family":"Thiele","given":"Christina J.","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Hudson","given":"Malcolm D.","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Russell","given":"Andrea E.","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Marine Pollution Bulletin","id":"ITEM-1","issued":{"date-parts":[["2019","5","1"]]},"page":"384-393","publisher":"Pergamon","title":"Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size","type":"article-journal","volume":"142"},"uris":["http://www.mendeley.com/documents/?uuid=ab655363-8f84-3030-9742-b859263aba9d"]}],"mendeley":{"formattedCitation":"(Thiele et al., 2019. Laboratory microplastic contamination was assessed using procedural blanks and airborne controls. Potential microplastics were enumerated using light microscopy (magnification ≤ 160x) and subsequently subjected to vibrational spectroscopy (Renishaw inVia, 785 nm) to ascertain plastic composition. Findings were corrected using 3x standard deviation of potential microplastic counts in blanks (Macdougall et al., 1980). Microplastics were reported as mean concentrations ± 1 standard deviation with median and 95% confidence interval (CI) range in brackets. A two-sample two-tailed t-test for unequal variances was performed.

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Results

Microplastic contamination detected in blanks led to a mean limit of detection of 1.7±2.2 for caged and 0.8±0.6 microplastics filter^{-1} for seabed specimens. Overall microplastic concentrations in oysters above LOD were low. None of the broodstock specimens contained microplastics, frequency of occurrence was 41.7% in seabed specimens with 2±1.2 (median 2, CI range 0.9-3.1) microplastics per microplastic-positive oyster. Seabed specimens contained significantly more microplastics than broodstock specimens from suspended cages (t-test, $p = 0.04$).

Discussion

This is the one of the few assessments of microplastics in O. edulis and the first from the Solent, UK, to our knowledge. Microplastic concentrations are low. Only specimens from the seabed contained microplastics. Their concentrations are in line with observations from C. gigas in the Salish Sea, USA, and the French Atlantic coast (Martinelli et al., 2020; Phuong et al., 2018) but potentially lower per specimen than in other bivalve species (Walkinshaw et al., 2020). This suggests that oysters either ingest less microplastics than other bivalve species or that their retention times are lower. None of the specimens assessed from the broodstock cages for restocking contained microplastics. Those specimens are held in cages suspended approximately 1 m below the water surface, suggesting that microplastic concentrations are lower in this habitat than near the seabed leading to lower exposure levels. If microplastics were to pose a hazard to reproduction in O. edulis as reported by Sussarellu et al. (2016) for C. gigas, their location in broodstock cages should ensure restocking efforts are not affected by microplastics.

Conclusion

Research output of microplastics in *Ostrea edulis* is low making it difficult to place our findings into broader context. Oyster species such as *O. edulis* seem to contain fewer microplastics than other bivalve species in general, but especially when suspended in broodstock cages at constant depth below the water surface. This suggests that interference of the restocking approach in the Solent through microplastic pollution should be negligible.

References

Arthur, C., Baker, J., Bamford, H. (Eds.), 2009. Proceedings of the International Research Workshop on the Occurrence, Effects , and Fate of Microplastic Marine Debris, in: NOAA Technical Memorandum NOS-OR&R-30. p. 49.

Green, D.S., 2016. Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. Environ. Pollut. 216, 95–103.

Helmer, L., Farrell, P., Hendy, I., Harding, S., Robertson, M., Preston, J., 2019. Active management is required to turn the tide for depleted *Ostrea edulis* stocks from the effects of overfishing, disease and invasive species. PeerJ 2019, e6431.

Macdougall, D., Francis, D.P.-C., Amore, J., Cox, G. V, Crosby, D.G., Estes, et al., 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. h

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.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., et al., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435.

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.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M., 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicol. Environ. Saf. 190, 110066.

CHARACTERIZATION OF SKIN, GILLS AND GUT BACTERIAL MICROBIOME OF MODIFIED ATMOSPHERE PACKED GILTHEAD SEABREAM USING NEXT-GENERATION SEQUENCING

Apollon Thomas[1,*], Spyros J. Konteles[1], Sotiris Ouzounis[2], Spyros Papatheodorou[1], Aliki Tsakni[1], Dimitra Houhoula[1], Theofania Tsironi[3]

[1]Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece
dhouhoula@uniwa.gr
[2]Medical Image and Signal Processing Laboratory, Department of Biomedical Engineering, University of West Attica, Athens, Greece
[3]Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
ftsironi@aua.gr

Introduction

The improvement of fish quality and the extension of shelf life has received increasing attention from academia and industry, at all stages of the production line and the supply chain (Tsironi et al., 2020). Modified atmosphere packaging (MAP) can effectively alter and delay the spoilage process and extend the shelf life of fresh fish. CO_2 inhibits the development of the respiratory organisms such as *Pseudomonas* spp. and *Shewanella putrefaciens* (Tsironi and Taoukis, 2018). Since the major cause of fish spoilage is of bacterial origin, the more detailed is the microbiological analyses, the more useful information may be obtained. One of the most powerful tools for microbiological analyses are the metagenomic studies using 16 S rRNA gene sequencing, that have been used to characterize several microbial communities (Jagadeesan, 2019). The introduction of Next Generation Sequencing (NGS) represents an important, fundamental technological advance in the biological sciences since the development of the polymerase chain reaction (PCR) in the mid-1980s. High performance and speed of NGS on 16S rRNA gene significantly reduce the cost and facilitates the research of metagenomics, providing a more complete assessment compared to a culture-based analysis. Few applications on NGS have been recently reported for the determination of microbial flora in fish (Silbande et al., 2018; Tsironi et al., 2019).

The aim of this study was to evaluate the effect of MAP on skin, gills and intestines bacterial microbiome using NGS.

Materials and methods

Fifty whole gilthead sea bream (*Sparus aurata*) samples (weight 380-420 g) were provided by Selonda S.A. and packed at aerobic conditions (AIR) or under MAP (60%CO_2/30%N_2/10%O_2) with gas-to-product volume ratio equal to 3:1 (1 fish per package). The pouches were packed in polystyrene boxes along with flaked ice and stored isothermally at 0°C. At days 0 and 10 of isothermal storage at 0°C, ten (10) whole, fish samples were randomly taken and analyzed regarding the bacterial microbiota of skin, gills, and intestines. DNA extraction was performed using the NucleoSpin Tissue kit. Extracted DNA was quantified using a spectrophotometer at 260nm and 280nm. After DNA extraction, 16S rRNA genes were amplified using domain-level bacterial primers that contain sequencing adapters and unique, sample-specific sequences. After generating amplicons, the Ion Plus™ Fragment Library Kit was used to ligate barcoded adapters and synthesize libraries. Barcoded libraries from all samples were pooled and templated on the OneTouch2™ system followed by 400bp sequencing on the Ion PGM. Automated analysis, annotation and taxonomic assignment occurs via the Ion Reporter Software pipeline. Classification of reads is through alignment to either the curated MicroSEQ ID or curated Green genes databases. Statistical analysis of the obtained data was performed using the R-package vegan (Oksanen, 2019).

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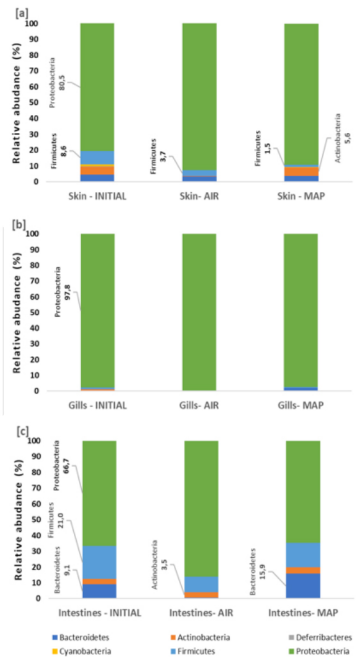


Figure 1. Phylum taxonomic composition of fish (a) skin, (b) gills and (c) gut microbiome, 24h after harvesting (INITIAL), as well as after 10d in ice (AIR or MAP).

Results

The results of the study indicated statistically significant differences in families' richness and diversity among the two types of packaging (i.e. AIR and MAP) as well as among the packaging type and the initial microbiome. The NGS analyses of the microbiota, at Phylum level, showed that the most persistent bacteria, both at the initial microbiome and at the end of shelf life, was *Proteobacteria*, either in AIR or MAP samples (Figure 1). As regards fish skin microbiota, the initially prevailing Families were *Comamonadaceae*, *Enterobacteriaceae*, and *Moraxellaceae* while on the intestines prevailed *Comamonadaceae*, *Anaplasmataceae*, *Bacillaceae* and *Enterobacteriaceae*. Statistical analyses indicated positive correlations between the skin and intestinal microbiota. By the end of shelf life, the composition of the initial microbiome was modified in both types of packaging (AIR, MAP). In general, several of the initially predominant families have been partially or completely replaced by psychotropic and psychrophilic families such as *Pseudoalteromonadaceae*, *Psychromonadaceae*, and *Shewanellaceae*. However, there were families, such as *Comamonadaceae*, persistent under MAP conditions. By the 8th day of isothermal storage at 0°C, fish under MAP exhibited higher sensory scorings than the respective AIR samples, indicating better retention of quality attributes of MAP fish.

Discussion and conclusion

MAP application immediately after slaughtering, as an alternative preservation method until further processing, has the potent to modify the original microbiome of fish. The prospect of extending the shelf life of fresh fish and modify their microbiological status until they reach the remote processing plant is tangible. However, the latter is strongly dependent on the aquaculture environment bacterial status. Thus, it is becoming evident that greater emphasis should be placed on the microbiological quality of aquaculture water and environment.

References

Jagadeesan B., Gerner-Smidt P., Allard M.W., Leuillet S., Winkler A., Xiao Y., Chaffron S., Van Der Vossen J., Tang S., Katase M., McClure P., Kimura B., Ching Chai L., Chapman J. & Grant K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. Food Microbiology, 79: 96-115.

Oksanen J., Blanchet F.Γ., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H. & Szoecs, E. (2014). Vegan: Community Ecology Package. R Package Version 2.2-0. http://CRAN.Rproject.org/package=vegan

Silbande A., Adenet S., Chopin C., Cornet J., Smith-Ravin J., Rochefort K. & Leroi F. (2018). Effect of vacuum and modified atmosphere packaging on the microbiological, chemical and sensory properties of tropical red drum (*Sciaenops ocellatus*) fillets stored at 4°C. International Journal of Food Micobiology, 266:31-41.

Tsironi T., Lougovois V., Simou V., Mexi A., Koussissis S., Tsakali E., Papatheodorou S., Stefanou V., Van Impe J. & Houhoula D. (2019b). Next Generation Sequencing (NGS) for the Determination of Fish Flesh Microbiota, Journal of Food Research, 8(4):101-110.

Tsironi T., Taoukis P. (2018). Current practice and innovations in fish packaging. Journal of Aquatic Food Product Technology, 27: 1024-1047.

DISTRIBUTION OF *npy* mRNA IN THE BRAIN OF ATLANTIC SALMON (*Salmo salar*, L.) AND RESPONSES TO GASTROINTESTINAL FULLNESS AND FASTING

Ingvill Tolås[1]*, Tharmini Kalananthan[1], Ana S. Gomes[1], Floriana Lai[1], Sissel Norland[1], Koji Murashita[2], Ivar Rønnestad[1]

[1]Department of Biological Sciences, University of Bergen. Thormøhlensgt. 53 A/B, 5006 Bergen
[2] Aquaculture Research Department, Fisheries Technology Institute, Fisheries Research and Education Agency, 224-1 Hiruda, Tamaki, Watarai, Mie 519-0423, JAPAN
Email: ingvill.tolas@uib.no

Balanced nutrition is essential to grow and maintain good health, and there is a continuous effort to improve our understanding of appetite and food intake in farmed salmon. As in mammals, feeding in fish is controlled by central feeding centers in the brain which receive and process endocrine signals from the brain and the periphery. The signals include hormones that increase (orexigenic) or inhibit (anorexigenic) appetite. Neuropeptide Y (NPY) is known as a potent orexigenic signal in vertebrates, but its role in teleosts is still unclear. *In silico* analysis revealed three *npy* paralogs, named *npya1, npya2 and npyb*, in the Atlantic salmon genome, and phylogenetic analysis confirmed that they clustered with the teleost homologues. All *npy* paralogs were found to be well conserved with the human homolog, with the predicted mature peptides sharing between 77 to 86% of amino acid sequence identity. We analyzed the mRNA expression of all *npy* paralogs in eight brain regions of Atlantic salmon post-smolt either fed normally or subjected to 4 days of fasting. *npya1* was predominantly expressed in the telencephalon but also highly expressed in the olfactory bulb and in the midbrain. *npya2* showed the highest expression in the hypothalamus and midbrain, while *npyb* was found to be highest expressed in the telencephalon, with very little expression in other brain regions. Fasting induced increased expression of *npya2* in the hypothalamus and midbrain but did not significantly influence expression level of *npya1* or *npyb* in any of the brain regions analyzed. Furthermore, a significant correlation was found between stomach filling and hypothalamic mRNA levels of *npya2*. Together these findings suggest that hypothalamic *npya2* acts as a central orexigenic signal in Atlantic salmon while the *npya1* and *npyb* may modulate other functions yet to be identified.

COMPARISON OF THE EFFECTIVENESS OF DIFFERENT TAGS IN THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

*N. Tourón[1], E. Paredes[2], S. Campos[1], D. Costas[1] and J.M. García-Estévez[1]

1 Centro de Investigación Mariña, Universidade de Vigo, ECIMAT, 36331 Vigo, Spain
Tel.: 722842627 *Corresponding author. ORCID: 0000-0001-6077-2801
Email: noelia.touron.besada@uvigo.es
2 Centro de Investigación Mariña – Universidade de Vigo, Laboratorio de Ecoloxía Costeira (ECOCOST) Departamento de Ecoloxía e Bioloxía Animal

The marking of sea urchins was implemented with the main objective of being able to individually identify the urchins in the natural environment once released. The possibility of identifying *P. lividus* specimens, both in captive conditions and released into the wild, by means of a reliable tag that has a high retention rate and doesn't affect the survival or growth of tagged individuals, is essential for the monitorization of sea urchins in growth studies, population dynamics studies, etc. and especially to check the success of partial or total repopulations performed in a given area. The identification of a brand with this characteristics would be a very useful tool for sea urchin aquaculture and especially of this species, which is highly overexploited and the most commercially appreciated in Europe, so it needs repopulation plans in a large part of the existing natural banks.

Numerous different marking methodologies have been tested for sea urchins, either by physical marking (external and internal labels) or by using chemical marking methods consisting of the use of fluorochromes, which adhere to the calcified structures of the urchin. Staining with these substances is performed using mainly two methodologies: inmersion (fluorochrome baths) or fluorochrome injection (tetracycline, calcein). Recently, polyfluorochromes (combinations of different fluorochromes) are also being used in order to achieve more visible marks. In this work, 5 different physical marks were used to mark 400 urchins of the *Paracentrotus lividus* species, which were kept for a month at the ECIMAT facilities in Toralla island. The efficacy of the methods used in each case was analysed, comparing the survival rate and the tag retention rate of the tagged urchins obtained with each tagging methodology.

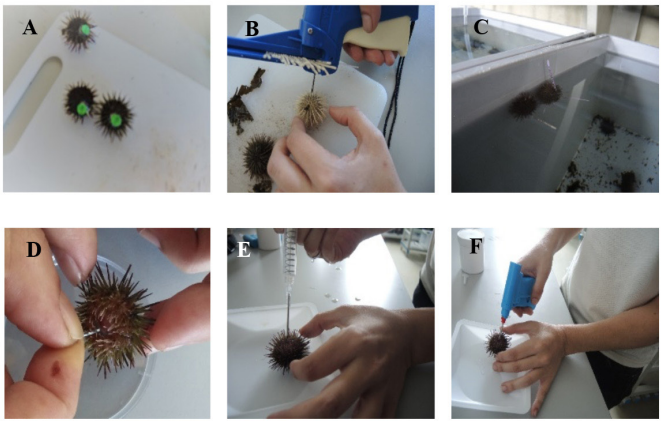
Material and Methods

In June 2020, 400 juvenile sea urchins (*P. lividus*) were received from the coast of Cangas do Morrazo (Pontevedra, Galicia): 42 ° 16′40 ″ N 8 ° 47′23 ″ W, with an average size of 20 mm in diameter and an average weight of 5.4 g. They were distributed in boxes (60 liters of capacity), at a density of 20 urchins per box, with filtered seawater in an open circuit, continuous aeration and feeding "ad libitum" with the brown macroalgae of the genus *Laminaria sp*. The duration of the experiment was one month (start on June 23, 2020, end on July 24, 2020). Five different types of sea urchin markings were used: Colored stickers, T-Bar labels, Minitransponder (Trovan brand), pieces of galvanized wire (3-4 mm long and 1 mm thick) and PIT Tags (Hallprint brand) with three replicates per mark and a control consisting of unmarked urchins.

Results

Minimum marking size

Wires: 11 mm diameter **Minitransponder:** 13 mm diameter



A.-Stickers adhered to the test of the urchins with Loctite glue; B.- Aboral introduction of T-Bar tags. C.- Urchins marked with T-Bar tags. D.- Introduction of the galvanized wire section through the peristomial membrane. E.- Introduction of Minitransponder (Trovan) with the help of a specific injector. F.- Introduction of PIT Tag (Hallprint) with its specific injector.

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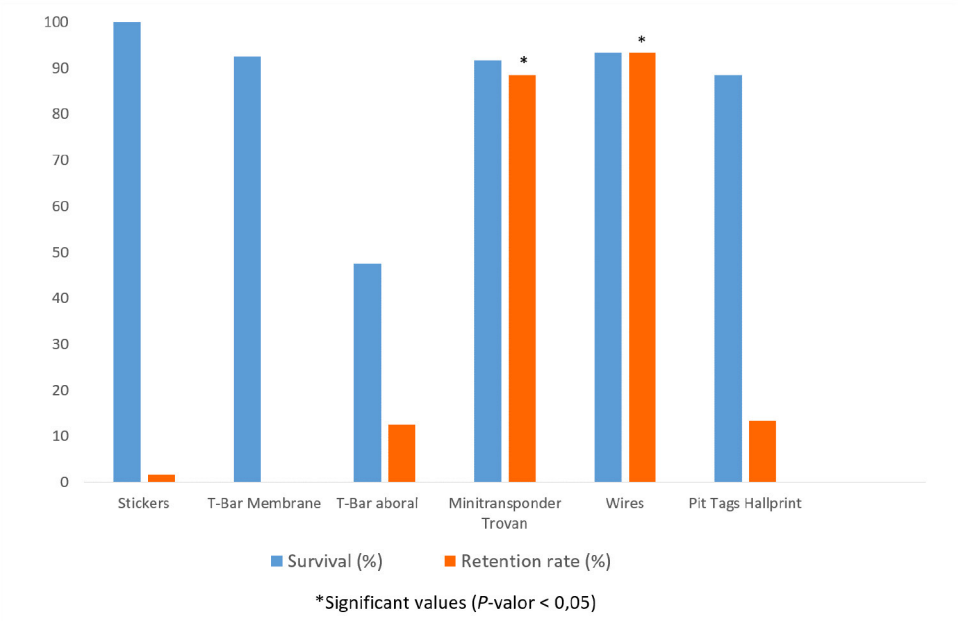

Conclusion

The highest tag retention and survival rates were obtained with Trovan Minitransponders and galvanized wire sections; both proved to be suitable marks for the identification of sea urchins in captive conditions, while waiting to obtain the results of the marked urchins released to the natural environment.

MACHINE LEARNING FOR AQUACULTURE, IMPROVING WATER QUALITY MANAGEMENT AND USE PREDICTIVE MAINTENANCE TO ENSURE DATA RELIABILITY

Dimitri Trotignon, Amine El-Farsi, Lucas Schaeffer, Maxime Lafont, Samuel Dupont, Charlotte Dupont

BIOCEANOR SAS, 1360 route des Dolines, 06560 Valbonne, France
dimitri.trotignon@bioceanor.com

Introduction

Water quality management in aquaculture is essential to ensure better production and food quality. It can help to optimize the costs, increase profits, and reduce the environmental pollution and impact. The availability of dissolved oxygen in water is an important parameter to follow for the industry. A too low rate in water could lead to an increase of animal stress, a decrease of growth and the death of an entire population in a farm. Therefore, Bioceanor have developed a predictive model to be able to anticipate the evolution of dissolved oxygen level in the future. A trustful model needs trustful data, so we needed to be confident in our data. As sensors are constantly immerged into water, they can be covered by biofouling or decalibrated. In this case, the data we collect is not precise anymore and its use to build a model can lead to unprecise predictions. This is why we decided to build a "predictive maintenance" model to ensure the quality of the data and to provide accurate predictions for the parameters we want to forecast.

Dissolved Oxygen prediction model

We decided to build our first model of dissolved oxygen forecasting for open sea farms. To build those models we used data from 6 areas different in term of localization and farming (fish or oyster production) in south of France. To build a robust model, we used meteorological data in addition to oxygen data to take into consideration the global environment (air temperature, wind, waves, etc...) on the oxygen availability in the water.

We have validated the model in the field in another area confirming the feasibility of the prediction of dissolved oxygen in open sea (Figure.1).

Predictive maintenance model

The issue of decalibration, showed us that there is an important need to monitor, not only the water but also the equipment used by the farmers. A lot of them do not have the time to check all the devices every day and can be misled by false data.

Knowing that, we have collected all the historical data we had from all the devices deployed with partners around the world. Our teams of experts and partners managed to create an important labelised dataset to detect unusual events such as decalibration or biofouling. Having an important dataset (more than 100 000 observations) we built deep learning model (LSTM) to classify those time series and be able to detect an issue on the sensor almost immediately after it happens. Then when an issue is detected, the farmer is directly alerted on our platform (Figure 2) and can go and check what is happening. This helps them to save a lot of time on their maintenance and stay focused on their production issues.

Our model detects the decalibration as soon as it appears, before it is easily detectable by human eye (Figure 2). The precision of our model is very high (> 0.94).

Discussion and conclusion

As shown by the results presented in this article, we have developed predictive algorithms for dissolved oxygen and open the possibility for predictive maintenance to allow farmers to trust the data they receive. It opens the feasibility to develop more and more models to assist farmers in their process.

The main goals of Bioceanor's data smart system that we developed are to:
• help the fish farmers to know better the environment of their production and anticipate the issues before it occurs.
• cut costs of feeding by giving advice for the right feeding time regarding the physico-chemical parameters.
• ensure traceability of the water quality of the farms.
• avoid losing time for equipment maintenance.
• ensure data quality provided by water quality probes.

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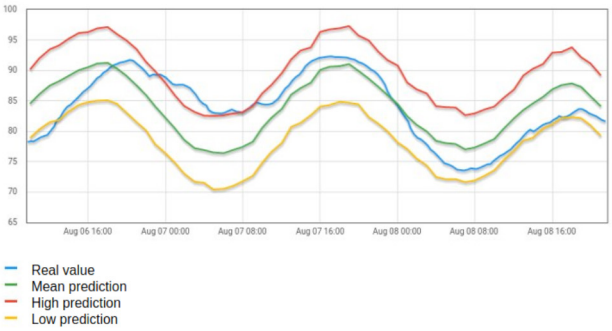

Figure 1 – Example of dissolved oxygen prediction in a fish farm in Mediterranean Sea. The blue line shows the real value that has been observed in the fish farm. The green line shows the mean value of the predicted dissolved oxygen level. The red and yellow line show respectively the high and low value of the predicted dissolved oxygen level.

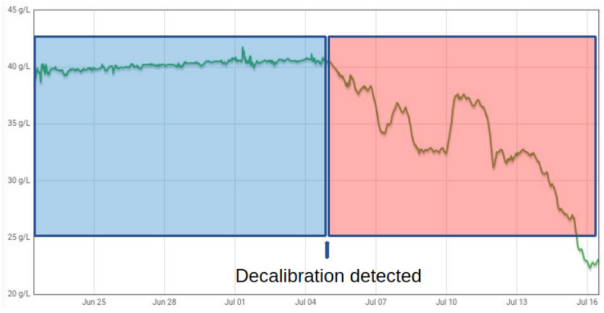

Figure 2 - Example of a salinity sensor normal behavior (blue) and decalibrated behavior (red). The predictive maintenance model detects the decalibration very early (blue tag).

Ensuring the data quality will give the opportunity for farmers to take their decisions based on real data and help companies such as Bioceanor to develop robust and usable features to continue to help aquaculturists to improve their production with restrained costs and a better environment management.

We are currently going further by adding remote sensing data to our model to be able to predict other phenomena like HAB (Harmful Algae Blooms). We are also integrating more devices for real-time data collection such as camera to analyze the fish behavior or the biomass in a net to improve our dissolved oxygen forecast and provide more tools for farmers.

SUPPLEMENTATION OF DIETARY INORGANIC ZINC ON SKELETAL ANOMALIES IN GILTHEAD SEA BREAM (*Sparus aurata*) LARVAE

Y. Tseng[1]*, D. Domínguez[1], U. Sivagurunathan[1], C.M. Hernández-Cruz[1], P. Eckhard Witten[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] Department of Biology, Ghent University, Ghent, Belgium
Email: tyiyen@gmail.com

Introduction

The occurrence of skeletal anomalies is still a constant problem that need to be solved in aquaculture. Besides, larval development stages are crucial for the recognition and identification of abnormalities in skeletal structures. Also, during this developmental phase, major changes occur in the skeletal system. Zinc (Zn) is an essential trace element that markedly affects ossification, bone development and lipid peroxidation (Watanabe et al., 1997). Previous studies have shown that increase in dietary Zn improves growth and survival in gilthead seabream larvae (Tseng et al., in prep.). However, the levels and range in Zn contents were very limited and there were no evidences of the effect of Zn on bone development or antioxidant functions. Therefore, the present study targeted the effect of 6 dietary Zn levels on the skeletal anomalies incidence and lipid peroxidation.

Materials and Methods

Diets. Six isoenergetic and isonitrogenous diets were supplemented with different levels of Zn at concentrations of 0, 20, 40, 60, 80 and 200 mg/kg. Thus, the analyzed dietary Zn content was 79, 98, 110, 130, 150 and 248 mg/kg, respectively. ***Fish and experimental conditions.*** Larvae (20 dph; initial length: 6.09 ± 0.30 mm; weight: 0.28 ± 0.04 mg) were randomly distributed into eighteen tanks (2100 individuals/ 200 L FRP tank) and were fed every 45 min from 8:00-20:00. ***Sampling.*** Growth was monitored by measuring the weight and length of larvae after 8 and 15 days (final sampling) of feeding. At day 8, samples were collected for gene expression relative and cartilage stain. At the final sampling, larvae (36 dph) were collected for histology, whole mount stain, gene expression, TBARS and remaining larvae for mineral analyse. Daily mortality was calculated for survival rate. Mineral analysis of diets was conducted using an inductively coupled plasma mass spectrometer (ICP-MS). ***Statistics.*** All data were tested for normality and homogeneity of variances and means compared by Ducan test and one-way ANOVA ($P < 0.05$).

Results

Malondialdehyde (MDA) in larvae fed by no supplement diet (Zn 79) showed the highest among the treatments (Figure 1.) ($P < 0.05$). Different diets fed by larvae showed no significantly different in the frequencies of severe anomalies (%) (Figure 2). The total length (mm) of larvae showed no significant difference among treatments.

Discussion and conclusion

Zinc inhibits free radical induced oxidative damage to cells and tissues (Lall, 2002). In agreement, the results showed that degradation products from lipid peroxidation, the MDA content, were significantly decreased in larvae by increasing dietary Zn content up to 130 mg/kg (Figure 1). However, further elevation of dietary Zn levels did not additionally reduced MDA contents, suggesting that 130 mg/kg of dietary Zn may be sufficient to fulfil the requirements of gilthead seabream larvae to prevent lipid peroxidation. In addition, zinc stimulates bone formation and inhibits bone resorption (Yamaguchi, 1998), hence it may prevent the incidence of skeletal anomalies and improve bone mineralization. In the present study, the highest incidence of severe skeletal anomalies was obtained in fish fed the lowest dietary Zn levels (33%) in comparison with fish fed 98-130 mg Zn/kg diet (16-23%)(Figure 2), followed by those fed Zn dietary levels higher than 130 mg/kg^{-1}(25-32%) but these values were not significantly different. In agreement with these results, Senegalese Sole (*Solea senegalensis*) post-larvae increase in dietary Mn at 90 mg kg^{-1} and Zn at 130 mg kg^{-1} decreased the severity of vertebral malformations (Viegas et al., 2020). However, in the zebrafish (*Danio rerio*) the Zn supplementation does not significantly impact the morphological anomalies (Roberto et al., 2017). These different results may be related to the different dietary Zn sources showed in those studies, as previously observed (Izquierdo et al., 2016). Further analysis are being conducted to better determine dietary Zn effects in larvae.

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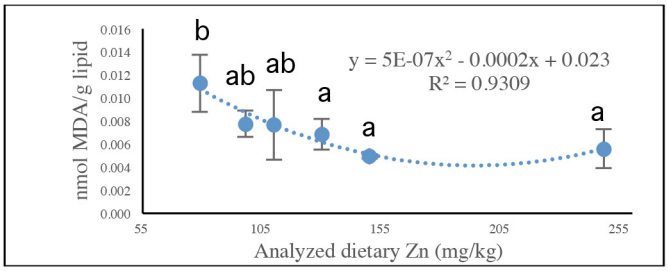

Figure 1. Thiobarbituric acid reactive substances (TBARS) – MDA of gilthead seabream larvae fed different diets for 17 days

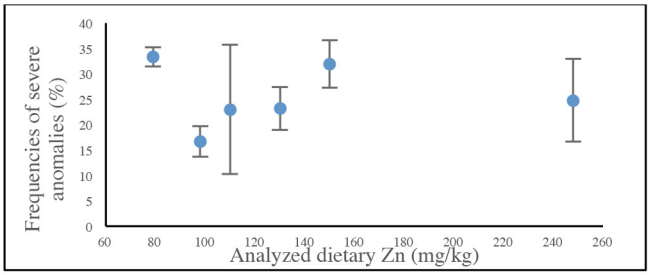

Figure 2. The frequencies of severe anomalies (%) in gilthead seabream larvae fed different diet for 17 days

Acknowledgment

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References

Izquierdo, M.S., Ghrab, W., Roo, J., Hamre, K., Hernandez-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). Aquacult. Res. doi:10.1111/are.13119.

Lall, S.P., 2002. In: Fish Nutrition. 3th edition, Halver, J.E., Hardy, R. (Eds.), Academic Press, San Diego, pp. 292-294.

Roberto VP, Martins G, Pereira A, Rodrigues S, Grenha A, Pinto W, Cancela ML, Dias J, Gavaia PJ (2018) Insights from dietary supplementation with zinc and strontium on the skeleton of zebrafish, Danio rerio (Hamilton, 1822) larvae: from morphological analysis to osteogenic markers. J Appl Ichthyol 34:512–523. https://doi.org/10.1111/jai.13664

Viegas, M.N., Salgado, M.A., Aguiar, C., Almeida, A., Gavaia, P., Dias, J., 2020. Effect of Dietary Manganese and Zinc Levels on Growth and Bone Status of Senegalese Sole (*Solea senegalensis*) Post-Larvae. Biol. Trace. Elem. Res. https://doi.org/10.1007/s12011-020-02307-4

Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. Aquaculture 151, 185–207.

Yamaguchi, M., 1998. Role of Zn in bone formation and bone resorption. J Trace Elem Exp Med; 11(2e3):119e35

THE ONTOGENETIC PATTERNS OF OXPHOS GENE EXPRESSION IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Andreas Tsipourlianos[a], Alexia Fytsili[a], Joao C. Cardoso[b], Zissis Mamuris[a], Deborah M. Power[b], Katerina A. Moutou[a]

a Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece
email: kmoutou@bio.uth.gr
b Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
email: dpower@ualg.pt

Introduction

Development and growth are highly energy-demanding processes, and efficient production of energy is expected to be crucial in early fish life stages. Oxidative phosphorylation (OXPHOS) is the central ATP producing mechanism and consists of five complexes of multimeric complexes, encoded by both the nuclear and the mitochondrial genome. The cross-talk between the two genomes and mechanisms shaping their polymorphism determine the efficiency of energy production. In the teleosts that have undergone a teleost-specific genome duplication (TGD), the fate and function of paralogues encoding the OXPHOS subunits is a further level of complexity determining function. This study aims to map the results of TGD on the genes encoding complex III of OXPHOS and to explore their ontogenetic profiles in commercially produced European sea bass. The correlation of gene expression levels with muscle development and growth is discussed.

Materials and methods

The genomes of nine teleost species and five other vertebrate genomes were investigated for homologues of the genes encoding for the subunits of ubiquinol-cytochrome c reductase (OXPHOS complex III) using the human sequences as the bait. Phylogenetic and synteny analysis was used to infer gene origin and evolution. The mitochondrial localization signal and cleavage position were predicted using MitoFates (1).

Samples from four developmental stages [first feeding (FF), flexion (FL), end of larva rearing (ELR) and mid metamorphosis (MM)] were collected from different commercial hatcheries and stored in RNAlater. Then RNA was isolated from whole larvae and cDNA synthesis was performed. Primers were designed for genes of interest and for two reference genes (fau, rpl13a), and the gene expression was quantified by real-time PCR. Significant differences between the expression level of each gene at different developmental stages were analyzed using a two-sided t-test. Differences were considered significant at $p<0.05$ level.

Results

Paralogues (*a* and *b*) were identified for four (*uqcrfs1*, *uqcrc2*, *uqcrh* and *uqcr11*) out of the nine genes of complex III and were the product of TGD. In all cases, the localization of the MPP cleavage site in the deduced proteins of the sea bass gene paralogs was predicted in the same position. Paralogue genes were expressed in all of the developmental stages analyzed, but the transcript abundance differed significantly between genes, paralogs, developmental stages and larvae batches. However, a consistent ontogenetic profile was observed across all the genes analyzed; FF exhibited high expression levels, followed by a decline in expression at stages FL and ELR and finally an upward trend in the MM stage. Expression abundance of genes in FF and MM stages was not significantly different. In addition, the expression of one paralogue (either *a* or *b*) was favoured over the other. The sum expression of both paralogues did not reflect the stoichiometry of the complex.

Discussion

Energy production and utilization is a crucial factor for fish development and growth. The understanding of the mechanisms that shape those fundamental processes is key in monitoring the progress of development and predicting the outcome of rearing practices and innovations. Our results suggest a) the subfunctionalization of the paralogue genes of the ubiquinol-cytochrome c reductase OXPHOS complex that derived from the TGD, and b) a common ontogenetic pattern of paralogue gene expression.

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Muscle is the biggest growing tissue in the developing larvae and changes in the relevant abundance of red and white muscle fibers between stages are expected to be reflected in the energy producing machine. In early developmental stages of European sea bass two types of muscle fibers are present; the red fibers responsible for slow swimming (cruising), and the white fibers responsible for fast swimming to support mainly prey-predator interactions. While both types of fibers are rich in mitochondria, the number of mitochondria is significantly higher in red fibers and could be almost double compared with the white fibers (2). At hatching, the white muscle consists of several layers of muscle fibers while the red muscle consists of a monolayer of fibers (3). At 28 days post hatch (dph) the red muscle starts to thicken at the regions closer to the lateral line by the addition of new layers of red fibers and dorsoventral extension, while further increasing its mitochondrial content (3). Thus, during stages FF, FL and almost up to ELR, the white muscle fibers might keep increasing in number and diameter in comparison to the red fibers leading to a decreasing fraction of mitochondrial volume. By MM, the increase in red fiber number could boost the mitochondrial volume and the transcription of OXPHOS genes.

The different pace in the development of white and red muscle is tuned to supporting the needs of fish larva regarding metabolic and developmental changes along with the adaptation to new tasks including swimming, exogenous feeding, and predator evasion. We suggest that the observed expression pattern of the OXPHOS CIII genes reflects the changes in mitochondria abundance with respect to the changes in the development and growth of red muscle fibers and the contribution of those in the total muscle mass. The collective value of the interindividual variability in gene expression at each developmental stage and how this links to differences in growth performance is explored.

Acknowledgements
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Bibliography

1. Fukasawa Y., Tsuji J., Fu S.-C., Tomii K., Horton P., Imai K. (2015). MitoFates: Improved prediction of mitochondrial targeting sequences and their cleavage sites. Mol Cell Proteomics 14, 1113-1126
2. Vieira V.L.A and Johnston I.A. (1992). Influence of temperature on muscle-fibre development in larvae of herring Clupea harengus. Mar Biol 112, 333–341.
3. Veggetti A., Mascarello F., Scapolo P. A. and Rowlerson A. (1990). Hyperplastic and hypertrophic growth of lateral muscle in Dicentrarchus labrax (L.). Anat Embryol 182, 1-10.

QUALITY EVALUATION OF PACKED PEARL OYSTER *Pinctada imbricata radiata* (Leach, 1814) FROM THE MEDITERRANEAN

Maria Makri[1], Xanthi Douvi[1], Alexios Ramfos[1], Efthimios Spinos[2], Theofania Tsironi[3], John A. Theodorou[1]

[1] Department of Animal Production, Fisheries & Aquaculture, University of Patras, 30200 Mesolongi, Greece
[2] Fisheries Department of Directorate of Agricultural Economy, Region of Western Greece, 26443 Patras, Greece
[3] Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece (ftsironi@aua.gr)

Introduction

Shellfish are an important component of global seafood production. In general, shellfish contain significant amounts of digestible proteins, essential amino acids, bioactive peptides, polyunsaturated long-chain fatty acids, astaxanthin and other carotenoids, vitamin B12 and other vitamins, including iodine, as well as other nutrients, which offer a variety of health benefits to the consumer. Although shellfish are generally safe for consumption, their exposure to different habitats, the nature of the shellfish filter such as oysters and mussels, as well as inappropriate culture conditions and handling practices may occasionally pose a health risk to consumers due to the various hazards (Venugopal and Gopakumar, 2017). *Pinctada imbricata radiata* is a benthic species that inhabits sandy bottoms and coral reefs (Strack, 2008), originates from the Indo-Pacific Ocean and has been reported in the Mediterranean as a non-endemic species since the 19th century (1874). the opening of the Suez Canal. Since then, the oyster has spread and settled in areas of the eastern Mediterranean with a significant presence in Sicily, Malta and the nearby islands, the Ionian Sea and the Adriatic (Theodorou et al., 2019). The objective of the study was the introduction of pearl oyster as an alternative seafood product and the evaluation of its nutritional value. Quality and shelf life was evaluated during refrigerated storage, investigating alternative packaging for transportation and display of the final products.

Materials and methods

Pearl oyster samples were captured from two regions in Greece, one of high (Saronic) and one of low (Evian) productivity, during four different seasonal samplings (spring, summer, autumn, winter). Samples were transferred to the laboratory for proximate evaluation (proteins, total lipids, moisture, ash) using appropriate AOAC methods.

Samples were packed in vacuum or in modified atmospheres and stored at isothermal, refrigerated conditions (2.5°C). Control samples were stored aerobically in non-sealed pouches, simulating conventional aerobic retail display facilities. Quality assessment was carried out immediately after packaging and at predetermined times of the storage period. Quality evaluation was based on microbiological analysis for monitoring of spoilage, pH, colour, lipid oxidation (TBARs method) and sensory evaluation.

Results

The proximate biochemical composition of the invasive pearl oyster Pinctada imbricata radiata in CE Greece coastline (Saronic & Evoikos Gulf) was examined in order to evaluate its nutritional value were:64.33% ± 2.40 for proteins, 11.41% ± 1.00 for fats, 10.56% for glycogen, 12.65% ± 2.27 for ash, and 79.97% ± 2.94 for moisture-water. The seasonal variation of the condition index (CI) ranged from 33.34% ± 7.62 (fall) to 42.35% ± 8.13 (winter) while the meat yield (MY) ranged from 24.95% ± 6.53 (fall) to 28.50% ± 4.10 (winter) indicating the suitability of pearl oyster as potential seafood for commercial exploitation for human consumption. Pearl oysters from the CE Greece was nutritious rich comparing with the other bivalve edible species exploited in CE Mediterranean, indicating its potential for human consumption.

Preliminary results show the potential to extend the shelf life of pearl oyster by appropriate packaging and storage conditions by at least 30%, using MAP and vacuum as alternative to conventional aerobic storage.

Discussion and conclusion

In conclusion, the pearl oyster is a non-endemic species that has spread with great success in the Mediterranean Sea, its growth is favored in waters with high temperature and salinity and low chlorophyll-a and has a great nutritional value such that it can be a species. target for fisheries. It is an easily adaptable species that spreads mainly in the eastern Mediterranean Sea. Optimized packaging conditions were selected based on microbial growth and sensory evaluation and shelf life extension.

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Acknowledgment

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References

Strack, E. (2008). Introduction. In: Southgate, P., Lucas, J., The Pearl Oyster. Elsevier, Oxford, pp. 1–35.

Theodorou A. J., Perdikaris C., Spinos E. (2019). On the occurrence of rayed pearl oyster *Pinctada imbricata radiata* (Leach, 1814) in Western Greece (Ionian Sea) and its biofouling potential. Biharean Biologist International Journal, 13(1), 4-7.

Venugopal V., Gopakumar K. (2017). Shellfish: Nutritive Value, Health Benefits, and Consumer Safety. Comprehensive Reviews in Food Science and Food Safety, 16, 1219-1242.

AQUAPONICS IN ACTION: PHYSIOLOGICAL RESPONSES OF SPINACH AND TILAPIA GROWTH

E. Tsoumalakou[1], E. Mente[2], N. Vlahos[3] and E. Levizou[1]

[1]University of Thessaly, School of Agricultural Sciences, Department of Crop Production and Rural Environment, Fytoko, GR-38446 Volos (Greece)
[2]University of Thessaly, School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, Fytoko, GR-38446 Volos (Greece)
[3]University of Patras, School of Agricultural Sciences, Department of Animal Production, Fisheries and Aquaculture, GR-30200 Mesolonghi (Greece)
E-mail: tsoumala@uth.gr

Introduction

Aquaponics is the production system that combines fish and plants cultivation through recirculating aquaculture system (RAS) and hydroponics. It is well documented that certain essential nutrients for plant growth, such as potassium (K) and iron (Fe) are present in sub-optimal concentrations in aquaponics (Delaide et al., 2016; Rafiee et al. 2019). Thus, most of the relevant studies have included extra nutrient supplementation to ensure maximum crop yield. Nevertheless, the additional fertilizers may to some extent compromise the sustainability of aquaponics system and its low input advantage. In this study we suggest that the balance between minimum input and maximum crop production should be based on the functional responses of plants under examination, which may identify possible system constrains and point to efficient management practices. We report several aspects of spinach performance and red tilapia growth when co-cultivated in aquaponic systems under three treatments, i.e. no fertilizers input, Fe and Fe+K supplementation.

Materials and Methods

The experiment was conducted in nine autonomous laboratory-scale aquaponic systems, of 135 l each, comprised of a fish tank (54l), a hydroponic unit (54l) and a biological sump filter (27l), as a raft method cultivation (1800cm^2). In total, 90 red tilapias, *Oreochromis mossambicus* (10 individuals/system) of 5.37±0.84g and 45 spinach plants, *Spinacia oleracea*, (5 plants/grow-bed) at the stage of four true leaves were cultivated under three treatments with three replicates each: a) control (C), where no fertilizer was added, b) Fe, where Fe-DTPA was added up to the target water concentration set by hydroponics (i.e. 40μmol l^{-1}) and c) Fe+K, with Fe as per the previous treatment along with K_2SO_4 (11 mmol l^{-1}). Above each grow-bed one HPS light bulb (400W) provided uniform light environment with PAR 300-400μmol m^{-2} sec^{-1} and photoperiod 10L:14D. Fish were fed twice a day (10:00 and 16:00) *ad libitum* for thirty minutes with commercial fish food of 47% protein content. The food was weighed before and after each meal and the daily amount administered was calculated. During the experimental period the physicochemical parameters of water were measured daily (i.e. pH and temperature, HQ40d, HACH). As for the plants, photosynthesis (Li-Cor 6400XT, Li-Cor Inc.) and chlorophyll a *in vivo* fluorescence (Handy-PEA, Hansatech) as an indicator of plant stress were measured on a weekly basis. The experiment lasted for 45 days after which the final harvest was performed, since plants had reached the marketable size. At the final harvest, several plant growth parameters were measured (leaves and roots fresh and dry weight, leaf area, biomass), the final weight of tilapias was recorded, and growth parameters were calculated, i.e. Specific Growth Rate (SGR %/day) and Weight Gain.

Results

Control plants showed the first signs of chlorosis after 10 days of cultivation, the severe expansion of which necessitated the early harvest of this plant group at day 20. At this time point all plant physiological and growth measurements confirmed the significant inferiority of C plants, yet fish weights were similar. Fish belonging to Control group were left to continue the experiment as typical aquaculture unit and their growth was evaluated again at the final harvest (day 45). Final weights of fish did not show statistically significant differences between treatments ($p>0.05$), while high survival was evident (97-100%). All tilapias finally reached 24g, exhibiting a specific growth rate (SGR) of 3.51±0.07, 3.57±0.06 and 3.53±0.06 %/day for C, Fe and Fe+K treatments respectively ($p>0.05$). The daily amount of fish food supplied was 4.51±0.18, 4.68±0.17 and 4.51±0.16g for C, Fe and Fe+K treatments respectively ($p>0.05$). Photosynthetic rates and chlorophyll fluorescence measurements during the experimental period were similar in spinach plants receiving either Fe or Fe+K supplementation and this was reflected at the yield parameters of the final harvest. Indeed, the leaves fresh weight was 37.18±5.12g and 43.41±5.94g for Fe and Fe+K treatments ($p>0.05$) and the same pattern was followed by roots, leaf area and biomass.

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Discussion and conclusions

The results of the present study highlight the inability of aquaponics to support spinach growth, mainly due to inherent Fe deficiency. Gas exchange as a sensitive indicator of plant function, and *in vivo* chlorophyll fluorescence as an early indicator of stress depicted well the impaired performance of spinach grown without nutrients input. The external supplementation of Fe resulted to similar growth and physiological responses of spinach compared to Fe+K addition. Thus, we may conclude that according to the minimal input concept, the addition of only Fe might be proved efficient for maximal spinach growth. Noteworthy is the fact that while K supplementation largely affect the electric conductivity (EC) of the circulating water, the relevant effect of Fe input is neutral. Vandam et al. (2017), corroborating the results of the present study reported that the supplementary fertilization in the Complemented aquaponic system (CAP) significantly promoted spinach growth when compared with aquaponic nutrient solution. In the present work, the fish growth was similar along all treatments, confirming that the supplementation of Fe may be the best option for the combined cultivation of spinach and tilapia in aquaponic systems.

References

Delaide B., S. Goddek, J. Gott, H. Soyeurt, and M. Jijakli. 2016. Lettuce (*Lactuca sativa* L. var. Sucrine) growth performance in complemented aquaponic solution outperforms hydroponics. Water 8.10: 467.

Rafiee G.R., C. Ros Saad, M.S. Kamarudin, M.R. Ismail, and K. Sijam. 2019. Effects of supplementary nutrient in an aquaponic system for production of ornamental red tilapia (*Oreochromis* Sp.) and lettuce (*Lactuca sativa* var longifolia). Survey in Fisheries Sciences, 5.2: 65-75.

Vandam, D. A., Anderson, T. S., De Villiers, D., & Timmons, M. B. 2017. Growth and tissue elemental composition response of spinach (Spinacia oleracea) to hydroponic and aquaponic water quality conditions. Horticulturae, 3(2), 32.

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5G VALIDATION TRIALS FOR THE AQUACULTURE INDUSTRY

Ioannis Tzanettis[1*], Panagiotis Vlacheas[1], Panagiotis Demestichas[1], Rakel Skaret-Thoresen[2], Mari Haukø[2], Andres Gonzalez[3] and Neil. Ruane[4]

[1]WINGS ICT Solutions, Athens, Greece; [2]Sealab AS, Trondheim 7042, Norway; [3]Telenor ASA, Fornebu 1331, Norway; [4]Marine Institute, Galway H91 R673, Ireland
*Email: gtzanettis@wings-ict-solutions.eu

Introduction
Recent technological advances such as the 'internet of things' (IoT), cloud computing, artificial intelligence have led to the coining of the phrase 'Agriculture 4.0 to describe the role these innovations can play in enhancing productivity and sustainability. Many new technologies have already been adopted in aquaculture through the development of intelligent management systems, sensor development and automated feeding systems. A key requirement for the success of these technologies is the ability of communication networks to deploy and cover rural areas efficiently. Technologies such as the legacy 2G network and current 3G/4G technologies are particularly suited for most current requirements but can be constrained in terms of coverage and data rate. More advanced use cases will require high reliability, low latency, global coverage, energy efficiency and the ability to connect a large number of sensors/devices without loss of performance. All these requirements should be met through the 5G infrastructure.

5G-HEART (5G for Health Aquaculture and Transport) is an EU project which aims to validate the use of 5G technologies for a range of industries including aquaculture. The project will trial 5G network solutions in two pilot sites in Greece and Norway.

Methodology
In Greece, the solution proposed aims to cover day-to-day operations such as fish health and welfare, optimal feeding and infrastructure integrity. The needs of the user translate into a series of network-specific requirements, including connection density for data collected by multiple water quality sensors, high bandwidth for collecting camera footage, (both underwater and security) and low latency for remote and autonomous operation of devices such as underwater drones.

In Norway, eight cages will be monitored by one camera in each cage, together with oxygen and salinity sensors. Using 5G technology, the ambition is to deliver the high uplink speed required to transfer the video streams and sensory data to an edge server with very low latency.

Expected Outputs
The project will install and validate integrated 5G network set-ups, linking on-site sensors, cameras and underwater drones with a 5G base station and measure the performance through pre-defined key performance indicators (KPI's).

Delivering high-quality video and sensor data to enable the industry to have continuous monitoring of important parameters including water quality, fish welfare, optimised feeding and reducing waste.

Acknowledgements
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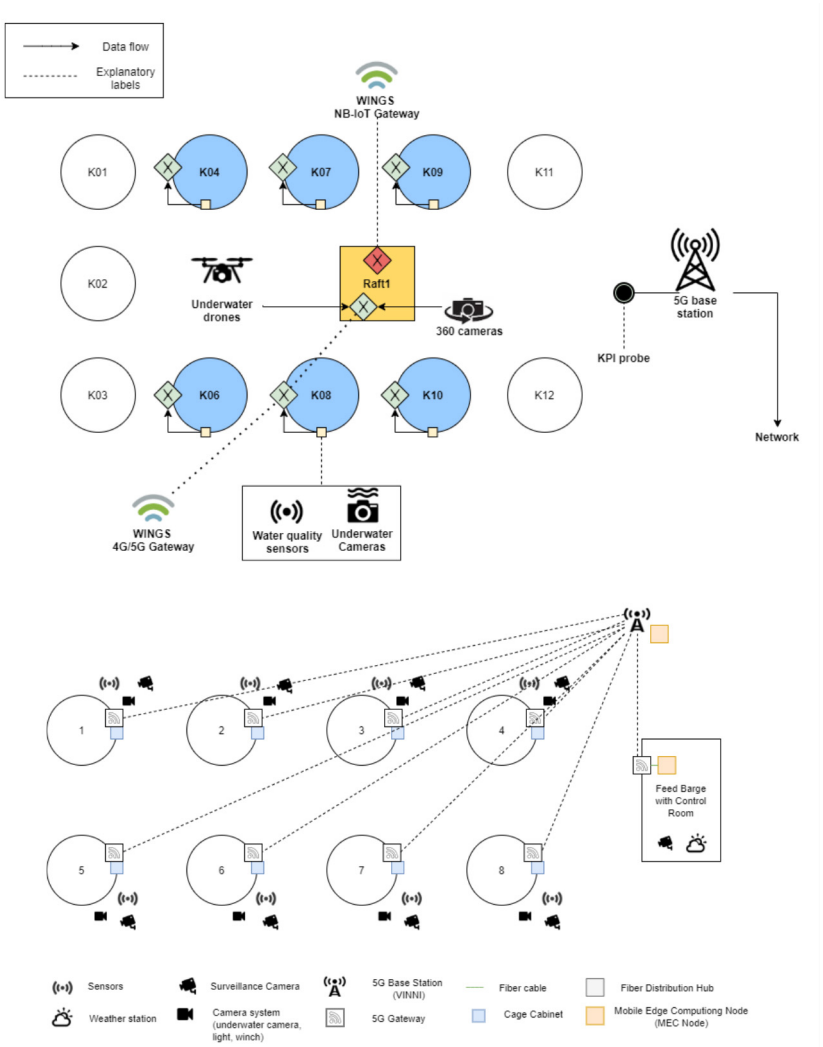

Illustration of the proposed 5G end-to-end solution at the Greek (top) and Norwegian (bottom) sites.

EFFECT OF DIETARY VITAMIN K IN GROWTH AND SKELETAL DEVELOPMENT OF GILTHEAD SEA BREAM LARVAE (*Sparus aurata*)

U. Sivagurunathan[1]*, D. Dominguez[1], Yiyen Tseng[1], J. Roo[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

Skeletal anomalies in cultured marine fish larvae are among major drawbacks in hatcheries to produce high quality juveniles at more effective production costs. Micronutrients which are nutritionally unbalanced may be partly responsible of these losses due to the scarce knowledge available regarding the optimum levels of these nutrients on larval diets. Vitamin K is a fat-soluble micronutrient that has various functions in bone development, calcium absorption, mineralization and blood clotting etc. Vitamin K3 (menadione) is the synthetically available form and is used in many animals feed stuff. Unfortunately, information regarding optimum dietary Vitamin K levels in microdiets and its effect in gilthead sea bream larvae is unavailable. Hence, considering this research gap, the main aim of the present study was to evaluate the effect of several levels of Vitamin K in practical microdiets on growth and skeletal development of gilthead sea bream larvae.

Materials and Methods

Diets: Six different isoenergetic and isonitrogenous supplemental diets were formulated with a varying level of Vitamin K (Menadione – vitamin K3) such as 0, 0.7, 7, 17, 35 and 70 mg vit K3/100g of diet. *Fish and experimental conditions:* 2100 gilthead sea bream larvae obtained from one single spawn from the GIA-Ecoaqua Progensa Fast Growing Selected Broodstock were randomly distributed into eighteen 170L (water carrying capacity) circular tanks. Initial (21-day post hatching (dph)) larvae had a mean total length of 6.26 ± 0.42 mm and a body weight of 0.28 ± 0.04 mg (mean ± s.d). *Sampling:* Growth was monitored by measuring the total length (TL) and body weight of larvae every 8 days. Daily mortality was registered and at the end of the study (37 dph) all the live larvae were counted and sampled for TL, body weight, histology, whole mount stain, gene expression and vitamin K analysis by High-Performance Liquid Chromatography (HPLC). *Statistics:* All data were tested for normality and homogeneity of variances and means compared by Tukey test ($P < 0.05$).

Results

After 2 weeks of feeding the experimental diets larvae fed 7 mg vit K/100g were larger in total length than larvae fed 35 and 70 mg vit K3/100g (Fig. 1). However, larvae fed with different levels of Vitamin K showed no significant differences in body weight. Besides, the percentage of completely mineralized vertebral axis was higher in larvae fed 7 and 17 vit K3/100g diet. Moreover, a high incidence of abdominal vertebral anomalies was observed in fish fed diet 35 and 70 mg vit K3/100g diet (Fig. 2). The relative gene expression of bone biomarkers *bmp2* and *oc* showed no significant difference among the groups.

Discussion and conclusion

~~Our~~ Increase in dietary levels up to 7 mg vit K3/100g diet led to the highest larval total length and the lowest incidence of skeletal anomalies, while providing a good vertebral axis mineralization not different than those of larvae fed 17 mg vit K3/100g. Therefore, a minimum level of 7-17 mg vit K3/100g diet are required in diets for gilthead seabream larvae for optimal growth and bone mineralization, what is higher than the requirements previously stablished for juveniles of other species (NERC, 2011). In mummichog larvae, even if the dietary vitamin K levels are sufficient to improve growth, they may be suboptimal for bone development. Moreover, in seabream larvae, supplementation with vit K up to 70 mg/100g diet lead to high abdominal vertebral kyphosis and increased the incidence of severe anomalies, suggesting some degree of hypervitaminosis. Nevertheless, larvae fed the diet non-supplemented with vitamin K did not showed a high incidence of skeletal anomalies, what could be related to the sufficient basal levels of this vitamin or the insufficient feeding period of the study, since deficient levels of vitamin K increase bone anomalies in other fish species (Udagawa 2001, 2004; Krossøy et al., 2009), such as vertebral fusion during both early and late larval development. Further analyses are being undergone to understand the effects of vitamin K supplementation.

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Figure 1: Total length (mm) of gilthead sea bream larvae fed diets with different level of Vitamin K

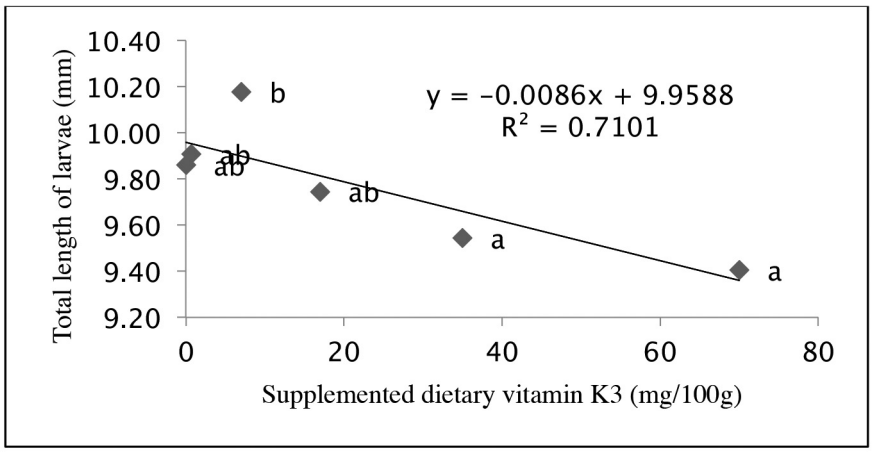
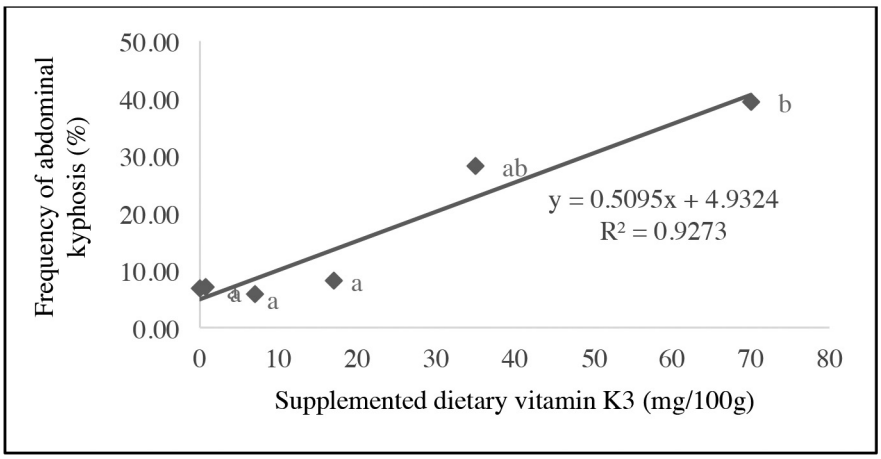


Figure 2: Incidence of abdominal vertebral kyphosis (%) in gilthead sea bream larvae fed diets with different level of vitamin K



Acknowledgment

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References

Krossøy, C., Waagbø, R., Fjelldal, P.G. et al. (2009) Dietary menadione nicotinamide bisulphite (vitamin K3) does not affect growth or bone health in first feeding fry of Atlantic salmon (Salmo salar L). Aquac. Nutr., 15, 638–649.

NRC, (2011) Nutrient Requirements of Fish and Shrimp. The National Academic Press, Washington D.C.

Udagawa, M. (2000) Physiological role of vitamin K in fish -review. Jpn. Agricult. Res. Quarter., 34, 279–284.

Udagawa, M. (2001) The effect of dietary vitamin K (phylloquinone and menadione) levels on the vertebral formation in mummichog *Fundulus heteroclitus*. Fisheries Sci., 67, 104–109.

Udagawa, M. (2004) The effect of parental vitamin K deficiency on bone structure in Mummichog *Fundulus heteroclitus*. J. World Aquac. Soc., 35, 366–371

BACTERIA AND PARASITE FAUNA OF RAINBOW TROUT (*Oncorhynchus mykiss)* WALBAUM, 1792) FROM A RACEWAY SYSTEM IN NORTHERN GERMANY

Patrick Unger[1*], Júlia Clols-Fuentes[1*] & Harry Wilhelm Palm[1]

[*] These authors contributed equally to the manuscript
[1]Aquaculture and Sea-Ranching, Faculty of Agricultural and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany
Email: patrick.unger@uni-rostock.de; Julia.fuentes@uni-rostock.de

Introduction

Reliable data on the parasite and bacterial diversity of freshwater fish from freshwater bodies in northern Germany is scarce. The aim of the present study was to determine the parasite fauna of cultured rainbow trouts (*Oncorhynchus mykiss* Walbaum, 1792) which were sampled from an open freshwater raceway aquaculture system at Lake Tollense, Germany in different seasons in 2018. Additionally, the presence of pathogenic bacteria and its affectation with fish raised in this aquaculture system was seasonally investigated. Ten different parasite species where isolated, belonging to the Monogenea (1), Digenea (5), Cestoda (2) and Crustacea (2). *Diplostomum spathaceum* (Rudolphi, 1819) Olsson, 1876, a potential pathogenic species, which was molecularly identified and found as a core species with prevalence's of 80.0 – 100.0% and high mean abundances of 42.8 to 55.0 in trout. Other taxa were found in the summer season exclusively, indicating strong seasonal variation of parasites. Statistical correlation was positively tested for coherences of eye fluke burden and fish performance between the seasons. Bacterial pathogens detected in rainbow trout mucosal samples were *Pseudomonas sp., Pseudomonas fluorescens, Acinetobacter sp., Aeromonas sp., Aeromonas hydrophila/veronii, Micrococcus endophyticus and Candida holmii*. Contribution percentages of species was higher in summer compared to fall season, when only three genera were detected, suggesting that water temperature increase was one of the factors that may have contributed to the apparition of bacterial infections in fish under thermal stress.

Material and Methods

For parasitological studies, fishes were taken from open freshwater raceway aquaculture system in Neubrandenburg, Mecklenburg-Western Pomerania at three samplings, in April, July and November 2018. Rainbow trout were taken alive from the farm, immediately killed, and smears of the gills were taken. The fishes were transferred to the laboratory at University of Rostock, and frosted at -20°C for subsequent parasitological studies (following Bush et al. 1997).
Bacterial fish skin-mucus communities were monthly sampled from June until October. Samples were collected from adult fishes and fingerlings by swabbing 3 cm^2 skin region with sterile swabs. Its pathogenic bacteria were isolated by selective plating techniques and identified using MALDI-Tof Mass Spectophotometry system and molecular analysis when necessary.

Results

The parasites fauna of rainbow trout differed through the year. In spring, four different parasite species were detected, including molecular records of *Diplostomum spathaceum* (n=19) and *D. pseudospathaceum* Niewiadomska, 1984 (n=1). The detected trematode larvae *Diplostomum* spp. (P=100%) and *Tylodelphys clavata* Niewiadomska, 1984 (P=60.9) being core species (Holmes and Price 1986). Additionally, the Monogenea *Gyrodactylus* sp. was found with prevalence of 9.4% in the gills of trout (see Table 1).

In summer, the number of parasites was significantly higher, as nine species could be detected. Here, only *Diplostomum* spp. was a core species with P=96.9% and other parasites found with low or moderate prevalence. In this subsample, only *D. spathaceum* (n=20) was identified. Two crustacean parasites, *Argulus foliaceus* (Linnaeus, 1758) and *Ergasilus sieboldi sieboldi* (Nordmann, 1832) were found parasitizing the gills of the studied rainbow trout. In case of the detected cestodes (*Eubothrium crassum*, *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793) and the larval trematode *Ichthyocotylurus variegatus* (Creplin, 1825) Odening, 1969 only single specimens were found in the aquaculture farm (P=1.6%). In autumn sampling, parasites variety was reduced again, consisting of four species as they are *Diplostomum spathaceum*, *D. pseudospathaceum*, *Tylodelphys clavata* and *T. podicipina* Kozicka & Niewiadomska, 1960. The prevalence and intensity of these constantly occurring species was comparable to those in spring and summer 2018. Only the prevalence of *T. clavata* was higher, being a core species with a prevalence of 73.8%.

(Continued on next page)

Table I. Isolated parasite species in the studied rainbow trout at three samplings; n=170. A, Abundance; I, Intensity; mI, mean Intensity; P, Prevalence.

		Spring sampling			Summer sampling			Autumn sampling		
		Oncorhynchus mykiss , n=64			*Oncorhynchus mykiss* , n=64			*Oncorhynchus mykiss* , n=42		
Parasite species	stage	P (%)	mI (I)	A	P (%)	mI (I)	A	P (%)	mI (I)	A
Monogenea										
Gyrodactylus sp.	a	9.4	1.5 (1-3)	0.1	3.1	1.5 (1-2)	0.1	-	-	-
Digenea										
Diplostomum spp.	l	100.0	47.3 (1-128)	47.3	96.9	50.0 (9-125)	47.3	92.9	35.3 (8-91)	32.7
Ichthyocotylurus variegatus	l	-	-	-	1.6	3.0 (3)	0.1	-	-	-
Tylodelphys clavata	l	60.9	3.4 (1-11)	2.1	28.1	4.6 (1-19)	1.3	73.8	1.8 (1-15)	1.3
Tylodelphys podicipina	l	-	-	-	1.6	1.0 (1)	0.1	1.6	1.0 (1)	0.1
Cestoda										
Eubothrium crassum	a	-	-	-	1.6	1.0 (1)	0.1	-	-	-
Triaenophorus nodulosus	l	-	-	-	1.6	1.0 (1)	0.1	-	-	-
Crustacea										
Argulus foliaceus	a	-	-	-	21.9	1.4 (1-4)	0.1	-	-	-
Ergasilus sieboldi	a	-	-	-	3.1	1.0 (1)	0.1	-	-	-

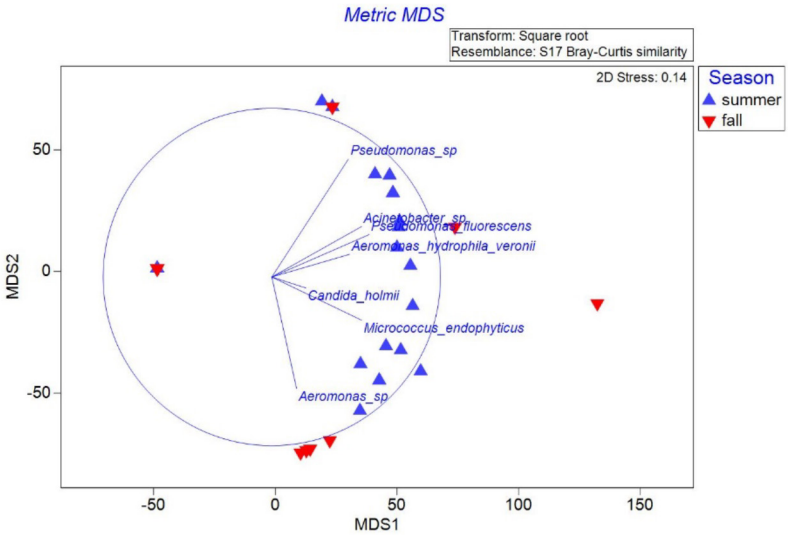

Figure 3. Metric Multidimensional scaling (mMDS) plot of mucosal bacteria detected on fish from Neubrandenburg. Each point represents one fish in which pathogenic bacteria was identified. The plot indicates simple matching similarity between samples as ordinated with dimensions k=2 and stress=0,14. Vectors are calculated based on Pearson correlation between samples. They represent the contribution percentage of each species (Pseudomonas sp., Pseudomonas fluorescens, Acinetobacter sp., Micrococcus endophyticus, Aeromonas sp and Aeromonas hydrophila/veronii) in all samples.

Bacterial pathogens detected in rainbow trout mucosal samples were *Pseudomonas sp*., *Pseudomonas fluorescens*, *Acinetobacter sp*., *Aeromonas sp*., *Aeromonas hydrophila/veronii*, *Micrococcus endophyticus* and *Candida holmii*. None of all isolations obtained from selective mediums corresponded to *Staphylococcus sp*. and *Streptococcus sp*. Total number of fish where pathogenic bacteria were detected was higher in summer compared to fall season. Pathogenic bacterial community composition was closer related in summer season, as most samples display less dissimilarity distance between them, in comparison with fall season samples (Figure 3). The average dissimilarity of bacterial species obtained from two-way analysis test using Bray-Curtis similarities was 95,62 between seasons and 84,80 between ages. Tests also revealed how contribution percentages of species was higher in summer compared to fall, being *Pseudomonas sp*., *Pseudomonas fluorescens* and *Micrococcus endophyticus* the dominant candidates in summer, ordered in descending percentage, and *Aeromonas sp*. in fall season.

Each symbol from the metric MDS graph represents a mucus sample obtained from one fish. Samples located at opposite sides of the metric graph space, and display same dissimilarity distances between them, correspond to those samples which detection of pathogenic bacteria was negative (figure 3).

(Continued on next page)

Independently of all results obtained from selective growth platting, a few fish with symptoms were reported in July. Skin ulcerations were observed on 5/32 fishes and 6/10 fingerlings showed other minor symptoms, such as fin necrosis or exophthalmia. Also, water temperature changes were observed, ranging from a maximum of 23°C at the beginning of July to 12,5°C in autumn.

Discussion and conclusion

The aim of the present study was to analyze the parasite fauna of rainbow trout from a flow through raceway system, displaying seasonal patterns and the transfer of pathogenic species from the wild. Additional subsequent tasks included to detect the presence of potentially pathogenic bacteria associated to the mucosal surface on fish skin. For rainbow trout, the parasite diversity with nine taxa was the highest in summer, while in spring and autumn, only four different parasite species were found. Similar tendencies were observed regarding bacterial studies, since seven pathogenic bacteria were identified in summer and only three in autumn. Thus, a strong seasonal variation is found within the parasite fauna and pathogenic bacteria community of rainbow trout.

Our results show that massive infections with *Diplostomum* spp. negatively affect the fish performance and accordingly the productivity of the aquaculture farm. In terms of host parasite – host interaction and host manipulation the metacercariae disrupt the lens of the fish, as well as its structure and therefore induce cataract to its host causing the disease Diplostomiasis (Avsever et al., 2016; Seppälä, 2011; Chappell, Hardie, & Secombes, 1994). Bacterial disease outbreaks in fish farms imply great economic losses by increasing mortality rates or devaluating fish with visible lesions (Keramat Amirkolaie, 2008). Bacterial studies revealed how temperature may be a decisive factor that influences the interaction between host and pathogenic agent, and indirectly contributes to the increase of infected fish. Indeed, diseased adults and fingerlings of rainbow trout appeared only in summer, when temperatures reached 23°C and overcame the optimal temperature range of this fish species (Quan J, 2021; Kang Y, 2019; Huang J, 2018; Shi KP, 2018).

References

Avsever, M. L., Selver, M. M., Yazicioglu, Ö., Toksen, E., Tay, S., Erdal, G., & Günen, M. Z. (2016). The first report of diplostomiasis from cultured rainbow trout (Oncorhynchus mykiss) in Turkey. Ankara University Veterinarian Fakulty Dergisi, 63, 377-381.

Bush O., Lafferty A.D., J.M. Lotz, and A.W. Shostak. 1997. Parasitology meets ecology on his own terms. Margolis et al. revisited. Journal of Parasitology 83: 575–583.

Chappell, L. H., Hardie, L. J., & Secombes, C. J. (1994). Diplostomiasis: the disease and host parasite interactions. In: Pike AW, Lewis JW, editors. Parasitic diseases of fish. Tresaith, Dyfed, UK: Samara Publishing Ltd.;. 59-86.

Holmes, J. C., & Price, P. W. (1986). Communities of parasites. Community ecology: pattern and process/edited by Jiro Kikkawa, Derek J. Anderson.

Huang, J., Li, Y., Liu, Z., Kang, Y., & Wang, J. (2018). Transcriptomic responses to heat stress in rainbow trout Oncorhynchus mykiss head kidney. Fish & shellfish immunology, 82, 32–40.

Kang, Y., Liu, Z., Shi, H., Wang, J., Huang, J., Li, Y., Li, J., & Wang, Y. (2019). Label-free quantification of protein expression in the rainbow trout (Oncorhynchus mykiss) in response to short-term exposure to heat stress. Comparative biochemistry and physiology. Part D, Genomics & proteomics, 30, 158–168.

Keramat Amirkolaie, 2008. Environmental Impact of Nutrient Discharged by Aquaculture Waste Water on the Haraz River. Journal of Fisheries and Aquatic Science, 3: 275-279

Quan, J., Kang, Y., Li, L., Zhao, G., Sun, J., & Liu, Z. (2021). Proteome analysis of rainbow trout (Oncorhynchus mykiss) liver responses to chronic heat stress using DIA/SWATH. Journal of proteomics, 233, 104079.

Seppälä, O. (2011). Manipulation of fish phenotype by parasites. In: Farrell, A.P. (Ed.), Encyclopedia of Fish Physiology: From Genome to Environment. Volume 1 The Senses, Supporting Tissues, Reproduction, and Behaviour. Amsterdam/ London: Elsevier Academic Press, 777-782.

Shi, K. P., Dong, S. L., Zhou, Y. G., Gao, Q. F., & Sun, D. J. (2018). Antioxidant responses of rainbow trout with different ploidies to acute temperature stress. Ying yong sheng tai xue bao = The journal of applied ecology, 29(9), 3102–3110.

MICROBIOME EARLY LIFE SENSITIVITY: OPPORTUNITIES FOR AQUACULTURE

Tamsyn Uren Webster*[1], Sonia Consuegra[1], Carlos Garcia de Leaniz[1]

[1.] Centre for Sustainable Aquatic Research, Biosciences, Swansea University, SA2 8PP, UK

Email: T.M.UrenWebster@swansea.ac.uk

The 'microbiome' collectively refers to a community of microorganisms and their genetic material, associated with a particular environment. There is increasing recognition of how animal-associated microbiomes (i.e. in the gut, on the skin or the gills) critically influence the health of their hosts in many different ways. Research on microbiomes in aquaculture has been accelerating and diversifying in recent years. A significant body of work now demonstrates the role of the microbiome in promoting gut health, animal performance and disease resilience.

Our recent research has focused on the differences between hatchery-reared and wild fish microbiomes, and on the impacts of aquaculture-related stressors on gut- and skin-associated microbial communities. We identified considerable differences in the diversity and structure of Atlantic salmon microbiomes between farmed and natural populations. Using reciprocal translocation, we then found that, although the salmon microbiome shows extensive plasticity, signatures of early rearing do persist. We have further demonstrated the sensitivity of the fish microbiome to environmental stressors in early life. Elevated fish cortisol production is directly linked with changes in the composition of the gut microbiome, including inhibition of typically probiotic taxa, while disruption of initial microbiome colonisation can promote an increased prevalence of opportunistic pathogens.

Going forward there are significant opportunities for innovative microbiome research to contribute to sustainable aquaculture development. For example, this may include early-life conditioning of animal and water microbiomes to promote health, welfare and performance, as well as the development of robust, non-invasive microbial screening tools for health assessment. Recent technological developments, particularly those that allow the characterisation of direct functional relationships between host and microbiome, will enable future cutting-edge research and the development of tools for industrial application.

GLOBAL CHANGE RESILIENCE iN AQUACULTURE (GLORiA): CAN AQUACULTURE COPE WITH EXTREME CLIMATIC EVENTS?

J.M. Valero-Rodriguez*[1]; F. Romero[1]; I. Abel-Abellán[1]; P. Sanchez Jerez[1]; J.L. Sánchez-Lizaso[1]; I. Sola[1]; D. Izquierdo-Gomez[2]; D. Fernandez-Jover[1]; A. Forcada[1]; P. Arechavala-Lopez[3]; V. Pardo-Saiz[1]; F.C. Marhuenda-Egea[4]; K. Toledo-Guedes[1]

[1]Department of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain
[2]S.T.E.E., University of Pau and the Adour Region (UPPA), France
[3]Department of Ecology and Marine Resources, Mediterranean Institute of Advanced Studies (IMEDEA – UIB/CSIC), Esporles, Spain
[4]Department of Agrochemistry and Biochemistry, University of Alicante, Alicante, Spain
* Email: Juanma.valero@ua.es

Background:
Climate change results in numerous damaging effects worldwide. One of such effects is the appearance of more frequent, adverse climatic conditions along the coastline. Strong storms could mess with local industries, affecting production, job positions, local ecosystems and even customers (Fig.1). Aquaculture industry could specially suffer from net damage and stock loss (De Silva & Soto, 2009). For example, the Storm 'Gloria' affected fish farms located at the south Eastern Spanish coast on January 2020, producing fish biomass losses and net damage (Amores *et al*, 2020).

What we want:
This project seeks to develop mitigation and contingency plans to improve prevention and management of escapes through work tables at various levels. Communication with local administration is essential. Our work also tackles the improvement of traceability methodologies. Our team will delve into their socioeconomic impacts (e.g.: damage for fisheries and market prices) and identify interactions of escapees with the surrounding environment. Ultimately, we will also establish a predictive model to get ahead escape events.

Why should we care?
The customers could experience involuntary scams when paying for mislabeled fish. In addition, other, more dangerous side- effects could arise from the escape events, such as high antibiotic concentrations in muscle (Arechavala-Lopez *et al*. 2018), genetic hybridization with wild counterparts, infections or competition.

GLORiA is a project 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".

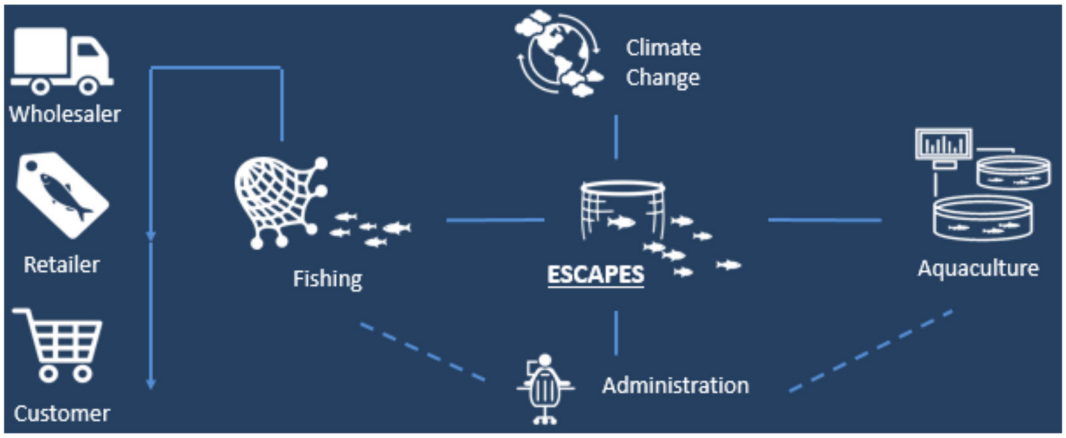

Fig. 1: Conceptual framework depicting the escapes problem.

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References:

Amores, A., Marcos, M., Carrió, D. S., & Gómez-Pujol, L. (2020). Coastal impacts of Storm Gloria (January 2020) over the north-western Mediterranean. *Natural Hazards and Earth System Sciences*, *20*(7), 1955-1968.

Arechavala-Lopez, P., Toledo-Guedes, K., Izquierdo-Gomez, D., Šegvić-Bubić, T., & Sanchez-Jerez, P. (2018). Implications of sea bream and sea bass escapes for sustainable aquaculture management: a review of interactions, risks and consequences. *Reviews in Fisheries Science & Aquaculture*, *26*(2), 214-234.

De Silva, S. S., & Soto, D. (2009). Climate change and aquaculture: potential impacts, adaptation and mitigation. *Climate change implications for fisheries and aquaculture: overview of current scientific knowledge*. *FAO Fisheries and Aquaculture Technical Paper*, *530*, 151-212.

COULD OFFSHORE AQUACULTURE MITIGATE EFFECTS OF GLOBAL WARMING?

López Mengual[1], P. Sanchez-Jerez[1], J. D. Ballester-Berman[2], *J. M. Valero-Rodriguez, and K. Toledo-Guedes[1]

[1] Department of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain
[2] Department of Physics, Systems Engineering And Signal Theory, University of Alicante, Alicante, Spain
E-mail: psanchez@ua.es

Introduction

Most production of intensive marine finfish aquaculture in the Mediterranean takes place today in semi-offshore floating cages (open sea system) relative near of the coast, at depths between 15 and 30 m. If mariculture have to play a major role in meeting the rising demand for fish, this sector needs to access to adequate areas for production in terms of thermal stability because the negative impact of temperature increases on aquaculture production (Reid et al 2019). Due to climate change and the competition for space with other activities in coastal waters available space for new mariculture development in coastal zones could be highly limited. Therefore, in a sort future, the success of an aquaculture project will depend to a large extent on the selection of an appropriate site to establish the farm, following a multi-criteria decision-making process (Sanchez-Jerez et al. 2016). The aim of the present study was to evaluate the spatial changes of thermal anomalies across a distance from the coast, up to 30 km, for the Spanish aquaculture facilities along the latitudinal gradient of 1000's km.

Material and methods

This work was carried out on a large scale covering the entire Mediterranean coast of the Iberian Peninsula. The study coordinates are -6 W, 5.7 E, 34 S, and 42.4 N. The data used in this study are publicly available and provided by the Copernicus Marine Environment Monitoring Service (CMEMS) working on operational mode since May 2015. In the present work Sea Surface Temperature (SST) products from the data set SST_MED_SST_L4_REP_OBSERVATIONS_010_021 were employed. The CNR-ISAC-GOS (Consiglio Nazionale delle Ricerche, Istituto di Scienze dell'Atmosfera e del Clima - Gruppo di Oceanografia da Satellite, Italy) has reprocessed Pathfinder V5.3 (PFV53) AVHRR data covering the 1981-2018 period and combined them with a bias-corrected version of the CMEMS NRT L4 data up to 2017 to provide a full time series of consistent daily gap-free maps (L4) at the original PFV53 resolution (0.0417° x 0.0417°).

Results and discussion

Results showed that offshore aquaculture will not have a positive effect mitigating the effects of global warming along a gradient of distance from the coast (Figure 1). In some case the magnitude of the anomaly as a function of distance from the coast (Site L and M). There is a clear latitudinal increase of thermal anomalies from North to South increasing from 0.4 ºC to 1.2 ºC in summer. In winter, all the locations except Site A, did not showed relevant thermal anomalies. Only Site R, affected by the upwelling of Alboran Sea showed a negative thermal anomaly in winter. Therefore, global warming is clearly affecting to aquaculture facilities across Spanish Mediterranean coast, increasing the temperature in summer, additionally to other negative effects linked to global changes (Rosa et al. 2012). The option of moving facilities up to 30 km away from the coast does not seem to be able to mitigate the effect of coastal warming.

This study is part of the project "GLORIA", which 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".

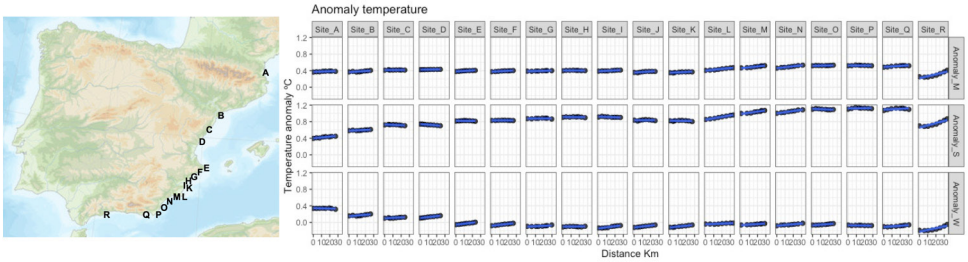

Figure 1. Temperature anomaly from North (A) to South (R) sites where aquaculture is already operating, across a gradient of distance to the coast (30 km): Mean value (Anomaly_M), summer (Anomaly_S) and winter (Anomaly_W).

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References

Reid, GK. Gurney-Smith HJ., Marcogliese D.J. Knowler D., Benfey, T., Garber AF, Forster I., Chopin T., Brewer-Dalton, K, Moccia, R.D, Flaherty M., Smith, CT, & De Silva S (2019) Climate change and aquaculture: considering biological response and resources. AQUACULTURE ENVIRONMENT INTERACTIONS. Vol. 11: 569–602, 2019 https://doi.org/10.3354/aei00332

Rosa R., Marques A. & Nunes ML. (2012) Impact of climate change in Mediterranean aquaculture. Reviews in Aquaculture 4, 163–177 doi: 10.1111/j.1753-5131.2012.01071.x

Sanchez-Jerez, P., Karakassis, I., Massa, F., Fezzard, D., Aguilar-Manjarrez, J., Soto, D., Chapela, R., Avila, P., Macias, J.C., Tomassetti, P., Marino, G., Borg, J.A., Franicevic, V., Yucel-Gier, G., Fleming, X., Biao, H., Nhhala, H., Hamza, A. Forcada, T. and Dempster (2016) Aquaculture's Struggle for Space: The Need I. A. 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. https://doi.org/10.3354/aei00161

TROPHIC EFFICIENCY IN MARINE SYSTEMS

Jaap van der Meer

Wageningen Marine Research (WMR) and Wageningen University, Aquaculture and Fisheries (WUR-AFI)
jaap.vandermeer@wur.nl

Trophic efficiency, that is the production at a trophic level divided by the production at the preceding level, is much higher in the plant-based food chains of marine systems compared to those in terrestrial systems. Whereas terrestrial systems such as forests and grasslands show an average efficiency of about 0.1%, the efficiency in marine systems is at least 6% (Van der Meer 2020). The success of agriculture in producing so much more food than a hunting-gathering strategy is mainly due to an increase in trophic efficiency, but similar gain will be much harder to achieve in marine systems. In this paper I will explore what factors are responsible for the variability in trophic efficiency among marine systems and speculate about possibilities to increase the efficiency for the benefit of human food production.

Van der Meer, J. (2020) Limits to food production from the seas. Nature Food 1(12): 672-674

THE ROLE OF AQUACULTURE IN CIRCULAR FOOD SYSTEMS

Anne-Jo van Riel[1,2*], Marit A.J. Nederlof[1], Geert F. Wiegertjes[1] and Imke J.M. de Boer[2]

[1]Aquaculture and Fisheries group, Wageningen University & Research, Wageningen, The Netherlands.
[2]Animal Production Systems group, Wageningen University & Research, Wageningen, The Netherlands.
Email: anne-jo.vanriel@wur.nl

Introduction

Feeding humanity within the planetary boundaries is one of the grand challenges over the coming decades (Foley et al., 2011; Godfray et al., 2010). Circular food systems are seen as a promising way to feed the rising population within these planetary boundaries (Ghisellini et al., 2016; Jurgilevich et al., 2016; Mak et al., 2019) One of the principles of a circular food system includes that each resource should be used in the way that is most valuable to the entire system. This implies that arable land should be used for production of human food and not for the production of animal feed. Using arable land to produce animal feed is less efficient due to metabolic losses of the animal that occur when converting feed into food. However, animals can play a valuable role in circular food systems due to their ability to convert by-products, unsuitable for human consumption, into high quality animal-source food (Figure 1) (Van Zanten et al., 2019). Next to by-product from crops, livestock and fisheries, animals can also upgrade plant-based former foodstuff and grass resources, unsuitable for human consumption.

In previous studies, the main focus has been on livestock (Van Zanten et al., 2019), while the role of aquaculture in circular food systems has been studied less. In aquaculture, circularity is often associated with integrated multitrophic food systems (IMTA). In these systems higher trophic level species (fed species) are combined with lower trophic level species (extractive species) to recycle nutrients within the system. But circularity is more than only the recycling of nutrients within a production system and must be viewed on a much larger scale, such as an entire food system. A recent study showed that fish can play an important role in a circular food system because they have an important role in the human diet (e.g. fatty acids) and, if harvested sustainably, capture fisheries can provide essential foods and aquaculture has additional advantages to livestock in the upcycling of by-products (Van Hal, 2020). The present study was restricted to two important species to represent aquaculture, a carnivorous (Atlantic salmon) and an omnivorous species (Tilapia), and did not include emissions associated to livestock or aquaculture. Unique characteristics of aquaculture, however, is that there are many species cultivated over a wide range of trophic levels, and that they are kept in a variety of housing systems. The variety of species cultivated and variety of production systems is expected to fulfil different roles in circular food systems.

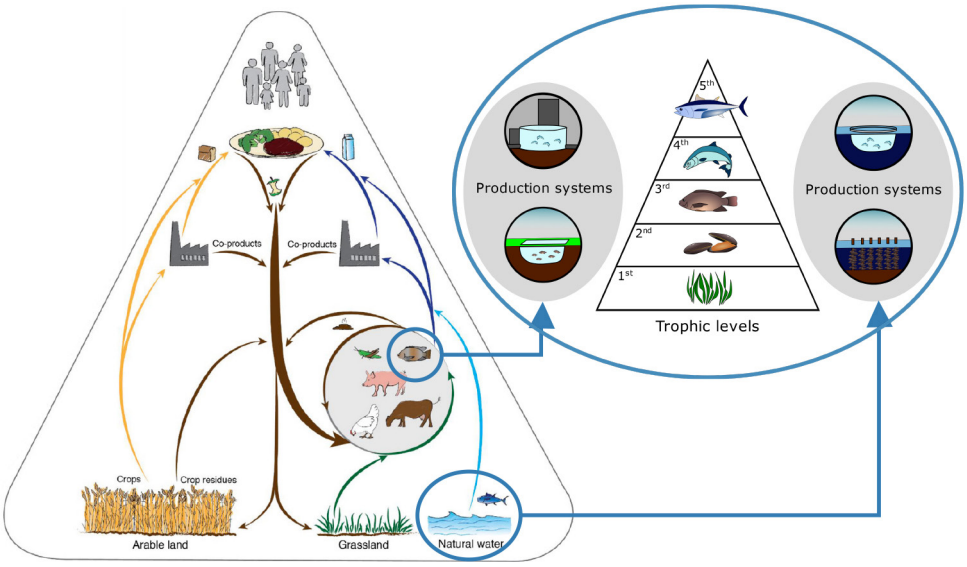

Figure 2 Visualisation of a circular food system (From: Van Zanten et al., 2019) with a special emphasis on the role of aquaculture

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The aim of this current project is to determine the role of aquaculture in circular food systems. We will use circular food system modelling to gain insights into what aquaculture species could be kept in which aquaculture systems, how much aquatic food could be produced and what by-products could be recycled as fish feed.

Materials and Methods

The theoretical framework used to determine the role of aquaculture in circular food systems will be presented, including the use of a model-based approach and relevant characteristics of aquaculture for a circular food system.

References

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., Mueller, N. D., O'Connell, C., Ray, D. K., West, P. C., Balzer, C., Bennett, E. M., Carpenter, S. R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., … Zaks, D. P. M. (2011). Solutions for a cultivated planet. *Nature*, *478*(7369), 337–342.

Ghisellini, P., Cialani, C., & Ulgiati, S. (2016). A review on circular economy: The expected transition to a balanced interplay of environmental and economic systems. *Journal of Cleaner Production*, *114*, 11–32.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. In *Science* (Vol. 327, Issue 5967).

Jurgilevich, A., Birge, T., Kentala-Lehtonen, J., Korhonen-Kurki, K., Pietikäinen, J., Saikku, L., & Schösler, H. (2016). Transition towards circular economy in the food system. *Sustainability (Switzerland)*, *8*(1), 1–14.

Mak, T. M. W., Xiong, X., Tsang, D. C. W., Yu, I. K. M., & Poon, C. S. (2019). *Sustainable food waste management towards circular bioeconomy: Policy review, limitations and opportunities*.

Van Hal, O. (2020). *Upcycling biomass in a circular food system – the role of livestock and fish –*. PhD thesis, Wageningen University, The Netherlands.

Van Zanten, H. H. E., Van Ittersum, M. K., & De Boer, I. J. M. (2019). The role of farm animals in a circular food system. *Global Food Security*, *21*, 18–22.

EVALUATION OF GENETIC GAIN IN RAINBOW TROUT FED STANDARD OR "FUTURE" DIET AFTER 10 GENERATIONS OF MULTI-TRAIT SELECTION IN THE AQUALANDE SELECTIVE BREEDING PROGRAM

Marc Vandeputte[1,2,*], Geneviève Corraze[3], Jérôme Doerflinger[4], Florian Enez[5], Frédéric Clota[1,2], Frédéric Terrier[3], Mathilde Horat[4], Laurence Larroquet[3], Nicolas Dechamp[1], Vincent Petit[4], Pierrick Haffray[5], Sandrine Skiba-Cassy[3], Mathilde Dupont-Nivet[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France
[2] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France
[3]INRAE, Univ Pau & Pays de l'Adour, E2S UPPA, NuMeA, Saint-Pée-sur-Nivelle, France
[4]SCEA les Sources de l'Avance, Pissos, France [5]SYSAAF Section Aquacole, Campus de Beaulieu, Rennes, France

Introduction

It is now widely admitted that selective breeding is a key driver for improved performance of aquaculture production. Selection for growth has been widely performed, but breeding goals tend to include more and more traits like processing yields, quality traits and disease resistance. Feed efficiency is also a key parameter for competitiveness and environmental suystainability, but cannot be easily selected for in aquaculture. However, it is often suggested that faster growing fish should tend to have an improved feed efficiency. Ideally, in order to study the impact of selection on a wide variety of traits (and not only the ones recorded in the breeding program), response to selection should be evaluated by comparing a selected line and an unselected control line at the same time, in similar conditions. Control lines are seldom available, hence few of these comparisons are available in the literature. It is also well known that the composition of feed is one of the main drivers of performance. In aquaculture, feeds are evolving quickly, with more and more limitation in the use of fish meal and fish oil, which are available only in a more or less fixed amount worldwide, while aquaculture production (and feed consumption) quickly increase. In this experiment, we compared the 10th generation of multi-trait selection line of Aqualande's breeding (G10) to a control line (G0), in a trial with a standard industry feed and a "future" feed, devoid of fish oil, fish meal and soya.

Material and methods

Broodstock used were females and neomales rainbow trout from the 10th generation (G10) of a commercial line (Aqualande, Pissos, France). This line has been line selected for fast late growth, body conformation, fat content and carcass yield to produce fillet at 3kg mean weight. We also had neomales of the initial G0 line (base population) propagated by random breeding without selection. Thirty G10 females were fertilized by 30 G10 or 30 G0 neomales, thus producing two offspring groups, G10 and G0xG10. At 84 dph (1g mean weight), they were placed in triplicated raceways until 264 dph, and measured for BW at 84, 147, 193, 250 and 264 dph. They were individually tagged, and split at 264 dph in 12 groups (2 lines x 2 feeds x 3 replicates) placed in cages in large raceways, to be fed either a standard industry diet (Aqualia, Arue, France) or a "future" diet, devoid of fish oil, fish meal and soya, and containing microalgae biomass to provide LC n-3 PUFA (INRAE, Donzacq, France). Both diets had similar crude protein (44.8-43.7%%), crude fat (19.4-21.8%) and energy content (24.2-24.5 kj/gDM).

At 374 dph, 40 fish per cage were slaughtered, weighed and measured for fat content and processing traits. A total of 90 G0xG10 fish were fed the Aqualia feed until 416 dph, and slaughtered when they reached the same average weight (1.5-1.6 kg) than the G10 fish at 374 dph.

Fish were hand fed twice daily until they were close to apparent satiation, and the amount of feed distributed in each raceway or cage was precisely recorded from 147 dph to 416 dph, so that Feed Conversion Ratio (FCR) could be estimated for each period, under the hypothesis that all feed distributed had been consumed.

Data were analysed by one way ANOVA, with raceway as the experimental unit and selection line as factor from 147 to 250 dph. Two-way ANOVA, with cage as the experimental unit and selection line and feed as factors was used during the feeding trial from 264 to 374 dph. The difference between groups measured only half of the selection gains, as only the sires were G0 or G10, while the dams were all G10 when creating the groups. Then, we estimated the expected performance of pure G0 by doubling the difference between G0xG10 and G10.

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	BW 374 (g)	DGC 264-374	FCR 264-374	Fillet fat (%)	Viscera Yield (%)	Fillet Yield (%)	Head Yield (%)
G10	1665	1.20	0.97	10.6	7.7	69.1	10.7
G0xG10	1356	1.01	1.08	9.0	9.5	68.4	10.8
G0 estimate	1048	0.82	1.19	7.3 %	11.3	67.7	10.9
Genetic gain	+59%	+46%	-19%	+44%	-32%	+2%	-2%

Results and discussion

The G10 line grew faster than the G10xG0 line (1687 vs 1375 g at 374 dph) and growth was better on the "future" feed (1521 vs 1481g on the industry feed). There was a significant genetics x feed interaction ($P<0.05$) on FCR, which was much higher on the future feed than on the industry feed (1.34 vs 1.03), and differed between G10 and G0 only on the industry feed. This was probably because the future feed was sinking and the ration was more difficult to adjust. In addition, due to data recording issues, we miss most processing data on the G0xG10 group fed future feed. Thus, we report detailed comparison of the G0 and G10 lines only with the industry feed in the table below. G0 estimate and genetic gain are estimated by doubling the difference between G10 and G0xG10 as explained before.

The ten generations of selection generated a gain of +59% on final body weight, with 19% reduction in FCR. Viscera yield was strongly reduced (-32%) by selection on conformation and carcass yield, but fillet yield was only little improved (+2%). When comparing fillet yield at the same size (374 dph G10 vs 416 dph G0xG10), the difference was almost zero (+0.3%). Global FCR on industry feed (from 50 g to 1.6 kg) was estimated to be 0.99 for G10 trout vs 1.25 for G0 trout. Interestingly, there was no reduction, and even an increase in bony tissues yields (+4% for head yield, + 39% for vertebral axis yield) when G0 and G10 were compared at the same weight of 1.6kg, which differed from expectations from previous research when only selecting only for growth, but conforms to what is expected from selection on carcass yield (Haffray et al., 2013). Specific selection for fillet yield (and not carcass yield, as initially done) may be necessary to improve fillet yield in the next generations, as has been demonstrated in another French trout population (Vandeputte et al., 2019).

Overall, these results show important effects of selective breeding on productivity, including correlated response on FCR (-19%). Although FCR results for the future feed remain questionable, growth was very satisfactory, showing the potential of total replacement of fish meal and fish oil, provided LC n-3 PUFA are provided in sufficient quantity (here by the micro-algal biomass).

Acknowledgements

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References

Haffray et al., 2013. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 368369: 145152.

MICROALGAE BY-PRODUCT IN DIETS FOR AMBERJACK (*Seriola dumerili,* Risso 1810); EFFECTS OF ON GROWTH, BODY COMPOSITION AND LIVER MORPHOLOPHY

A. Ventura-Castellano*[1], C. Monzón-Rivero[1], S. Ramírez-Bolaños[1], R. Quirós-Pozo[1], J. Roo-Filgueira,, 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
E-mail: anais.venturacastellano@gmail.com

Introduction

Currently, *Isochrysis* sp. is a marine microalga widely used in aquaculture due to its high nutritional value, antioxidant potential, and high content of fucoxanthin as the main carotenoid (Sun *et al*., 2019). Several studies of *Isochrysis* sp. in fish feeds have shown different results. Although a promising raw material, its inclusion in the diets should be controlled, as high percentages may interfere by lowering protein against high-fat levels in fish (Vizcaino *et al*., 2016), as well as reducing growth performance and increasing greenish pigmentation of fish skin (Tibaldi *et al*., 2015). Fact of innovative products from this microalga for animal, human and cosmetic purposes moreover generate different valuable wastes, which may represent novel secondary raw materials for sustainable feed production. Therefore, in the present study, it is proposed to evaluate the whole *Isochrysis* in front to a by-product obtained after defatting of *Isochrysis* sp. (CO-ISO) for its inclusion in dry feed for *Seriola dumerili* (Risso, 1810), a fast-growing species of great Mediterranean interest.

Materials and method

-Fish and experimental diets: Five experimental diets were prepared (Control, ISO-3%, CO-ISO 3%, CO-ISO 6%, and CO-ISO 12%). Seventy-five fish per tank were randomly distributed triplicated groups in 500-l fibreglass tanks. The fish (initial body weight of 0.5 ± 0.07 g) were fed the experimental diets to apparent satiation every hour from 08.00 to 19.00 h, 7 days a week for 30 days. Survival, water temperature and dissolved oxygen were monitored every day (20.9 to 21.23 ° C and 4.9 to 5.0 mg l-1 respectively).

-Analysed parameters: The growth performance was reported in all fish along with the trial. At the end of the experimental period whole body, liver and gut from five fish per tank were removed and stored at -80 °C until the different chemical, histological qualitative analysis.

Results and discussion

The highest growth values were found for those fish fed with Control, 3% and 6% CO-ISO diets ($P<0.05$, table 1). In the case of the specific growth rate (SGR), larvae amberjack fed 3% and 6% CO-ISO diets give equal responses with respect to the control diet ($P<0.05$). Regarding feed conversion responses (FCR) the values were in parallel to final weight and SGR parameters ($P>0.05$), fish fed Control, 3% and 6% CO-ISO registered the lowest results.

Biochemical whole body and liver results showed the highest differences for the ISO 3% fish. The total lipid result shows very similar among between CO-ISO-3%, CO-ISO 6% CO-ISO 12% and Control diet (table 2 and 3). In the case of the gut, no differences in lipids were observed (table 3). The relationship for these CO-ISO and ISO diets results inversely to that observed in the liver which would corroborate the poor total lipid that may reach the liver and other important tissues as a consequence of the decrease of total lipids in CO-ISO diets. In summary, ISO product seems to poorer affect fish concerning its byproduct CO-ISO for larvae amberjack. Besides, Control fish biochemical results were close to that for CO-ISO 3% and CO-ISO 6% which from this point of view will represent the appropriate level for larvae amberjack with the Control.

Histological qualitative analysis evidenced an effect on liver fat content between the dietary treatments. There is a tendency to decrease the amount of fat in hepatocytes in those diets with higher inclusion of CO-ISO, which may indicate a mobilization or unavailability of fat in the liver.

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Table 1: Growth performance and survival of larvae amberjack fed with different experimental diets

	Diet				
	Control	3% ISO	3% CO-ISO	6% CO-ISO	12% CO-ISO
Initial weight (g)	0.49 ± 0.07	0.49 ± 0.07	0.49 ± 0.07	0.49 ± 0.07	0.49 ± 0.07
Final weight (g)	1.96^{a} ±0.06	1.58 ^{b} ±0.15	1.88 ^{a} ±0.02	1.86 ^{a} ±0.03	1.30^{b} ± 0.0.3
Final survival (%)	95.11±5.04	91.55±0.76	95.11±2.03	95.11±2.06	94.66 ± 1.33
SGR (% day-1)	6.88^{a}±0.14	5.78^{b} ± 0.46	6.67 ^{a} ±0.06	6.62$^{a\,b}$±0.08	4.82^{c}±0.13
FCR	0.45^{c}±0.02	0.62^{b}±0.08	0.48^{c}±0.01	0.48^{c}±0.0.01	0.81^{a}±0.04

Values represent mean ± SE. Means followed by the same superscript do not differ at P < 0.05. Specific growth rate (% day)1), SGR 1⁄4 ln (final weight/initial weight)/days -1; Feed intake ratio (% lw), FIR 1⁄4 100 · feed consumption (g)/average biomass (g) · days-1 and Feed conversion ratio, FCR 1⁄4 feed consumption (g)/biomass gain (g).

Table 2: Whole body composition (% DW) of greater amberjack

	Diet				
	CONTROL	ISO-3%	CO-ISO 3%	CO-ISO 6%	CO-ISO 12%
Lipid	15.32±2.02	16.94±4.03	15.96±5.66	14.96±2.02	14.54±4.03
Protein	77.44±3.37 ^{bc}	76.35±3.15 ^{bc}	68.81±2.23 ^{a}	74.72±3.37 ^{b}	79.90±1.54 ^{c}
Moisture	83.99±0.60 ^{bc}	84.47±1.08 ^{c}	81.32±0.88 ^{a}	82.67±0.76 ^{ab}	84.91±1.98 ^{c}

fed the different treatments after 30 days

Values (mean ± SD) with different superscript letters in the same row denote significant differences (P < 0.05).

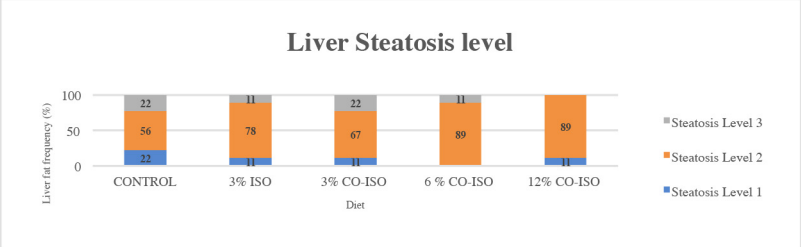

Figure 1: Frequencies according to the degree of lipid accumulation in hepatocytes of fish fed the different diets

Acknowledgments

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References

Sun, Z., Wang, X., and Liu, J. (2019). Screening of Isochrysis strains for simultaneous production of docosahexaenoic acid and fucoxanthin. Algal Research, 41, 101545.

Tibaldi, E., Zittelli, G. C., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., Venturini, S., Tredici, M. R. and Poli, B. M. (2015). Growth performance and quality traits of European sea bass (*D. labrax*) fed diets including increasing levels of freeze-dried Isochrysis sp. (T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivatives. Aquaculture, 440, 60-68.

Vizcaino, A. J., Saéz, M. I., López, G., Arizcun, M., Abellán, E., Martínez, T. F., Cerón-García, M. C. and Alarcón, F. J. (2016). *Tetraselmis suecia* and *Tisochrysis lutea* meal as dietary ingredients for gilthead sea bream (*Sparus aurata* L.) fry. Journal of applied phycology, 28 (5), 2843-2855.

OPTIMIZING RESOURCE USE IN POND ECOSYSTEMS

Marc Verdegem*, Kazi Kabir, Tran Huu Tinh, Tran Ngoc Hai, Mike Phillips, Johan Verreth, Johan Schrama

E-mail: marc.verdegem@wur.nl

Introduction

Aquaculture is intensifying and so is pond aquaculture. Whereas before 1990, production in a stagnant pond with aeration was seldom higher than 10000 kg/ha/crop, today productions of 15000 – 20000 kg/ha/crop are reported, mainly due to the use of high quality pelleted feeds. Productions can be further enhanced by raising primary production in partitioned aquaculture systems, in-pond raceways or split ponds. Increasing production is also possible through water exchange to remove metabolites and particulate wastes. However, discharge of untreated effluents is not desirable. One alternative approach is the nutritious pond concept that aims to improve the in-pond (re-) utilization of fish wastes.

Nutritious pond concept

The nutritious pond concept aims to stimulate the microbial mineralization of fish waste and the production of natural food. This concurs with optimizing nutrient (e.g. nitrogen (N) and phosphorous (P)) fluxes through the pond's food web. Fish waste is rich in N, P and micronutrients, but low in energy. Fish feed contains sufficient energy for the fish to digest the feed, but not for microorganisms to mineralize the waste. This leads to incomplete mineralization and accumulation of waste.

The concept of nutritious pond is to balance the input of energy and nutrients at pond level to be able to maximize waste utilization and thus minimize waste driven pollution. This approach can be executed by adding more energy (i.e. Carbon) and by manipulating the type of energy/carbon supplied.

A. Extra carbohydrate

One solution is to formulate pelleted feed that targets both the fish and the pond's food web. This can be done by providing an easily digestible carbohydrate (CHO) as energy source for the pond's food web. Two ways of administrating the CHO were tested. The first treatment consisted of a common commercial diet and a carbohydrate (CHO) that were administrated side by side to the pond (Feed+CHO). In the 2nd treatment the dietary ingredients and the CHO were mixed prior to pelleting (FeedCHO). The two treatments were fed to Pacific white shrimp raised in biofloc mesocosm tanks. The nitrogen retention efficiency was 46% with the Feed+CHO treatment compared to 38% with the FeedCHO treatment, while the carbon retention efficiency was 12 and 10%, respectively (unpublished data; $P < 0.001$). Hence, the best result was obtained when applying feed and CHO separately to the pond. A disadvantage of adding CHO and feed separately is that the carbon-footprint of the production system increases (Figure 1).

B. Carbohydrate that is difficult to digest by the fish

Another approach is to increase the fraction of non-starch polysaccharide in the diet. Fish lack the enzymes to digest NSP. As a result, a large fraction of the NSP will end up in the faeces. Once in the pond, bacteria can digest the NSP. In consequence, a diet with a higher dietary NSP content, will stimulate the natural food web more than a control diet with a normal NSP content (Figure 2) (Kabir et al., 2019).

The type of NSP in the diet influences the contribution of natural food to tilapia production. A pectin + hemicellulose (PecHem) diet with quick/easy bio-degradable NSP was compared to a lignin + cellulose (LigCel) diet with slow/difficult bio-degradable NSP, to monitor performance of Nile tilapia in small experimental ponds. Both diets had similar C:N ratios. The LigCel diet enhanced the contribution of natural food to fish production, more than the PecHem diet, resulting in better Nile tilapia performance with the LigCel diet (Table 1). Results suggest that the digestibility of the carbon source affects the food web productivity (Kabir et al., 2020).

Results show that paying attention to the contribution of dietary carbon sources to nutrient cycling and fluxes through the food web can significantly reduce the environmental impact of pond farming, while improving the utilization efficiency of fed nutrients and reducing production costs.

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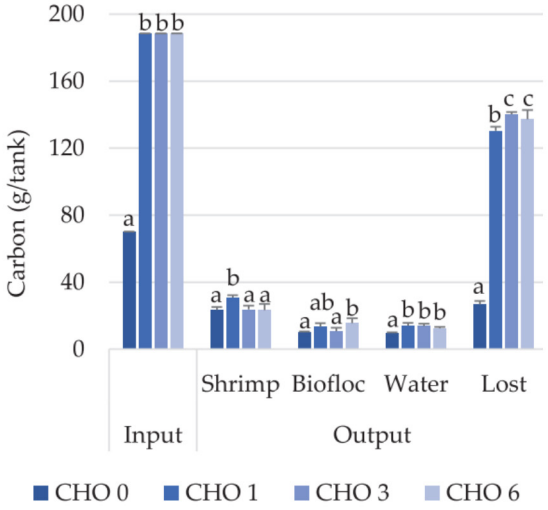

Figure 1:
Carbon budget for a 6-week biofloc mesocosm study in which a commercial diet was fed to Pacific white shrimp with no CHO addition (CHO 0) and 3 treatments in which the same diet was fed besides a daily CHO addition. The amount of CHO fed was always the same, but administrated in 1 (CHO 1), 3 (CHO 3) or 6 (CHO 6) equal daily portions. There were 3 replicates per treatment. Error bars are standard deviation. Bars in a group with different superscripts are statistically different ($P < 0.05$) (Tinh et al., 2021).

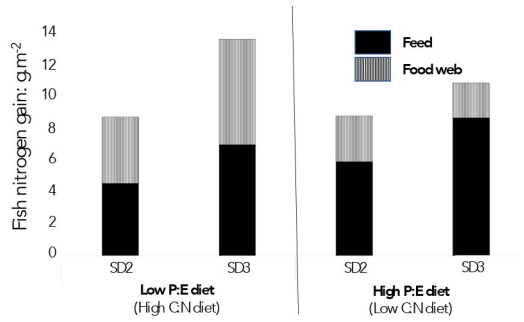

Figure 2:
N gain per m^2 of Nile tilapia (*Oreochromis niloticus*) fed a high NSP (Low P:E) diet or a control diet with a low NSP content (High P:E diet). The stocking density of tilapia were 2 (SD2) or 3 (SD3) fish per m^2. The stacked bars give the total N-gain by fish per m^2, split into feed (black) or natural food (stripes) based weight gain. P:E is protein to energy ratio, C:N is carbon to nitrogen ratio.

		Diet		P-value
	Units	PecHem	LigCel	
Biomass gain	g/m^2	146	173	< 0.05
Survival	%	85	88	ns
FCR	g/g	2.0	1.5	< 0.05
Growth Rate	g/d	1.4	1.5	< 0.001

Table 1: Performance of Nile tilapia fed a Pectine + Hemicellulose (PecHem) diet or a Lignin + Cellulose (LigCel) diet. Fish were grown for 57 days in 45-m^2 ponds, with 6 replicates per diet (Kabir et al., 2020).

References

Kabir, K.A., Verdegem, M.C.J., Verreth, J.A.J., Phillips, M.J., Schrama, J.W., 2019. Effect of dietary protein to energy ratio, stocking density and feeding level on performance of Nile tilapia in pond aquaculture. Aquaculture. 511.

Kabir, K.A., Verdegem, M.C.J., Verreth, J.A.J., Phillips, M.J., Schrama, J.W., 2020. Dietary non-starch polysaccharides influenced natural food web and fish production in semi-intensive pond culture of Nile tilapia. Aquaculture. 528.

Tinh, T.H., Hai, T.N., Verreth, J.A.J., Verdegem, M.C.J., 2021. Effects of carbohydrate addition frequencies on biofloc culture of Pacific white shrimp (Litopenaeus vannamei). Aquaculture. 534.

BIOFLOC AND POND-BASED IMTA: NEW SUSTAINABLE SYSTEMS FOR AQUACULTURE

F.N. Vieira[1]*, M.A. Martins[1], W.C. Valenti[2], P.C. Abreu[3], M. Kafensztok[4], J. Kimpara[5]

[1] Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Laboratório de Camarões Marinhos, Florianópolis, SC, Brazil
[2] Universidade Estadual Paulista - UNESP, Centro de Aquicultura, Jaboticabal, Brazil
[3] Universidade Federal de Rio Grande – FURG, Instituto de Oceanografia, Rio Grande, Brazil
[4] Primar Orgânica, Tibau do Sul, Brazil
[5] Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA meio norte, Parnaíba, Brazil.

* Presenting author: felipe.vieira@ufsc.br

Introduction

FAO forecasts that aquaculture must continue to grow rapidly because of the intensification of production systems, diversification of species and introduction of innovations and technologies to make production more efficient. Among the applied technologies in the intensification and diversification of the production, stands out the biofloc technology (BFT) and Integrated Multi-Trophic Aquaculture (IMTA).

BFT systems have been used in the production of Pacific white shrimp with high stocking densities with minimum water exchange rates, considerably reducing the production areas and water resources compared to traditional semi-intensive system. The BFT system is based on the presence of microbial aggregates. The manipulation of the carbon: nitrogen (C: N) ratio on the water favour the development of heterotrophic bacteria. This manipulation occurs by adding organic carbon based on the nitrogen concentration present in the feed and water. The manipulation of the C:N ratio favours the conversion of inorganic nitrogen in microbial biomass, maintaining water quality reducing the need for water exchange and increasing biosecurity. In addition, microbial aggregates can be used as a food source for animals. However, the adjustment of production parameters, like C:N ratio and water turbulence, can improve shrimp production.

IMTA is a production system that integrates species from different trophic levels in the same growing environment, resulting in converting the residues from one species into a food source or fertilizer for another (Chopin et al., 2001). The application of IMTA to the rearing of Pacific white shrimp in biofloc could contribute to increase productivity based on the diversification of the production, which can positively affect the profitability. In addition, the use of species of different trophic levels would allow the maximum utilization of the nutrients present in the solids generated in the biofloc system. Also, IMTA is an alternative to increase the profitability of farms, as it is possible to diversify the production and synergic effects of organisms living in the same space results in overall higher productivity. For example, seaweeds assimilate potentially polluting inorganic nutrients in the water, releases compounds that may increase shrimp immunity and is a high-value product in the nutraceutical market; oysters are filtering organisms that may improve water quality and also provide high-value biomass. The transition from monoculture to IMTA is a demand from the shrimp industry to match sustainability, but the lack of commercial-scale pond-based IMTA research has limited the adoption IMTA systems by farmers.

The present work aims: 1) Enhance the Biofloc Technology for shrimp rearing by adjustments aeration flow rate and the use of biofilm; 2) Evaluation shrimp integration with mullet and seaweeds (*Ulva ohnoi*) in biofloc system; 3) Design a shrimp - seaweed - oyster IMTA production system on a commercial scale to enhance profitability, ensure sustainability and promote the circular economy of aquaculture enterprises besides conserving natural resources.

Preliminary results

The nitrification process was more efficient in tanks with biofilm and higher air flow rate (33.75 L/min), presenting smaller concentrations of ammonia and nitrite than the BFT treatment. Similarly, treatments with biofilm and stronger flow rate had better shrimp growth performance.

Both inclusions of mullet and seaweed to shrimp rearing in biofloc system increased the yield e system efficiency. The presence of mullet resulted in lower biofloc concentrations, compared to the monoculture of shrimp, thus confirming that mullet can be used to control solids concentrations originating from shrimp production in a BFT system. The best stocking density for *U. ohnoi* is 2g L^{-1}, resulting in a higher specific growth rate.

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An IMTA system prototype was conceived, combining the culture of the Pacific white shrimp, *L. vannamei*, with Brazilian native oyster, *Crassostrea gazar*, and algae, *Ulva* sp. Tests of this prototype are running on the Primar Organic farm placed in Northeast Brazil, a tropical region. Preliminary results showed that the species is compatible, but some adjustments in the density and proportion of species are needed.

Acknowledgements
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AquaVitae website: https://aquavitaeproject.eu

References

Samocha T.M., Schveitzer R., Krummenaeur D., Morris T.C., 2012. Recent advances in super-intensive, zero-exchange shrimp raceway systems. Glob. Aquacult. Advocate 15, 70–71.

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.

WATER QUALITY MODELLING IN INTENSIVE CULTURE USING A WEIGHTED FUZZY INFERENCE SYSTEM

*Midory Esmeralda Vigueras Velázquez
Corresponding autor- midory.vigueras@gmail.com
Jose Juan Carbajal Hernández,Luis Pastor Sánchez Fernández, Margarita Hernández Martínez

The technology is used in all areas and aquaculture is not the exception. It has developed rapidly in the last fifty years. This work proposes a new computational model for water quality assessment in freshwater intensive cultured ponds, by using Weighted Fuzzy Inference Systems through a rule categorization process to preserve and help the growing and fish production.

Each species unique and its requirements for habitat, environment, and nutrition must adapt to these, but there are physical, chemical parameters which are necessary for coexisting and interact in any aquatic environment, as temperature, dissolved oxygen, pH, total ammonia and non-ionized ammonia. For this reason, those parameters were considered and measured

Water quality assessment

To show the proposed index performance, a comparison of CWI index against that proposed by the National Sanitation Foundation (NSF) and the Canadian Council of Ministers of the Environment (CCME) was performed. Those indexes are the most representative models for water assessment and can be adjusted to the species.

First, the NSF provides a good basis for freshwater quality assessment when weight is assigned to each parameter, and according to their importance in the ecosystem. However, the NSF score shows high results of water quality although some parameters are not in optimal conditions. In the same way, CCME constantly shows good water quality. This is because, in its calculation, an average of the parameter set compensates the bad conditions of one parameter with the good of another. In Fig. 2, it is possible to appreciate a great similarity between CWI, NSF and CCME.

In the first three row of Table 1, it has been observed that the dissolved oxygen level is low, and the other models do not punish it as severely, despite the importance of this parameter. On the other hand, there are cases where all the parameters are in the optimal range, but in the temperature parameter in row 5 the temperature is higher, and high temperature produces more chemical reactions, and the DO concentration decreased, the CWI had a better assessment of this situation. In the other indexes, this situation is less severely punished resulting overall a little difference between both evaluations.

Overall, the results of NSF and CCME in this context show a good result, but the values obtained are not entirely satisfactory, because they do not contemplate the importance of each parameter. CWI was designed for that purpose, enabling the monitoring and the assessment of high-accuracy for the studied species. This model emerges as an alternative, suitable and reliable tool for the aquaculture of any aquatic species, only ranges must be set up for each species.

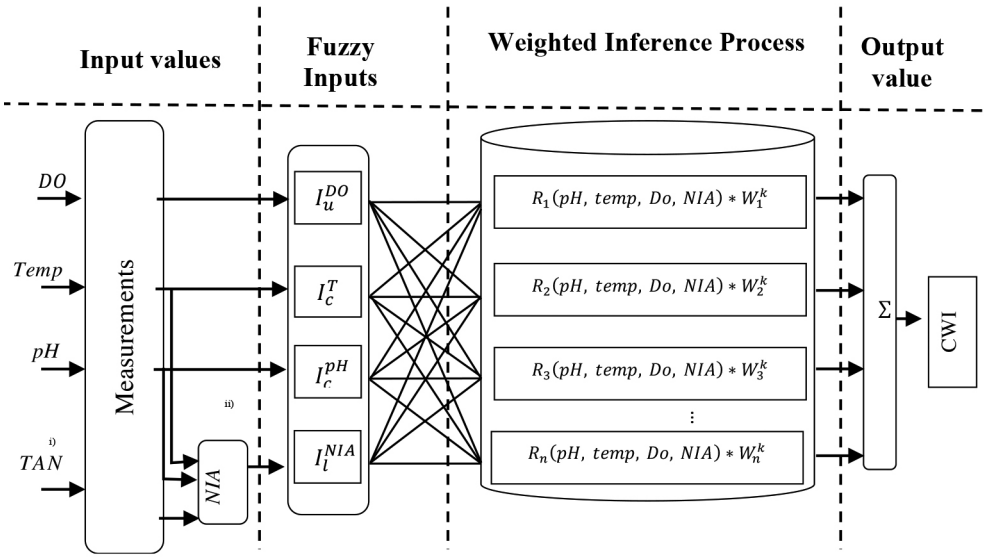

Fig. 1. TAN (i) and NIA (ii) are obtained by using the Nessler method and the correlation between pH and temperature proposed. W_n^k denotes the importance weights that are computed according the rule impact in the habitat.

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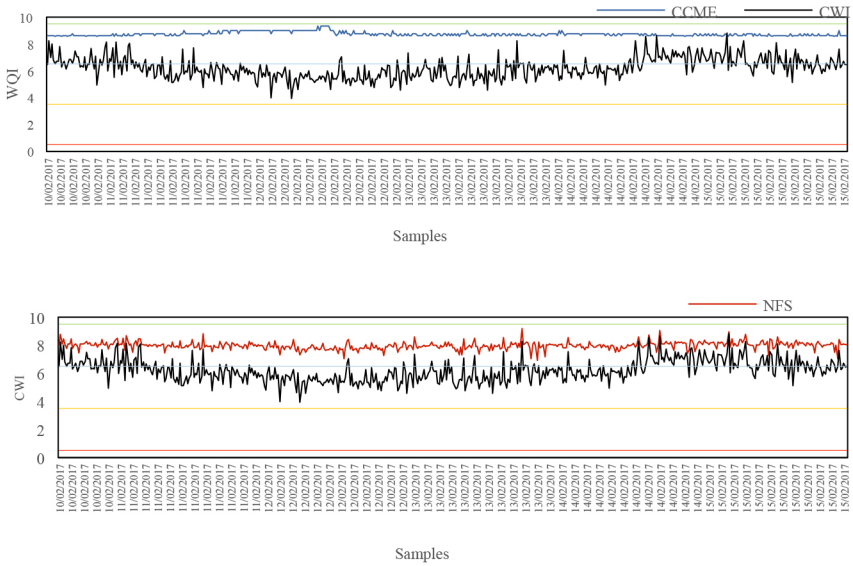
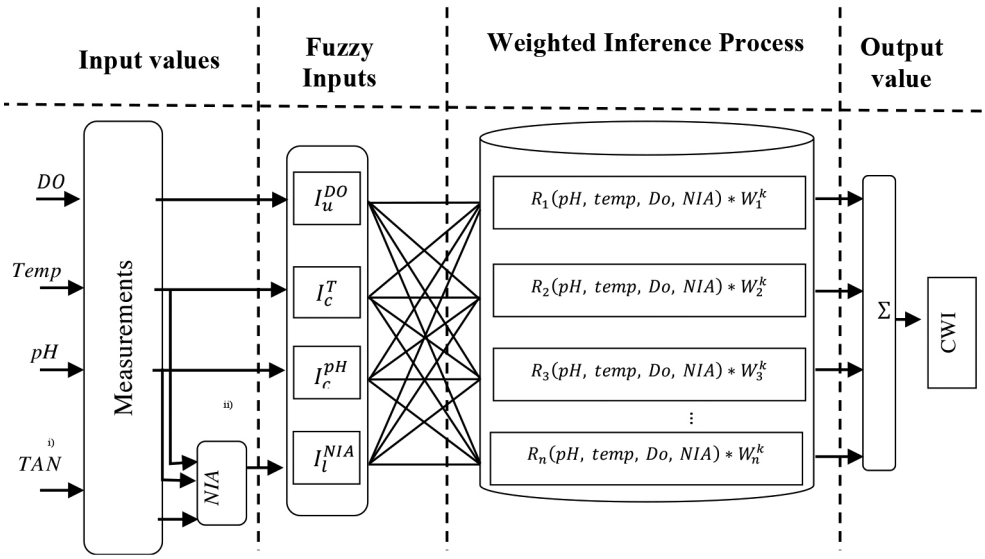


Fig. 2. Comparison between the CWI and CCME and NSF

Table 1. Numerical comparison between water quality indexes.

Date		Parameter measurements				Water Quality Indexes			
		pH	DO	Temp	NIA	NFS	CCME	FIS	CWI
12/02/17	1	7.069	2.97	21.48	0.005711	7.673	8.171	4.369	2.781
12/02/17	2	6.987	2.85	21.48	0.005536	7.622	8.518	4.310	2.730
13/02/17	3	7.347	4.29	20.51	0.010873	8.148	7.896	5.564	5.431
15/02/17	4	7.491	5.3	***21.48***	0.011419	8.404	7.733	6.131	5.973
15/02/17	5	7.465	5.46	***19.53***	0.011239	7.955	7.678	6.060	7.213



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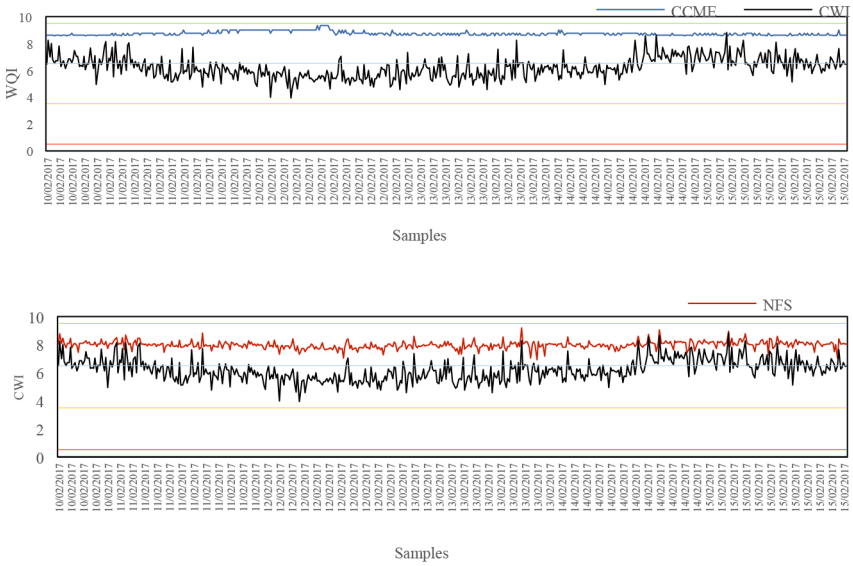

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15/02/17	5	7.465	5.46	***19.53***	0.011239	7.955	7.678	6.060	7.213

MODELLING INTERACTIONS AND FEEDBACKS BETWEEN INTEGRATED MULTI-TROPHIC AQUACULTURE AND THE RECEIVING ENVIRONMENT IN THE NORTH AND AEGEAN SEAS

Lauriane Vilmin and Luca A. van Duren*

Deltares, 2600 MH Delft, The Netherlands
E-mail: luca.vanduren@deltares.nl

Introduction

Integrated multi-trophic aquaculture (IMTA) has been identified as a promising solution for the sustainable development of aquaculture in many recent studies (Chopin et al., 2012). Benefits include waste recovery or transformation, increased growth and diversity of produced species and improved use of coastal area (Reid et al., 2011). These depend, however, on the scale of its application and on the characteristics inherent to the receiving environment. IMTA models come in crucial at the stage of designing IMTA setups to understand and predict their benefits and limitations.

In the scope of the EU H2020 IMPAQT project, we developed two far-field models (called herein North Sea model and Aegean Sea model) to investigate the effects and benefits of IMTA faming at two pilot sites that contrast in their farm settings and natural environment. The first site produces *Sacharina latissima* and blue mussels (*Mytilus edulis*) off the Dutch coast, in the Rhine region of freshwater influence, i.e., a nutrient rich environment with high throughflow. The second site is located in a more sheltered and highly oligotrophic environment, off the Turkish coast, and tests the benefits of growing *Ulva rigida* and mussels (*Mytilus galloprovincialis*) in the vicinity of seabass cages. We present here results of first scenario runs and discuss insights gained through these experiences.

Material and Methods

The two IMTA models are set up using coupled hydrodynamic and water quality modules from the Delft3D Flexible Mesh Suite (https://www.deltares.nl/en/software/delft3d-flexible-mesh-suite/). The horizontal and vertical grid resolutions are adapted to correctly resolve local flow patterns and stratification and capture the effects of the farms on water quality. The hydrodynamic module simulates currents, water levels, salinity and temperature. The water quality module incorporates biogeochemical processes affecting the growth of seaweed and mussels, and that can be affected by the dynamics of these living species (i.e., nutrient cycling, primary production and air-water exchanges). Special attention was paid to representation of phytoplankton dynamics to account for competition with seaweed species in nutrient-poor environments. The effects of fish cages are represented as additional nutrient and organic carbon loads. Seaweed and mussel metabolism dynamics are explicitly modelled, allowing for the simulation of feedback processes in the food chain, which is pivotal to IMTA.

Results and discussion

With the current model implementation, the North Sea results show that seaweed and mussel production can be increased to 10 tons/ha of seaweed and 5 tons/ha of mussels within a 6 km^2 farm, with very little environmental impact (in terms of nutrient depletion and oxygen concentrations and phytoplankton biomass). Upscaling of IMTA farming to potential future designated areas, further offshore and outside of the influence area of the Rhine plume would be more fruitful in terms of seaweed production, and less in terms of mussel production. This is due to higher nutrient turnover due to higher mixing, and lower phytoplankton biomasses (hence lower food availability for mussel cultivation). The environmental impacts of IMTA development in these areas, such as decrease of nutrient concentrations and of chlorophyll-a, is more visible, especially where water velocities are the lowest and hence retention times highest. Results also show advantages of IMTA farming, since, in the tested scenarios, mussel oxygen consumption is overcompensated by production by seaweed photosynthesis.

Results from the Aegean Sea model show that in such an oligotrophic environment, the additional nutrient input provided by fish production (and subsequent increase in phytoplankton biomass) is indispensable to be able to produce mussels. According to the model, the current IMTA setup does not allow for a reduction in nutrient emissions (and associated environmental impact) compared to fish monoculture. The level of nutrient concentrations remains too low for the survival of *Ulva rigida*, which is unable to extract the extra nutrient loads from the system. In the future, the Aegean Sea model could be used to test the potential cultivation of seaweed species better adapted to oligotrophic environments (provided nutrient uptake and growth parameters are available for these species), and its benefits for nutrient reduction.

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While the North Sea model was validated against routine water quality measurements throughout its domain, this step has not been yet carried out for the Aegean Sea model due to the lack of available data. Before being fit for operational use, these IMTA models should be also validated against temporal data describing farm dynamics (e.g., mussel and seaweed biomass time series and time series of nutrient emissions).

In a next step, these models could be refined by including feedback effects of seaweed fronds on the flow (increase in drag). These are at the moment not implemented, which might lead to an overestimation of nutrient flows through the farms, when seaweed densities are high, and thus to an overestimation of seaweed growth at the larger scale.

Conclusion

This work shows the different responses of (and interactions between) IMTA farm components and their footprint on the environment in both nutrient-rich and nutrient-poor environments. It also helps identifying controlling processes, which should be incorporated in future models, to improve their predictive value.

The models are currently valuable research tools to get more general insight in how ecosystems react to aquaculture and to assess the response of individual species to an increase in competition or an increase in food availability. It is clear that the current IMTA models still need some further development before they can be fully relied upon to take decisions, either regarding the management of a farm or the management of an ecosystem by regulators. However, the first developments are encouraging and open up various application potentials, both for farmers and for regulators.

References

Chopin, T. et al. 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Reviews in Aquaculture 4:209-220.

Reid, G. K. et al. 2011. Open-water integrated multi-trophic aquaculture (IMTA): modelling the shellfish component. Bull Aquacult Assoc Canada 109:3-12.

Acknowledgments

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SCALE CORTISOL IS POSITIVELY CORRELATED TO FIN INJURIES IN RAINBOW TROUT (*Oncorhynchus mykiss*) REARED IN COMMERCIAL FLOW THROUGH SYSTEMS

L. Weirup[1,2]*, C. Schulz[1,2], H. Seibel[1,2], J. Aerts[3,4]

[1]Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum (Germany)
[2]Institute of Animal Breeding and Husbandry, Kiel University, Hermann-Rodewald-Strasse 6, 24118 Kiel (Germany)
[3]Stress Physiology Research Group, Faculty of Sciences, Ghent University, Wetenschapspark 1, 8400 Ostend (Belgium)
[4]Stress Physiology Research Group, Animal Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food, Wetenschapspark 1, 8400 Ostend (Belgium)

E-Mail: weirup@gma-buesum.de

Introduction

A suboptimal farm management can lead to (chronic) stress and can result in external morphological damage, which both can have deleterious effects on fish performance and welfare (Iwama et al., 1997; Noble et al., 2012). As cortisol accumulates in elasmoid scales of teleost fish, scale cortisol has been shown as a biomarker for chronic stress (Aerts et al., 2015), while external morphological damage are generally accepted as indicators for impaired welfare (Noble et al., 2012 and 2018). Aim of this study was to investigate a potential correlation between chronic stress and the occurrence of external morphological damage and to measure the effect of management and water quality parameters on welfare of rainbow trout (*Oncorhynchus mykiss*) reared in commercial flow through systems.

Materials and methods

Welfare assessment and tissue sampling, on eight farms with rainbow trout during grow, was performed from May 14th to June 3rd of 2019. Data on management (water supply and -exchange, stocking density, feeding frequency) was provided by the farmers and water quality was analysed on site. Measured external morphological damage of fish (n=10 / farm) included eye-, skin- and fin injuries, as well as deformities and emaciation. Severity of each external morphological damage was scored using severity grades (SG) ranging from 0 (none) to 3 (severe) according to and partly revised after Hoyle et al., (2007), Noble et al. (2018) and Bass and Wall (Undated). Fifty ontogenetic scales per fish (n=10 / farm) were sampled and analysed using UPLC-MS/MS, as described in Aerts et al. (2015).

Results

Lower water supply and -exchange, higher stocking density and higher feeding frequencies resulted in higher outlet water ammonium / ammonia, nitrite, nitrate, pH, turbidity and temperature and reduced oxygen water content. Measured eye- and skin injuries, deformities and emaciation occurred less frequently, while fin injuries were common and differed significantly in severity between farms. Results on all fish across farms showed a highly positive correlation between scale cortisol and fin injuries. Scale cortisol and fin injuries were both positively correlated to the duration of a complete water exchange in the tank and negatively correlated to the quantity of water supplied and the average water supply per kg fish, while fin injuries were further positively correlated to stocking density and feeding frequency. Scale cortisol was positively correlated to ammonium / ammonia, nitrite, nitrate, pH and temperature in the outlet water, while fin injuries were positively correlated to ammonium / ammonia, nitrite and turbidity in the outlet water.

Discussion and conclusion

Findings on a lower water supply / -exchange, low velocity, a decreased water quality, high stocking density and / or high feeding frequency correlating to fin injuries and / or stress are in accordance with other studies. The observed significant positive correlation of scale cortisol and fin injuries indicates that the occurrence of fin injures is potentially associated with stress. Scale cortisol findings demonstrate fin injuries to be a highly relevant welfare issue and indicator on farms. A combined effect of injuries occurring in relation to stress or vice versa potentially amplifies the effect on welfare, compared to stress or fin injuries occurring solely. Further the observed significant positive correlation of management and water quality with both scale cortisol and fin injuries, point out the impact of rearing conditions on the stress level and external appearance of fish and therefore likely welfare in general.

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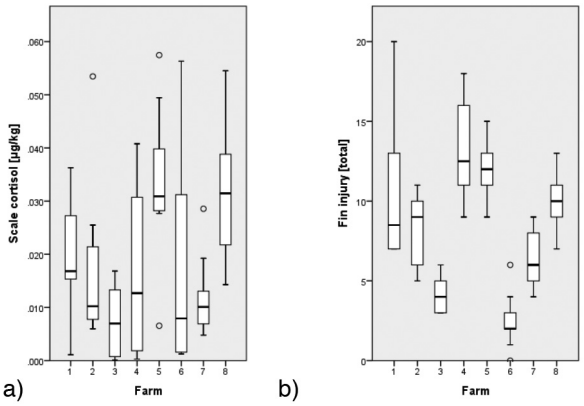

a) b)

Figure 1 Per farm a) scale cortisol in µg/kg; b) total fin injury. Number of fish per farm is n=10, (for graphical reasons only, Figure a: Farm 1 n=8 and Farm 8 n=9).

References

Aerts J., Metz J.R., Ampe B., Decostere A., Flik G., Saeger S.D. 2015. Scales Tell a Story on the Stress History of Fish. PLoS One 10, 1–17. https://doi.org/10.1371/journal.pone.0123411.

Bass N., Wall T., Undated. A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, p. 2.

Hoyle I., Oidtmann B., Ellis T. Turnbull J., North B., Nikolaidis J., Knowles T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 270, 142–148. https://doi.org/10.1016/j.aquaculture.2007.03.037.

Iwama G.K., Pickering A.D., Sumpter J.P., Schreck C.B. 1997. Fish Stress and Health in Aquaculture. Society for Experimental Biology Seminar Series: 62. Cambridge University Press, pp. 278. ISBN: 9780521281706.

Noble C., Gismervik K., Iversen M.H., Kolarevic J., Nilsson J., Stien L.H., Turnbull J.F. 2018. Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare, pp. 351. ISBN: 978-82-8296-556-9.

Noble C., Hernán A., Cañon Jones H.A., Damsgård B., Flood M.J., Midling K.O. Roque A., Steinar Sæther B., Yue Cottee S. 2012. Injuries and Deformities in Fish: Their Potential Impacts upon Aquacultural Production and Welfare. Fish Physiol. Biochem. 38 (1), 61–83. https://doi.org/10.1007/s10695-011-9557-1.

FISH WELFARE EVALUATION INDEX BASED ON THE PREVALENCE AND SEVERITY OF EXTERNAL MORPHOLOGICAL DAMAGE

L. Weirup[1,2]*, C. Schulz[1,2], H. Seibel[1,2]

[1]Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum (Germany)
[2]Institute of Animal Breeding and Husbandry, Kiel University, Hermann-Rodewald-Strasse 6, 24118 Kiel (Germany)

E-Mail: weirup@gma-buesum.de

Introduction
Scientific studies regarding fish being sentient with cognitive capacities and pain perception, raised the need to quantify fish welfare accurately by implementing standard procedures for welfare assessment in aquaculture. Aim of this study was to define on-farm welfare indicators that are reliable, valid and practicable to set up a welfare evaluation index tested by the example of rainbow trout (*Oncorhynchus mykiss*) in commercial flow-through systems in Germany. Potential on-farm welfare indicators tested included management (tank design, water supply and -exchange, stocking density, feeding frequency), water quality (temperature, oxygen, pH, nitrogen compounds, turbidity), behaviour and health observations (disease- and low welfare indicating behaviours, social interactions, schooling, activity level, crowding, external morphological damage), external morphological damage (eye haemorrhages, cataract, exophthalmia, scale loss, skin haemorrhages, skin- and snout wounds, dorsal-, caudal-, pectoral-, anal- and pelvic fin injuries, operculum shortage, vertebral- as well as upper- and lower jaw deformities, emaciation), macroscopic organ health (liver colour) and growth parameters (CF, SSI, HSI, CSI). To gain additional in-depth insights into the effects caused by differences in management and water quality, various health and stress parameters (osmolality, histology, scale cortisol, molecular marker) were assessed and correlated to the on-farm welfare indicators.

Materials and methods
Welfare assessment and tissue sampling, on nine farms with rainbow trout during grow- out, was performed from May 14th to June 3rd of 2019. Data on management was provided by the farmers and water quality was analysed on site. Behaviour and health observations were conducted by three independent observers. Measured external morphological damage was assessed on 30 fish per farm. Severity of each external morphological damage was scored using photographic images with severity grades (SG) ranging from 0 (no damage) to 3 (severe damage) according to and partly revised after Hoyle et al., (2007), Noble et al. (2018) and Bass and Wall (Undated). Further 10 fish per farm were sampled for blood to analyse molecular markers by Fluidigm Biomark HD-System, for organs (liver, spleen, kidney, head kidney, heart and gills) to conduct histological analysis, and for ontogenetic scales to analyse scale cortisol by UPLC-MS/MS.

Results and discussion
Management, water quality and behaviour correlated to external morphological damage, indicating that suboptimal rearing conditions result in a visibly degraded external appearance of fish. Results further showed a positive correlation of scale cortisol as an indicator for chronic stress as well as the sum of pathohistological changes as an indicator for health, in relation to the sum of external morphological damage. These findings demonstrate external morphological damage to be highly relevant welfare indicators.

Management, water quality and behaviour are welfare indicators of an extremely high relevance, which should be monitored closely on farms and be implemented according to recommendations and best practices in aquaculture to prevent welfare impairment of any kind in advance. For the final welfare evaluation index however, management, water quality, behaviour, liver colour and growth parameters were excluded as they were challenging in terms of reliability, validity and / or practicability. Further, external morphological indicators were excluded that occurred rarely on the nine farms (vertebral-, upper- and lower jaw deformities) or were potentially induced by netting (scale loss, eye haemorrhages). The remaining external morphological indicators (cataract, exophthalmia, skin haemorrhages, skin- and snout wounds, dorsal-, caudal-, pectoral-, anal- and pelvic fin injuries, operculum shortage, emaciation) can be assessed by using photographic images that depict the different severity grades of impairment. Such method will be easy and fast to learn and to apply, without incurring material costs. The welfare evaluation index summarizes the external morphological damage including severity and prevalence, resulting in categories of good, moderate and poor welfare. The welfare evaluation index can be employed by fish farmers, and by monitoring- or certification programs to determine and control the state of fish welfare. The index serves as a first step in assessing the welfare state of fish, and can be followed by further measurements and adjustments to determine and eliminate the causes behind fish welfare impairments.

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References

Bass N., Wall T., Undated. A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, p. 2.

Hoyle I., Oidtmann B., Ellis T. Turnbull J., North B., Nikolaidis J., Knowles T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 270, 142–148. https://doi.org/10.1016/j.aquaculture.2007.03.037.

Noble C., Gismervik K., Iversen M.H., Kolarevic J., Nilsson J., Stien L.H., Turnbull J.F. 2018. Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare, pp. 351. ISBN: 978-82-8296-556-9.

TRANSPORTATION OF LIVING FISH –OPTIMISATION OF RELEVANT WATER QUALITY PARAMETERS DURING COMMERCIAL TROUT TRANSPORT WITH AND WITHOUT AERATION

Tamara Wind[a]*, Stephan Hofer[b], Alexander Brinker[a,c], Mark Schumann[a]

[a] Fisheries Research Station of Baden-Württemberg, Argenweg 50/1, 88085 Langenargen, Germany
[b] Hofer Forellen GmbH, Stuttgarter Straße 66, 78727 Oberndorf a. N.
[c] University of Constance, Institute for Limnology, Mainaustraße 252, 78464 Konstanz, Germany
*Email: Tamara.Wind@lazbw.bwl.de

Introduction

Transport of live fish is a regular requirement in fish farming which represents an extraordinary situation for fish. Fish transports commonly take place in closed tanks with oxygen supply, no water exchange and distinctly elevated stocking densities compared to husbandry situation, resulting in a significant change of important fishrelevant water quality parameters. An air ventilation system could provide options for targeted control of those gas parameters.

To adequately address the general development of decisive water quality parameters during transport and their potential to detrimentally affect fish welfare, this research project investigated the development of a set of important fish-relevant water quality parameters during commercial transports of rainbow trout. Further, the control options by aeration using an onboard-system to measure gas parameters and their complex interactions with other water quality parameters were examined.

Materials and methods

Commercial transports of rainbow trout *Oncorhynchus mykiss* with an average transport distance of 180 km and a transportation time of around 3.5 h were accompanied. The transportation tanks had a volume of circa 2 m³ and loading weight was about 400500 kg. In the ventilated transports, ventilation was added shortly after loading and adjusted to 11 L/ min of atmospheric air. Trout were fasted two days prior to transport.

During transports, the pH (pH 320 pH Electrode SenTix 41, WTW, Weilheim, Germany), CO_2 and oxygen as well as total gas pressure (CO_2 portable and HandyPolaris, OxyGuard International A/S, Farum, Denmark) were directly measured every 30 min. Samples for nitrate, nitrite and ammonia were taken by manually pumping water out of the tank (fuel pump with pump ball). Furthermore, 0.16 g of nitrification inhibitor (formula 2533, TCMP, Hach, Düsseldorf) was added to inhibit possible ongoing nitrification. Later on in the lab, the probes were filtered and total ammonia nitrogen, nitrate and nitrite (Nitrate LCK 339, Hach GmbH, Weinheim, Germany, Nitrite-test 1.14776.0001, Merck KGaA, Darmstadt, Germany) were determined photometrically (IGKB 2000/ DR 6000 UV-VIS Spectrophotometer, Hach GmbH, Weinheim, Germany). NH_3 was calculated with the "Ablaufwasser-Rechner" (www.lazbw.de), while alkalinity was determined by titration (ISO 9963-1:1994) and hardness was analyzed photometrically (Hardness LCK 327, Hach GmbH, Weinheim, Germany).

To analyze stress parameters, 2 mL of venous blood from 10 fish pre- and posttransport were taken. Blood glucose was determined immediately afterwards (ACCU CHEK Aviva, Roche Diagnostics GmbH, Mannheim, Germany). A part of the blood was filled in Lithium-Heparin tubules (25l U/mL Blood, Sarstedt AG & Co. KG, Numbrecht, Germany). Samples for hematocrit were taken from this blood and centrifuged on-site (10 min, 12.500 rpm, HEMATOCRIT 210, Andreas Hettich GmbH und Co. KG, Tuttlingen, Germany). The rest of the sample was also centrifuged and the gained plasma extracted with a pipette and frozen at -20°C (10 min, 3100 rpm, Mini Fuge PLUS, STARLAB International GmbH, Hamburg, Germany). The urea concentration of the blood plasma was analyzed in the lab (LT-UR 0100, Labor und Technik LT-Sys, Berlin, Germany; Spectrophotometer ND-2000 UV-Vis, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

Results & Discussion

During transportation of trout various water quality parameters increased strongly. In the unventilated transports, CO_2 accumulated nearly exponentially and reached values up to 80 mg/L, while ammonia increased linearly to 5 mg/L. Oxygen was oversaturated and total gas pressure below saturation. pH decreased in correlation with increasing CO_2, resulting in a decelerated increase of ammonia.

During the ventilated transports the CO_2-concentration decreased to 20 mg/L, while oxygen saturation was more consistent overall. The decrease in CO_2 led to an increase of pH resulting in a final ammonia-concentration three-times higher than in unventilated transports.

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The analysis of stress parameters did not reflect the differences occurring in the two transportation variants or any critical values for animal welfare. However, a reliable estimation of the effects these values have on trout is hardly possible with current diagnosis tools since exposure time is short compared to the chronic exposure for which they are developed. However, as extreme concentrations should be avoided generally the study offer some insights: the aeration system, as well as the initial water quality parameters, had a predictable impact on the development of water quality parameters during transport. High initial pH-values favored an excessive enrichment of ammonia. To keep ammonia toxicity low a certain increase of CO_2 is desirable, for which reason the aeration should be kept low. On the other hand, if initial pH is low, a higher aeration could help prevent a further decrease of pH and, simultaneously, control the CO_2concentration. With the developed knowledge and a flexible aeration system at hand, unwanted peaks of relevant water quality parameters during transport can be efficiently anticipated and avoided.

NATURAL PLANT EXTRACTS MODULATE GROWTH PERFORMANCE, OXIDATIVE STATUS AND STRESS RESISTANCE OF SENEGALESE SOLE POSTLARVAE

Maria 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

Aquaculture is the fastest-growing animal production industry and one of the main sources of aquatic protein for human consumption (FAO, 2020). Nutrition is recognized as a key factor to shape fish growth and health status, which are essential to achieve a more sustainable and competitive industry. Plant based extracts such as curcumin, green tea, and grape seeds are known for their high amount and diversity of polyphenols. These bioactive molecules have high antioxidant capacity making them attractive additives to include in fish diets, to potentially improve the antioxidative status of fish, hence, enhancing growth and stress resistance of farmed fish (Abbas, 2017). The aim of this study was to assess the effect of those natural antioxidants in growth performance, muscle morphometry, oxidative status and thermal stress resistance of Senegalese sole postlarvae.

A commercial diet was used as control (CTRL) and then supplemented with curcumin (CC), green tea (GT) and grape seeds (GS) extracts. These four diets were fed to sole postlarvae for 25 days, when fish were sampled to evaluate growth performance, muscle cellularity and oxidative status. The expression of genes related to growth were analysed in muscle and those implicated in antioxidant defences were analysed in the digestive cavity. The remaining fish were then submitted to a thermal stress after which oxidative status of the fish was evaluated, as previous described.

CC and GS diets significantly improved sole growth performance when compared to the CTRL (Fig. 1). Sole fed CC diet had larger muscle cross sectional area, a higher number of muscle fibres and an increased proportion of large-sized fibres (>25 μm) compared to those fed the CTRL. Concomitantly, the dietary inclusion of curcumin resulted in a significant up-regulation of the *myogenic differentiation 2* (*myod2*) and the *myomaker* (*mymk*) transcripts in the muscle.

Moreover, sole fed CC showed a decrease in the levels of oxidative stress related biomarkers (heat shock protein 70 and glutathione-S-transferase (GST)) compared to the CTRL fish; this might be due to direct antioxidant capacity of this additive. In opposition, sole fed GT and GS diets presented higher lipid peroxidation and protein carbonylation (PC), suggesting a potential pro-oxidant effect of these dietary supplements. However, after thermal stress, fish from GT and GS diets were able to revert such pro-oxidant effect and showed improved oxidative status by preventing an increase of PC and decrease of antioxidant glutathione (GSH) in sole as observed in the CTRL and CC fish (Fig. 2).

Overall, these results demonstrate that the dietary supplementation with curcumin and grape seed extract promoted Senegalese sole growth. In the case of curcumin, enhanced growth was associated with both hyperplasia and hypertrophy of muscle fibres, through an up regulation of myogenic factors. However, the use of green tea and grape seed extracts in fish diets will require further evaluation to fully explore the potential of these natural antioxidants and identify the most adequate inclusion level for Senegalese sole diets.

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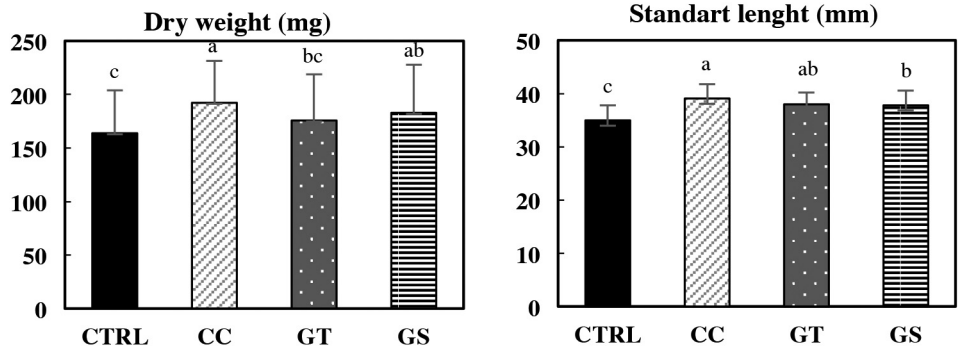

Figure 1. Growth performance indicators of Senegalese sole postlarvae fed the experimental diets (CTRL, CC, GT and GS) at the end of the growth trial (70 DAH). Values are presented means ± SD. Different superscript letters indicate significant differences (P < 0.05, 1-way ANOVA) between the dietary treatments.

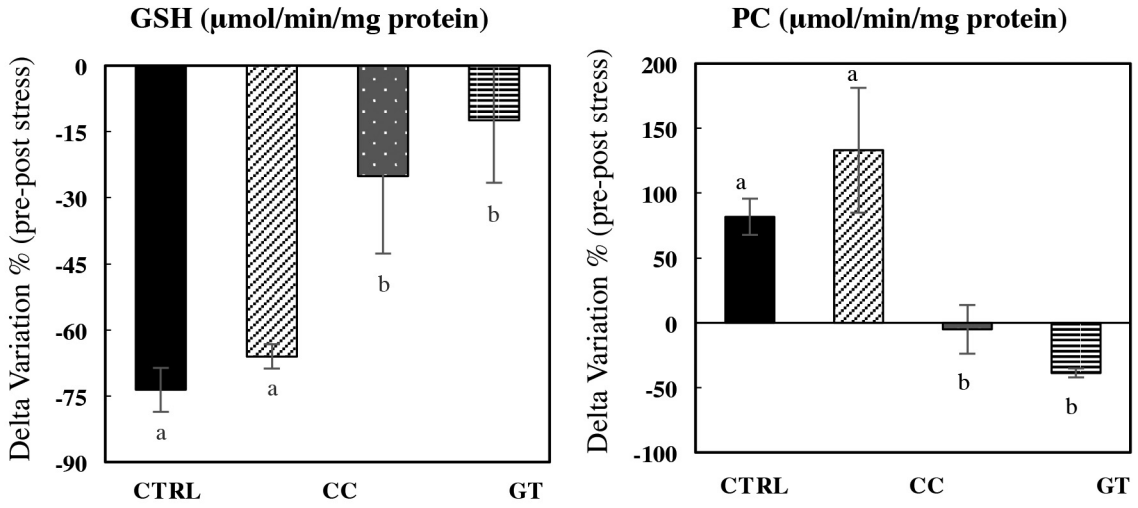

Figure 2. Delta variation (%) of the response of oxidative stress-related biomarkers from standard to acute stress conditions. Values are presented means ± SD. Different superscript letters indicate significant differences (P < 0.05, 1-way ANOVA) between the dietary treatments.

Acknowledgements

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References

Abbas, M. et al. (2017). Natural polyphenols: An overview. Int. J. Food Prop. 20, 1689–1699.

FAO (2020). The State of World Fisheries and Aquaculture. Sustainability in action. Rome.

ASIAN SEA BASS *Lates calcarifer* AQUACULTURE IN THE SAUDI ARABIA

B. C. Young [1, 2*], R. H. Alfaggeh [1] and I. AlMoutiri [1]

[1] National Fisheries Center, Ministry of Environment, Water & Agriculture, Post Office Box 9612, Jeddah 21423, Kingdom of Saudi Arabia
[2] Taiwan International Cooperation and Development Fund, Taipei 11047, Taiwan
[*]Corresponding author
E-mail address: bensimmonsnpust@gmail.com

Introduction

Presently, the total aquaculture production of Saudi Arabia is ~72,000 tons, and shrimp farming accounts for ~70% of the total aquaculture production. The primary farmed fish species Sabaki tilapia (*Oreochromis spilurus*), Nile tilapia (*Oreochromis niloticus*), Asian sea bass, and gilt-head bream (*Sparus aurata*). Because of cultural technology support, and environmental factors, Asian sea bass farming has been on the rise in Saudi Arabia since the 2010s. It is noteworthy that the Asian sea bass industry in Saudi Arabia is still popular with market demand and artificial propagation. By contrast, the fingerling source of gilt-head bream depends on imports (Young et al., 2020).

Despite Asian sea bass becoming more prevalent in aquaculture production in Saudi Arabia, there is a lack of information on the operations costs. Therefore, we conducted a farm survey that focused on the types of Asian sea bass aquaculture practices in Saudi Arabia. This study aimed to obtain a better understanding of the current business situation and operating costs that limit the Asian sea bass farming industry process in Saudi Arabia.

Methods

Purposeful sampling is used to select survey participants. The survey was conducted in 2019. A total of 65 Asian sea bass aquaculture-related personnel were sampled from all primary aquaculture companies in Saudi Arabia. In addition to the primary content in questionnaires, the operating costs were analyzed.

Results

Table 1. Annual average proportional costs of the Asian sea bass culture

Items	Small-scale (<10 ha)	Medium-scale (10–25 ha)	Industrial-scale (>25 ha)
1. Fry/fingerling	5.24%	5.41%	5.29%
2. Feed	70.33%	60.76%	50.06%
3. Fertilizer	0.80%	0.65%	0.36%
4. Labor	15.51%	15.66%	18.98%
5. Harvesting and marketing costs	0.23%	2.14%	8.81%
6. Utilities	1.50%	2.95%	4.06%
7. Administrative costs, [a]	2.29%	4.92%	5.81%
8. Depreciation	6.1%	7.51%	6.63%

[a] Administrative costs include equipment, medicine, and rent.

Discussion

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Discussion

Our results indicate that the primary costs of Asian sea bass farming in Saudi Arabia were feed, labor, and fry costs, which are in agreement with studies on Asian sea bass in Asia and Australia. Chithambaran (2019) suggested polyculture with high profitability, our result reported that 98.5% respondents would apply to polyculture. Several studies concluded that Saudi Arabian aquaculture producers face several problems regarding the lack of a broader aquaculture development strategy, absence of local hatchery facilities or inadequate production, diseases, and a shortage of well-trained personnel. We reported that difficulties in farming management are primarily attributed to high production costs, price instability, and disease problems.

References

Chithambaran S. 2019. Growth and Predator-prey interaction of Asian seabass, Lates calcarifer and Sabaki Tilapia, Oreochromis spilurus in Polyculture System. Thalassas 35: 215–221.

Young BC, Alfaggeh RH, and Al Moutiri I. 2020 Larviculture of Snubnose Pompano under conditions of high salinity. North American Journal of Aquaculture 10.1002/naaq.10173

ENERGY AND NUTRIENT BALANCE OF *Ulva* sp. AND EUROPEAN SEA BASS CO-CULTURE IN RAS UNDER TWO DIFFERENT DIETS

C. Zantioti*[1], E. Chatzoglou[1] K. Kousouli[1], N. Tsaoussi[1], H. Miliou[1]

[1]Agricultural University of Athens, Laboratory of Applied Hydrobiology, Iera Odos 75, 11855 Athens, Greece
E-mail: xristinazant95@gmail.com

Introduction

Nitrogen (N) and phosphorus (P) are the main pollutants dominating intensive aquaculture. Feed is the main N, P and energy source in aquaculture systems. In Recirculating Aquaculture System (RAS), a successive oxidation of ammonia to nitrite and finally to nitrates occurs in the biological filter. Thus, in RAS, nitrates and phosphates could accumulate over time and may harm fish growth and health. *Ulva* has the ability to simultaneously uptake N and P when integrated into intensive fish cultures. (Neori et al, 1996). In this study, European sea bass (*Dicentrarchus labrax*) and *Ulva* sp. were co-cultured in an indoor RAS, in order to assess the role of *Ulva* in N and P uptake, as well as the resulting energy flow. Two diets containing either vegetable oils (VO) in Trial 1, or fish oil from unavoidable unwanted sardines (FO) in Trial 2, were used. The co-culture of sea bass and *Ulva* in IMTA-RAS was a case study of the IMTA-EFFECT (Integrated MultiTrophic Aquaculture for EFFiciency and Environmental ConservaTion) project.

Materials and Methods

In each Trial, the RAS consisted of 3 lower-level tanks for seabass rearing and 3 upper- level tanks for *Ulva* cultivation (*Ulva* -RAS), whereas a similar RAS was used as a control with 3 tanks without *Ulva* (Control-RAS). Seawater flowed from each upper tank, either containing *Ulva* or not, to a fish tank and then to a gravel bed biofilter. From the outlet of bacterial biofilter, the seawater was pumped and inserted to the 3 upper tanks by separate inlets with a constant flow rate (F). Rearing duration was 12 weeks and culture conditions were similar in both Trials. Two iso-energetic and iso-protein diets, containing 10% of vegetable oils (rapeseed and palm oil 1:1) in Trial 1 and 10% sardine oil in Trial 2, were used. *Ulva* was collected from the Saronic gulf, and transferred to the upper tanks of *Ulva*-RAS with a density of 0,5-1 kg m^{-2}. *Ulva* was harvested and renewed weekly (Trial 2) or biweekly (Trial 1). In both RAS, water samples were collected (a) every morning before feeding from all tanks, the inlet and the outlet of the gravel biofilter, and (b) once a week before and after the cleaning of the tanks. More details about experimental conditions and nutrient analyses in water, feed, *Ulva* and fish tissues, as well as gross energy calculations are given in Chatzoglou et al. (2020). Total N in water (mg/L) was calculated as: Nitrite + Total Ammonia + Nitrate + Organic Nitrogen. Loss by cleaning for N or P was calculated as g/RAS = sum (N or P before cleaning – N or P after cleaning)/1000. Bacterial biofilter retention for N or P was estimated as g/RAS = [sum (N or P in biofilter inlet - N or P in biofilter output) 3 x F x 24]/1000. Digestible energy intake (DEI), branchial and urinary nitrogen loss (BUN), as well as fecal nitrogen loss, were estimated using nutrient digestibility coefficients given by Strand (2005) for European sea bass. Metabolisable energy intake (MEI) was calculated as the difference between DEI and the branchial and urinary energy loss (BUE). BUE= BUN x 24.85, where 24.85 is the amount of energy (in kJ) equivalent to 1 g excreted nitrogen, assuming that all nitrogen is excreted as NH3–N. The energy of N in RAS water was estimated with the same assumption. Retained energy (RE) was calculated as the difference between gross energy content of final and initial fish carcass. The total heat production (H) was calculated as the difference between MEI and RE according to Saravanan et al. (2012). Sankey diagrams were used to determine N, P and energy balance for each RAS. In each Sankey diagram, input data were N(g), P(g) or E(MJ) in initial RAS water and of the initial total fish biomass, as well as of the total consumed diet and total wild *Ulva* biomass (*Ulva* -RAS). The output included N(g), P(g) or E(MJ) in final RAS water, loss by cleaning, biofilter retention and fecal loss (N, E), as well as of final total fish and total cultured *Ulva* biomass (*Ulva*-RAS).

Results

In Sankey diagrams, P of fish and cultivated *Ulva* biomass was higher in the output than in the input, while unrecorded P was lower in *Ulva*-RAS compared to Control-RAS. In both Trials, regardless the fish diet, sea bass reared in *Ulva*-RAS showed increased P retention and lipid content compared to Control-RAS. Specifically, the dietary P that was excreted by sea bass in *Ulva* -RAS was 61,53% in Trial 1 and 64,88% in Trial 2, whereas in Control-RAS was 67,12% and 68,4%, respectively. Bacterial biofilter has no effect on P removal. However, it is evident that the final P in RAS water was higher in *Ulva*-RAS compared to Control-RAS in both Trials. N of fish and cultivated *Ulva* biomass was higher in the output than in the input, while in RAS water was lower in both Trials, however, the seaweed energy was reduced. The heat loss energy, was lower in *Ulva*-RAS compared to Control-RAS, i.e., 48,65% vs 42,47% (Trial 1) and 53,26% vs 51,43% (Trial 2) of the dietary energy. The RE was 44,51% (Trial 1) and 33,62% (Trial 2) of the dietary energy, in *Ulva*-RAS, whereas 38,11% and 32,09% in Control-RAS, respectively.

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Conclusions

Lipid content and retained energy were increased in sea bass reared in *Ulva*-RAS. Previous studies indicate that fish co-cultured with *Ulva* (Chatzoglou et al., 2020) or fed a diet supplemented with *Ulva*, may result in accumulation of lipid in muscles (Nakagawa et al., 1987). Moreover, the estimated heat loss decreased in *Ulva*-RAS compared to Control-RAS. This was more evident in sea bass fed the VO-diet, although a chronic stress may have occurred due to FO replacement. Nevertheless, it has been demonstrated that the storage lipids of fish fed algae to be active and readily mobilized to energy prior to muscle protein degradation in response to energy requirements, resulting to a suppression of body weight loss (Nakagawa et al., 1987). In this study, sea bass appeared to be able to consume *Ulva* fragments that passed from its cultivation tanks to fish rearing tanks through the RAS water. The increased P retention in fish co-cultured with *Ulva*, may be associated with the relatively increased lipid content. Despite the P retention from fish and *Ulva,* P in *Ulva*-RAS water was increased probably due to seaweed decomposition. *Ulva* absorbed N from water, demonstrating its supplementary role to the bacterial biofilter in N removal. It is known that N-limited seaweeds can take up large quantities of ammonia in fish pond effluents (Cohen & Neori, 1991). Differences in cultivated *Ulva* pigmentation were also observed, with the cultivated *Ulva* having a dark green color. These indications, along with the seaweed energy loss, may be linked to photosynthesis, a biochemical process that requires energy. It is concluded that *Ulva* contributes to a suppression of heat energy loss and removed N and P from the water, therefore improving the IMTA-RAS efficiency and contributing to a sustainable aquaculture. Nevertheless, further elucidation is required on the effects of *Ulva* culture on phosphorus accumulation in the aquatic environment.

References

Chatzoglou E., Kechagia P., Tsopelakos A., Miliou H., 2020. Co-culture of *Ulva* sp. and *Dicentrarchus labrax* in Recirculating Aquaculture System: effects on growth, retention of nutrients and fatty acid profile. Aquat. Living Resour., 33(19), 13 pp.

Cohen I., Neori A., 1991. *Ulva lactuca* biofilters for marine fishpond effluents I. Ammonia uptake kinetics and nitrogen content. Bot. Mar. 34, 475-482.

Nakagawa H., Kasahara S., Sugiyama T., 1987. Effect of *Ulva* meal supplementation on lipid metabolism of black sea bream, *Acanthopagrus schlegeli* (Bleeker). Aquaculture 62, 109-121.

Neori A., .Krom D.M.,. Ellner P.S., Boyd E.C., Popper D., Rabinovitch R., Davison J.P., Dvir O., Zuber D., Ucko M., Angel D., Gordina H. 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. Aquaculture 141, 183-199.

Saravanan S., Schrama J.W., Figueiredo-Silva A.C., Kaushik S.J., Verreth J.A.J., Geurden I., 2012. Constraints on energy intake in fish: The link between diet composition, energy metabolism, and energy intake in rainbow trout. PLoS ONE 7(4): e34743.

Strand A.A. (2005). Growth- and bioenergetic models and their application in aquaculture of perch (*Perca fluviatilis*). Vattenbruksinstitutionen, SLU, 901 83 Umeå

FEASIBILITY OF BLACK SCALLOP *Mimachlamys varia* (L., 1758) CULTURE IN SUSPENDED CONDITIONS IN WATERS OF THE BASQUE COAST (SE BAY OF BISCAY)

I. Zorita*, J.G. Rodríguez, J.M. Garmendia.

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

Introduction

Offshore aquaculture is an emerging activity in waters of the Basque Country (SE Bay of Biscay). Since 2011 the feasibility of the mussel (*Mytilus galloprovincialis*) culture has been demonstrated in open waters, but there is also interest in diversifying the bivalve mollusc production by cultivating new species with higher commercial value. Pectinids and especially the black scallop *Mimachlamys varia* (L., 1758) could be an alternative to the mussel production. Thus, the aim of the present study was to assess the growth and survival of black scallop, during the grow-out culture phase, starting with medium size organisms, up to commercial size in suspended aquaculture facilities of the Basque coast using different growing systems.

Material and methods

The study was conducted in two experimental sites of the Basque coast. The raft was installed in a sheltered area in the harbor of Mutriku and the offshore long-line system was located, in Mendexa, at two miles off the coast. Black scallop spat was obtained from a commercial hatchery in Brest (France), with an initial mean length of 23.6 mm and an initial mean body weight of 2.0 g. Black scallops were distributed in oyster cages and pots. In each pot 350 individuals were introduced while in oyster cages 160 black scallops were introduced. The experiment was run from June 2019 to March 2020. Mortality and growth (length and weight) were determined at each sampling time. At the end of the study, a one-way Kruskal Wallis followed by the Mann Whitney U-test was applied to determine differences in shell length, total weight and mortality among different growing systems and sites (alpha = 0.05).

Results

The survival of black scallops at both sites decreased throughout the exposure period but especially during the first 3 months (Fig. 1). Although in the last sampling black scallops reared in cages in offshore waters presented significantly higher survival values than those of pots, higher survivals were detected using pots than cages throughout the experiment. In contrast, in the sheltered area, no significant differences were detected between the two growing systems. As for site differences, black scallops reared in pots showed significantly higher survival values in offshore waters than in the sheltered area, but no significant differences were observed in black scallops reared in cages at different sites. Overall, the survival was low throughout the experiment, especially at the end of the study (5.6-28.1%) (Fig. 1). As for the growth, black scallops showed a progressive increase in the length and weight reaching a maximum value after 10 months of immersion (Fig. 2). Black scallops from offshore waters presented higher growth values than those reared at the sheltered area. Accordingly, higher growth was recorded when using pots as growing system. Black scallops reached a mean size and mean weight ranging from 40.5 to 49.9 mm and from 8.8 to 18.6 g, respectively (Fig. 2).

Discussion and conclusion

The cultivation of *M. varia* in waters of the Basque coast is biologically feasible. The most promising growth and survival values were obtained in black scallops suspended in the long-line system of Mendexa, in offshore waters. The best system for growing black scallops was the use of pots instead of cages. This was probably because in pots organisms had more attachment surface and more space, while in cages black scallops were overstocked. Black scallops of a mean size of 23.6 mm reached commercial size (*i.e.*, a mean size of 40 mm) in 10 months which is a relative short time period and comparable to previously recorded values in other areas (Cano et al., 2006, Rathman et al., 2017). However, survival values were low throughout the experiment suggesting that mortality should be reduced to make this species attractive from the commercial point of view. Hence, it should be noted that cleaning and maintenance works of the pots and the cages were not carried out, and this could have negatively affected growth and survival. Finally, this work provided relevant data for the diversification of bivalve mollusc production in the Basque coast.

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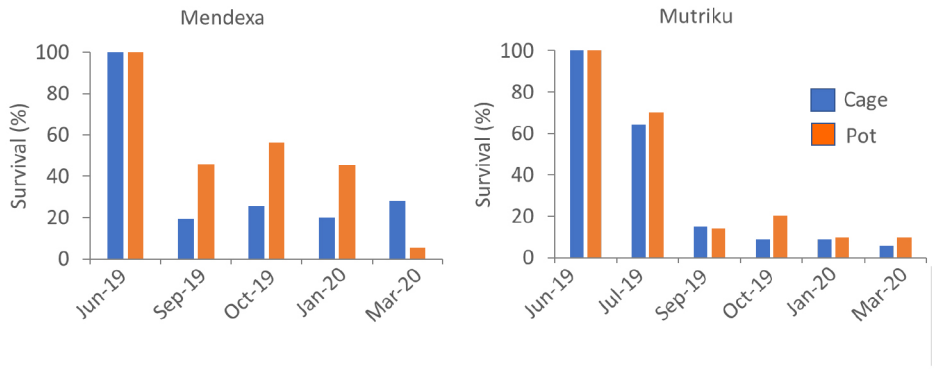

Fig. 1. Survival percentage (%) of black scallops at each sampling time in Mendexa (offshore waters) and Mutriku (sheltered area).

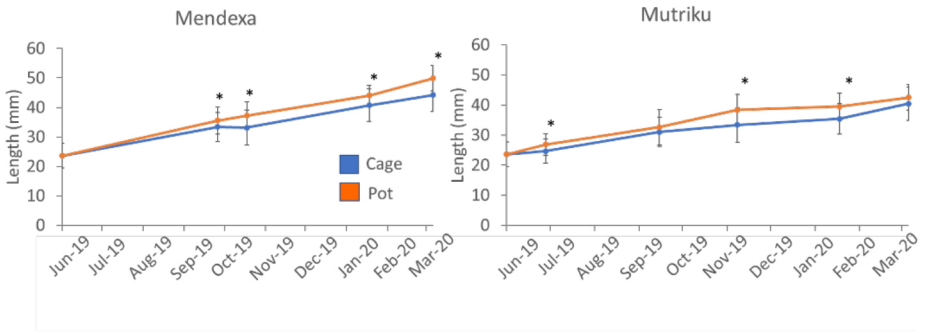

Fig. 2. Mean length of black scallops reared in Mendexa (offshore waters) and Mutriku (sheltered area).

References

Cano, J., Campos, M.J., López, F.J., Saavedra, M., 2006. Utilización de distintos sistemas de engorde en el cultivo de vieira *Pecten maximus* (L., 1758), zamburiña *Mimachlamys varia* (L., 1758) y volandeira *Aequipecten opercularis* (L., 1758) en Andalucía (España). Boletín del Instituto Español de Oceanografía 21, 283-291.

Rathman, M., Bolotin, J., Glavić, N., Barišić, J., 2017. Influence of water depth on growth and mortality of *Chlamys varia* (Linnaeus, 1758): implications for cage culture in Mali Ston Bay, Croatia. Aquaculture International 25, 135-146.